A Family of Serine Proteases Expressed Exclusively in Myelo-Monocytic Cells Specifically Processes the Nuclear Factor-κB Subunit p65 In Vitro and May Impair Human Immunodeficiency Virus Replication in These Cells

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Summary

Two groups of U937 promonocytic cells were obtained by limiting dilution cloning which differed strikingly in their ability to support human immunodeficiency virus 1 (HIV-1) replication. "Plus" clones replicated the virus efficiently, whereas "minus" clones did not. We examined these clones for differences in nuclear factor (NF)-κB activity which might account for the observed phenomenon. Stimulation of plus clones liberated the classical p50–p65 complex from cytoplasmic pools, whereas minus clones produced an apparently novel, faster-migrating complex, as judged by electrophoretic mobility shift assays. It is surprising that the faster-migrating complex was composed also of p50 and p65. However, the p65 subunit was COOH-terminally truncated, as shown by immunoprecipitation. The truncation resulted from limited proteolysis of p65 during cellular extraction which released particular lysosomal serine proteases, such as elastase, cathepsin G, and proteinase 3. These specific proteases are coordinately expressed and were present exclusively in the minus U937 clones, but not in the plus clones, as demonstrated in the case of cathepsin G. In addition, these proteases were detected in certain subclones of THP-1 and HL-60 cells and in primary monocytes, in each case correlating with the truncated form of p65. We demonstrate in vitro cleavage of p65 by purified elastase and cathepsin G. It is possible that particular serine proteases may have inhibiting effects on the replication of HIV-1 in myelo-monocytic cells. The data also demonstrate that special precautions must be taken when making extracts from myelo-monocytic cells.

The nuclear factor (NF)[1]-κB/Rel family of transcription factors is involved in the regulated expression of a large number of genes, particularly those participating in immune functions and acute phase reactions. Furthermore, several viruses, including HIV-1, are regulated by NF-κB/Rel. NF-κB is retained in the cytoplasm of unstimulated cells by the inhibitory IκB-α protein and can translocate into the nucleus in response to a number of stimuli (for reviews see references 1, 2). Activation of NF-κB involves phosphorylation and proteolytic digestion of IκB-α (3, 4). The NF-κB/Rel proteins, which include p50 (NF-κB1), p52 (NF-κB2), p65 (RelA), RelB, and c-Rel, can form many distinct homo- and heterodimeric complexes; those containing c-Rel, p65, or RelB can be potent transactivators, whereas homodimers of the p50 and p52 subunits generally are not (1, 2, 5–8).

NF-κB is a strong activator of HIV-1 transcription, particularly in mononuclear phagocytic cells (9–11). This is demonstrated by the inability of mutant, κB-deleted human and simian immunodeficiency viruses to replicate in these cells (G. Poli, and A. S. Fauci, unpublished observations; 12). Monocytes and macrophages seem to differ in their ability to support HIV replication (13–15). Whereas certain populations of tissue macrophages can produce high amounts of virions (16–21), only a small percentage of circulating monocytes harbor and release HIV, in vivo (22–25). Furthermore, primary monocytes are variably infectable in vitro, depending in part on whether they were maintained with GM-CSF or

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[1] *Abbreviations used in this paper:* DFP, diisopropyl fluorophosphate; EMSA, electrophoretic mobility shift assay; NF, nuclear factor; PR-3, proteinase 3; TPA, Tetradeconoylphorbol 13-acetate.

IFN-γ (26–31), supporting the notion that HIV replication is enhanced in more differentiated host cells (14, 29, 30, 32). Variability in HIV replication was observed also within different subclones of monocytic cell lines, such as U937 (33) and THP-1 (34, 35). In the latter case, it was speculated that the NF-κB complex was somehow specifically inhibited in those THP-1 cells that supported HIV replication poorly (34).

We report here the analysis of a number of U937 promonocytic cell subclones that differ in their ability to support HIV-1 replication. Whereas some clones ("plus") allow an efficient growth of HIV, "minus" clones provide an unfavorable environment for the virus. We demonstrate that "minus" clones, but not "plus" clones, contain a faster-migrating NF-κB complex, detectable also in certain other myelo-monocytic cells. This unusual complex is characterized by the presence of a COOH-terminal truncation of the p65 subunit, which is generated in vitro during extraction, due to the release of cell- and stage-specific lysosomal proteases such as cathepsin G (36), elastase (37, 38), and proteinase 3 (PR-3) (39). The faster-migrating NF-κB complex correlates with the presence of these proteases and correlates also with the inability of HIV to grow well in minus clone cells. Our data suggest the intriguing possibility that such serine proteases may negatively affect the life cycle of HIV-1. We show that treatment of these cells with macrophage differentiating agents, which converts them into productive hosts for HIV replication (26, 27, 29–31), also dramatically downregulates the proteases.

Materials and Methods

Cell Culture, Stimulations, and HIV Infections. The HL-60, THP-1, and U937 cell lines were obtained from the American Type Culture Collection (Rockville, MD). SB and AK represent two separate U937 cell cultures that were maintained in the laboratory. The NTera-2 cells (5, 6, 8) and the isolation of the U937 clones from the parental cell line SB (40) have been previously described. Cells were cultured in RPMI-1640 supplemented with antibiotics and 10% FCS (GIBCO-BRL, Gaithersburg, MD) for the U937 clones and THP-1 cells, or 20% FCS in the case of HL-60 cells. NTera-2 cells were maintained in DMEM (10% FCS). Monocytes were isolated from healthy donors and purified by elutriation, according to standard procedures (41). Cells were subsequently either harvested immediately, or put in culture in DMEM (10% FCS).

For the acute HIV infections, cells were plated in duplicate in 48-well plates at a concentration of 1–2.5 × 10^5 ml. The MN virus was added at a final concentration of 1X (1,000-fold dilution of a stock preparation containing ~10^{10} virus particles per ml). Culture supernatants were harvested twice a week and stored at −70°C until used. HIV infection was assessed by detection of Mg^{2+}-dependent reverse transcriptase (RT) activity in the culture supernatants, as described previously (42).

For the experiments shown in Figs. 2 and 5, U937 and HL-60 cells (0.5 × 10^6/ml) were stimulated with 200 U/ml of TNF-α (Genzyme Corp., Cambridge, MA) for the indicated period of time. In the other cases, cells were treated for 3–5 d as follows: (*a*) HL-60 cells (0.3 × 10^6/ml): 10^{-6} M retinoic acid (all-*trans*; Sigma Chemical Co., St. Louis, MO); 1.25% DMSO (Mallinckrodt Specialty Chemicals, Chesterfield, MO); 100 U/ml TNF-α; 16 ng/ml 12-O-Tetradecanoylphorbol 13-acetate (TPA, Sigma Chemical Co.); (*b*) U937 clone 12 (0.3 × 10^6/ml): 16 ng/ml TPA; (*c*) monocytes (0.5 × 10^6/ml): 10 ng/ml IFN-γ (Genzyme Corp.); 20 ng/ml GM-CSF (Genzyme Corp.); 200 ng/ml LPS (Sigma Chemical Co.); and 5 ng/ml TPA.

Expression Vectors, Cell Extracts, and Electrophoretic Mobility Shift Assay (EMSA). Whole cell extracts were prepared in buffer C supplemented with protease inhibitors, as previously described (5, 6). The protease inhibitors included: PMSF, 0.5 mM; antipain-dihydrochloride, 50 μg/ml; 4-amidinophenyl-methanesulfonylfluoride (APMSF), 40 μg/ml; aprotinin, 10 μg/ml; bestatin, 40 μg/ml; chymostatin, 20 μg/ml; E-64, 0.4 mg/ml; leupeptin, 0.5 μg/ml; pepstatin, 0.7 μg/ml; and phosphoramidon, 100 μg/ml. To obtain nuclear extracts, U937 cells were lysed with 0.2% NP-40 and NTera-2 cells with a Dounce homogenizer, as reported elsewhere (3, 5, 6, 8), and the resulting nuclei were salt extracted in buffer C. For the experiments shown in Fig. 5, whole cell extracts were made by resuspending the cells in a modified lysis buffer (50 mM Tris, pH 7.4, 100 mM NaCl, 50 mM NaF, 1 mM NaVO4, 30 mM pyrophosphate, 0.5% NP-40, and 0.5 mM PMSF) containing the protease inhibitor diisopropyl fluorophosphate (DFP; Sigma Chemical Co., 30 mM), and subsequent ultracentrifugation. The PMT2T-p50 and PMT2T-p65 expression plasmids and the PMT2T vector, the transfection protocol, the EMSA and antibody-supershift procedures, the palindromic-κB probe (PD-κB), and the Ig κ light chain-κB probe (Ig-κ-LC-κB), have all been reported (3, 5–8).

NF-κB heterodimers used in Figs. 4 and 7 were obtained from nuclear extracts of NTera-2 cells transfected with p50 and p65 expression vectors (4 μg each). For the experiment shown in Fig. 4 *A*, 1 μl of the NTera-2 nuclear extract was premixed with 1 μl of whole cell extract prepared from NTera-2 cells transfected with the insertless PMT2T vector (8 μg) or comparable amounts (as determined by measuring the protein concentration) of unstimulated clone 10 and clone 12 cells. The probe was added after 10 min of preincubation at room temperature.

For the experiment shown in Fig. 4 *B*, protease inhibitors were premixed (15 min at room temperature) with clone 12 cell extract before being added to the NTera-2 extract, as described above (see Fig. 4 *A*). The following final concentrations were used: bestatin, 2 mM; calpain inhibitor I, 3 mM; calpain inhibitor II (all from Boehringer, Indianapolis, IN), 3 mM; *N*-tosyl-phenylalanyl-chloromethyl ketone (TPCK, Sigma Chemical Co.), 0.5 mM; APMSF, 2 μg/μl; aprotinin (both from Beohringer), 0.6 μg/μl; *N*-tosyl-lysyl-chloromethyl ketone (TLCK, Sigma Chemical Co.), 3 mM; antipain-dihydrochloride, 2 mM; pepstatin, 0.8 μg/μl; leupeptin, 2 mM; phosphoramidon, 5 μg/μl; 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF; all from Boehringer), 2 mM; *N*-CBZ-L-phenylalanine-chloromethyl ketone (ZPCK), 1 mM; iodoacetamide (IAA), 3 mM; *N*-ethylmaleimide (NTM; all from Sigma Chemical Co.), 1 mM; BSA fraction V (Pentex, Kankakee, IL), 5 μg/μl; rabbit preimmune serum, 10%; DFP, 3 mM; 3,4-dichloroisocoumarin (DIC; both from Sigma Chemical Co.), 0.5 mM; α1-antitrypsin (Calbiochem-Novabiochem Corp., La Jolla, CA), 3 μg/μl; and chymostatin (Boehringer), 6 mM.

Purified elastase and cathepsin G enzymes were purchased as powder (all from Calbiochem Novabiochem Corp.) and resuspended in PBS (with 15% glycerol) at a 1 mg/ml stock concentration.

Immunoprecipitation and Western Blot. Immunoprecipitations were performed with rabbit anti-p65 antibodies on whole cell extracts (10^7 cells/reaction), as described (6, 8). The antigen–antibody complexes were precipitated with protein A–Sepharose, separated on a polyacrylamide gel, and then subjected to Western blot analysis: the primary antibody was an anti-p65 mouse polyclonal antibody directed against amino acids 276 to 502, and the secondary

antibody was a sheep anti-mouse ^{125}I-labeled antibody (Amersham Corp., Arlington Heights, IL).

The same standard conditions were used for the direct Western blot analysis, except that less extract was used (corresponding to 3×10^6 cells/lane) and the antigen–antibody reaction was developed with ^{125}I-labeled protein A (Amersham Corp.).

Antibodies. The antibodies used were: (*a*) a rabbit p65 NH_2-terminal peptide antibody (Fig. 2, *B* and *C*, Fig. 3, and Fig. 5 *B*; [5]); (*b*) a rabbit antibody directed to a COOH-terminal peptide of p65 (Figs. 3 and 5 *B*); (*c*) a mouse and a rabbit p65 polyclonal antibody directed against amino acids 276 to 502 (Figs. 3 and 6 *A*); (*d*) a p50 NH_2-terminal peptide antibody (Fig. 2, *B* and *C*; [5]); (*e*) an antibody recognizing an NH_2-terminal peptide of p52 (Fig. 2, *B* and *C*; [8]); (*f*) a c-Rel COOH-terminal antipeptide antibody (Fig. 2, *B* and *C*; [43]); and (*g*) a commercially available anticathepsin G antibody (Fig. 6 *B*; obtained from Calbiochem-Novabiochem Corp).

Results

Subclones of U937 Cells Differ in Their Ability to Support HIV-1 Replication. 45 cell clones were obtained by limiting dilution from the parental promonocytic cell line U937 SB (40). Two distinct groups of clones could be discerned based on their ability to support HIV-1 replication (Fig. 1). One group of clones (plus) exhibited kinetics of infection comparable with that of the parental U937 cell line (Fig. 1 *A*), whereas another group (minus clones) consistently exhibited markedly delayed kinetics (Fig. 1 *B*). The impaired ability of HIV to efficiently replicate in minus clones was not explained by the lack or reduced expression of membrane CD4 molecules in these cells (data not shown), in agreement with another study (33).

Extracts From U937 Minus Subclones Contain a Modified NF-κB Complex. We sought differences in U937 subclones that might account for the HIV growth data. Individual subclones and the U937 clone AK were treated with TNF-α or left untreated. Extracts from all cells gave rise to a single, constitutive band in EMSAs which was due to homodimers of the p50 subunit of NF-κB (▷, Fig. 2 *A*), as reported previously for another U937 clone (5; see also below). Stimulation with TNF-α induced an additional NF-κB binding activity, but individual clones differed in the appearance of this induced band shift: plus clones (Nos. *30*, *10*, *31*, and the parental line *SB*, Fig. 2 *A*) yielded the traditional p50-p65 NF-κB species (►, *left*, Fig. 2 *A*) which migrated more slowly than p50 homodimers, whereas minus clones (Nos. *34*, *12*, and the *AK* line, Fig. 2 *A*) yielded an induced and apparently novel, more diffuse complex which largely comigrated with the p50 homodimers (◄, *right*, Fig. 2 *A*). Of interest, an HL-60 subline stimulated with TNF-α also exhibited this faster-migrating complex (data not shown; see below also). Despite the difference in the mobilities of the TNF-α–induced complexes generated from the two types of clones, both complexes contained p50 and p65 polypeptides and not some other Rel-related proteins, as demonstrated with supershifting antibodies specific to these NF-κB subunits (5, 8, 43) (Fig. 2, *B* and *C*). The inducible, faster-migrating complex from

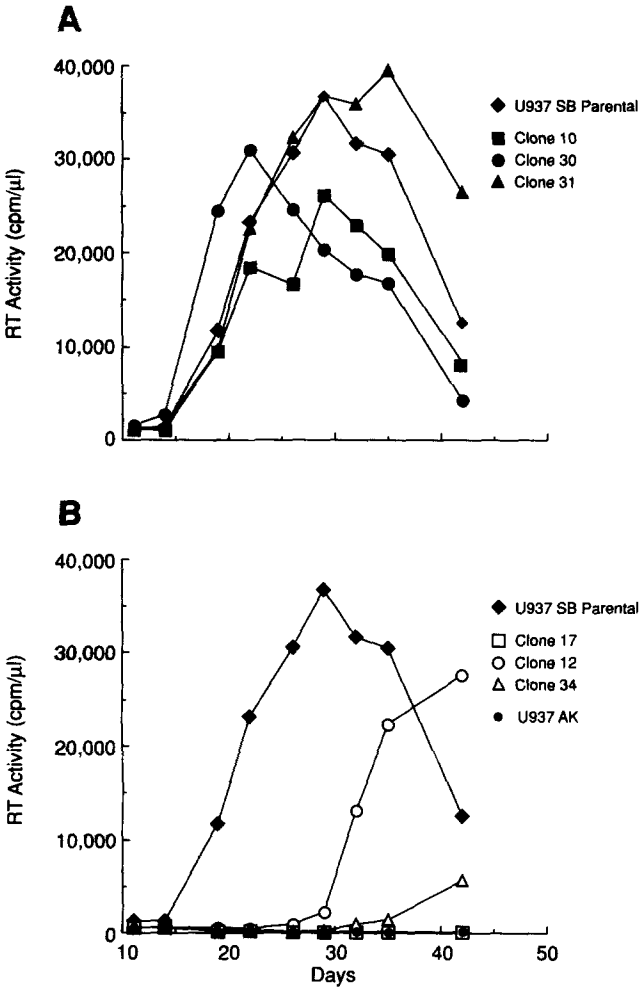


Figure 1. U937-derived cell clones differentially support HIV replication. Infections of plus (*A*) and minus (*B*) U937 cell clones in vitro, with the MN strain of HIV-1 (Advanced Biotechnology Inc., Columbia, MD). Experiments were repeated four times. The result of a representative infection is shown. Similar results were obtained also with the IIIB strain (data not shown). The parental U937 cell line SB and the U937-derived clones were negative for mycoplasma contamination as determined by two sequential screenings.

HL-60 cells was also predominantly composed of p50 and p65 (data not shown). The modified NF-κB complex was seen in cells of the myelo-monocytic lineage, but was not observed in many other cells including T, B, and nonhematopoietic cells (data not shown).

The Modified NF-κB Complex Contains a Truncated p65 Subunit. To characterize the novel NF-κB complex, extracts from various U937 clones were immunoprecipitated with three different antibodies directed against p65 and subsequently analyzed by Western blot with a polyclonal p65 antibody (Fig. 3). All three antibodies precipitated a protein with an apparent molecular mass of ~69 kD when extracts obtained from the parental line SB and clone 30 were used. This protein comigrated with p65 from other cell types (data not shown) and its immunoprecipitation was blocked by specific

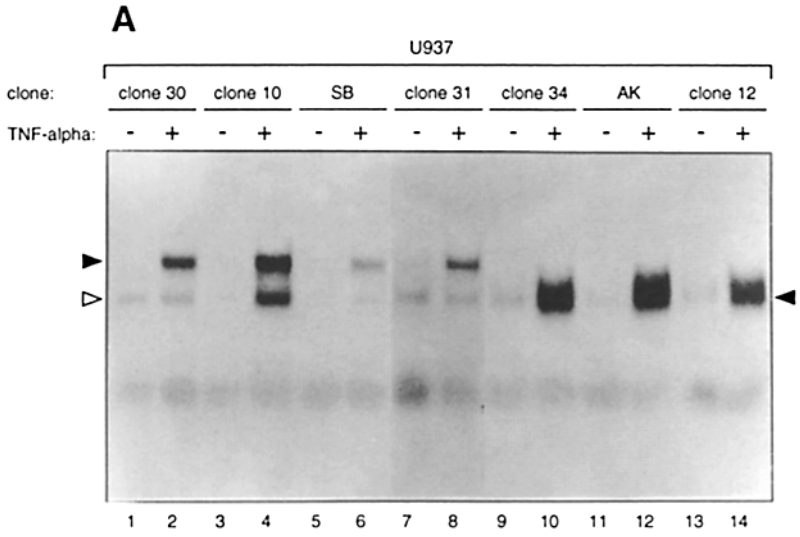
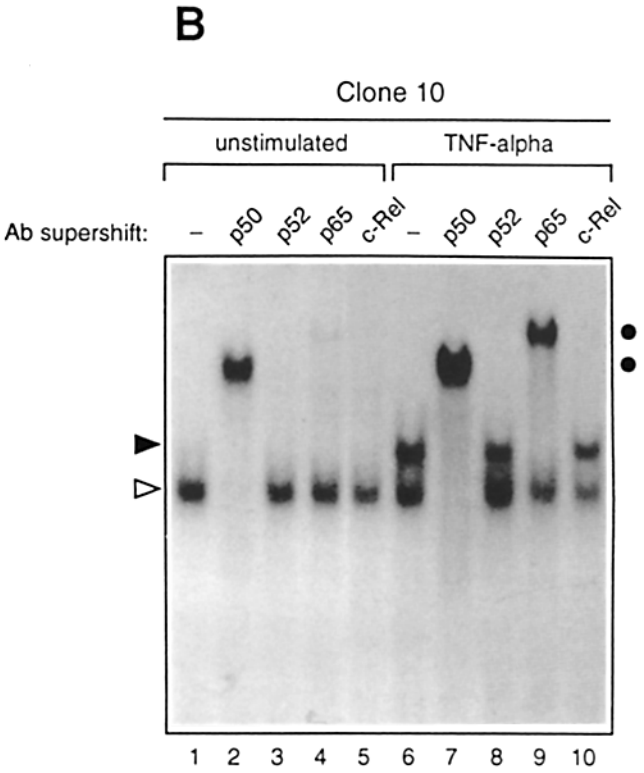
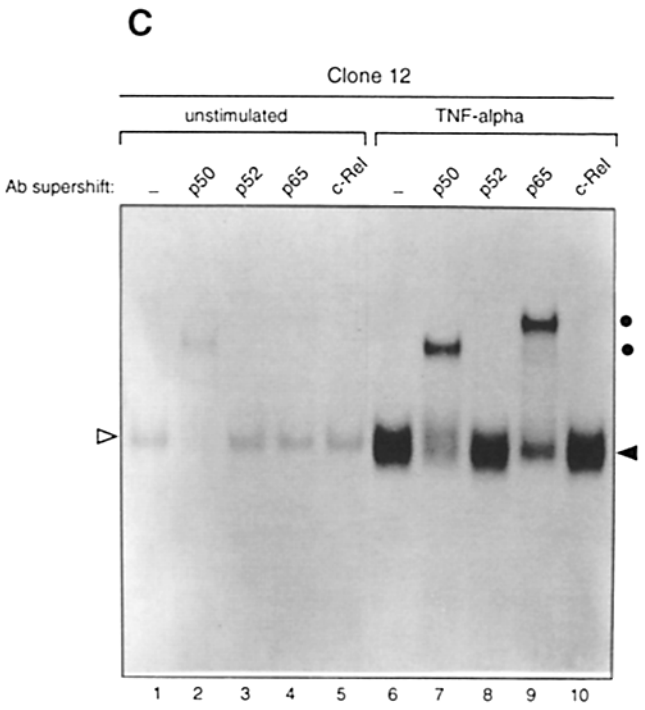


Figure 2. Extracts from different U937 subclones contain distinct TNF-α inducible NF-κB complexes, yet both are composed of p50 and p65. (*A*) EMSAs of whole cell extracts from unstimulated (*odd-numbered lanes*) or TNF-α–activated (*even-numbered lanes*) U937 clones. (▷) Specific constitutive and (►, ◄) TNF-α–induced complexes. (*B* and *C*) Supershift analysis on nuclear extracts of the representative clones 10 (*B*) and 12 (*C*) were performed with previously described antibodies, directed against the NH_2-terminal peptides of p50 (*B* and *C*, lanes *2* and *7*) (5), p50B (lanes *3* and *8*) (8), p65 (lanes *4* and *9*) (5) or a COOH-terminal peptide of the human c-Rel protein (lanes *5* and *10*), as indicated. Cells were either untreated (lanes *1–5*) or treated with TNF-α (lanes *6–10*). (►, ◄) p50-p65 heterodimers and (Δ) p50 homodimers. The two dots on the right side show the supershifts. The amount of extract used in each lane was normalized for protein concentration. The probe was the Ig-κLC-κB probe (6).





p65 peptide competition (Fig. 3). In contrast, when cell extracts from clone 12 and 34 were tested, the polyclonal antibody and the NH_2-terminal peptide antibody predominantly recognized a shorter form of p65 (about 50 kD), whereas the COOH-terminal peptide antibody failed to detect any band. This suggested that the NF-κB complex observed in these cell lines contained a form of p65 that was truncated at the COOH-terminus. Given the size of the resulting protein, it is likely that the truncation occurred just after the Rel-homology domain (RHD), preserving the ability of this protein to dimerize and bind DNA (as shown above), but eliminating the transactivating domain (44, 45). This conclusion was supported by gel shift analysis since anti-p65 antibodies directed against the COOH-terminal peptide did not supershift the novel complex induced in clone 12 cells or in our HL-60 cell line (data not shown).

The Truncated p65 Subunit Is Generated Via Proteolytic Processing. To directly test for the presence of proteases capable of truncating p65, we mixed unstimulated U937 clones 10 and 12 whole cell extracts (which did not contain appreciable amounts of DNA binding heterodimers; see Fig. 2 *A*) with nuclear extracts obtained from NTera-2 cells transfected with

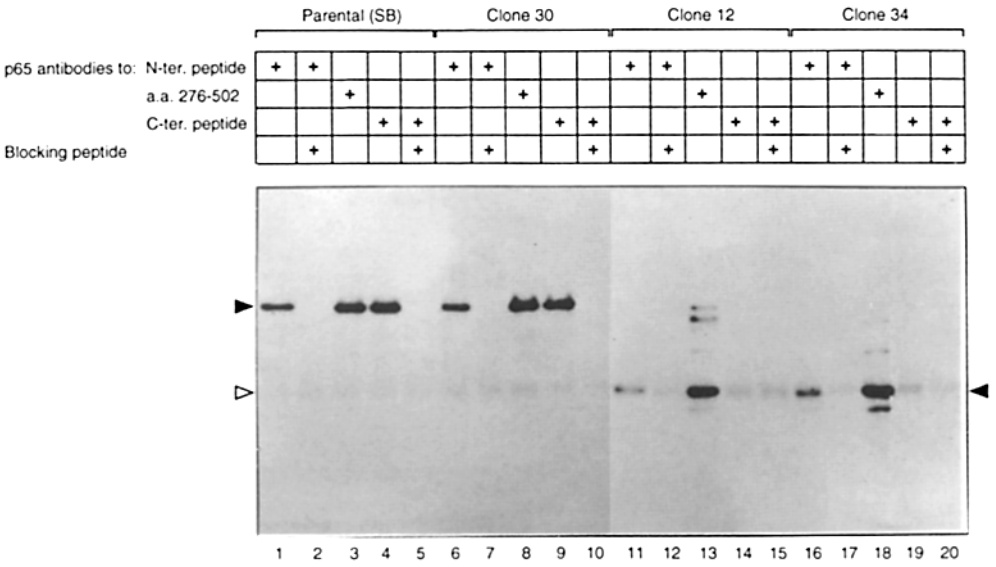


Figure 3. Demonstration of a truncated form of the p65 subunit in certain U937 clone extracts. Immunoprecipitation with a panel of p65-specific antibodies on whole cell extracts of representative U937 clones followed by Western blot with a mouse polyclonal p65 antibody. (►, ◄) Specific bands; (▷) points to the background due to the presence of the heavy chain of the antibodies used for the immunoprecipitation. Immunoprecipitations performed with peptide antibodies were blocked with 1 μg of the p65 peptide used to generate the antibody (*N-ter. peptide*: lanes *2, 7, 12,* and *17*; *C-ter. peptide*: lanes *5, 10, 15,* and *20*). Extracts from the various U937 clones and the antibodies were used as indicated.

p50 and p65 expression vectors (5, 6, 8). The transfected NTera-2 cells alone gave rise to both p50 homodimers and p50-p65 heterodimers, as previously reported (5, 6) (Fig. 4 *A*). Preincubation with extracts from unstimulated cells of clone 12 (minus), but not of clone 10 (plus), generated a faster-moving complex comigrating with the novel complex seen with stimulated minus clones (Fig. 4 *A*, lanes *3* and *2*, respectively). Thus, active proteases were present in lysates of clone 12, which converted a normal p50-p65 complex into a faster-migrating one, whereas no such proteases were present in extracts of clone 10, a clone that gave rise to normal NF-κB complexes when stimulated.

In an attempt to identify this protease(s), we assayed a number of protease inhibitors (including many not normally present in the extraction cocktail; see Materials and Methods) for their ability to prevent clone 12 lysates from converting the normal NF-κB complex into the faster-migrating form (Fig. 4 *B*). Of note, the protease inhibitors bestatin, APMSF, aprotinin, antipain-dihydrochloride, pepstatin, leupeptin, phosphoramidon, AEBSF, and chymostatin are normally included in the cocktail used during extraction. Among these, only chymostatin has some activity towards these proteases, but its concentration in normal extraction procedures is far lower than that required for efficient inhibition. DIC, DFP, and α1-antitrypsin efficiently blocked the truncation of p65 (Fig. 4 *B*, lanes *19*, *20*, and *21*, respectively). Rabbit preimmune serum, but not BSA (Fig. 4 *B*, lanes *18* and *17*, respectively), could also weakly inhibit p65 proteolysis, probably because of naturally present protease inhibitors in serum such as α1-antitrypsin and α2-macroglobulin (46). All of these inhibitors target serine proteases, although with different fine specificities.

The Truncation of p65 Occurs In Vitro During the Extraction Procedure. Cell extracts of clone 12 and HL60 cells in the presence of the protease inhibitor DFP allowed the detection of wild-type p50-p65 heterodimers (Fig. 5 *A*), as well as of full-length p65. This was demonstrated directly by the use of supershifting antibodies recognizing the COOH-terminal peptide of p65 (Fig. 5 *B*) and by immunoprecipitation experiments (data not shown). Therefore, truncation of p65 occurs during extraction rather than in living cells. In addition, the strong induction of IκB-α synthesis, observed after TNF-α stimulation of clone 10 and clone 12 cells, provides further indirect evidence for the presence of wild-type p65 in all these cells (data not shown). Truncated p65 lacks transactivation domains and thus would not be expected to activate transcription of IκB-α, a gene whose promoter has been shown to depend on functional NF-κB (3, 4, 47, 48).

A Family of Serine Proteases Are Likely Responsible for the In Vitro Degradation of p65. To investigate the expression pattern of the presumed serine proteases responsible for the in vitro truncation of p65, we tested other cells, using the proteolysis of p65 as an indicator of protease activity. Truncated form(s) of p65 were not restricted to certain U937 clones, but could also be observed in extracts of several other cells, including a HL-60 cell line (as discussed above), peripheral blood monocytes, and, to a lesser extent, THP-1 cells. On the other hand, PBLs contained primarily a full-length p65 protein (data not shown; 5). It is surprising that full-length p65 could be induced in all U937 minus clones, HL-60 cell lines, and in primary monocytes when these cells were differentiated for several days with agents such as phorbol ester, TNF-α, IFN-γ, GM-CSF, or LPS (Fig. 6 *A*). Moreover, extracts obtained from clone 12 cells differentiated with TPA for several days were no longer able to process p65 or change the mobility of the p50-p65 heterodimers derived from NTera-2 cells (data not shown), confirming that TPA downregulated the activity of the implicated proteases. In contrast, treatment of the HL-60 cells with retinoic acid or DMSO, which promote differentiation into granulocytes as opposed to macrophages (49), did not downregulate the proteases. It has been reported that a family of serine proteases including elastase

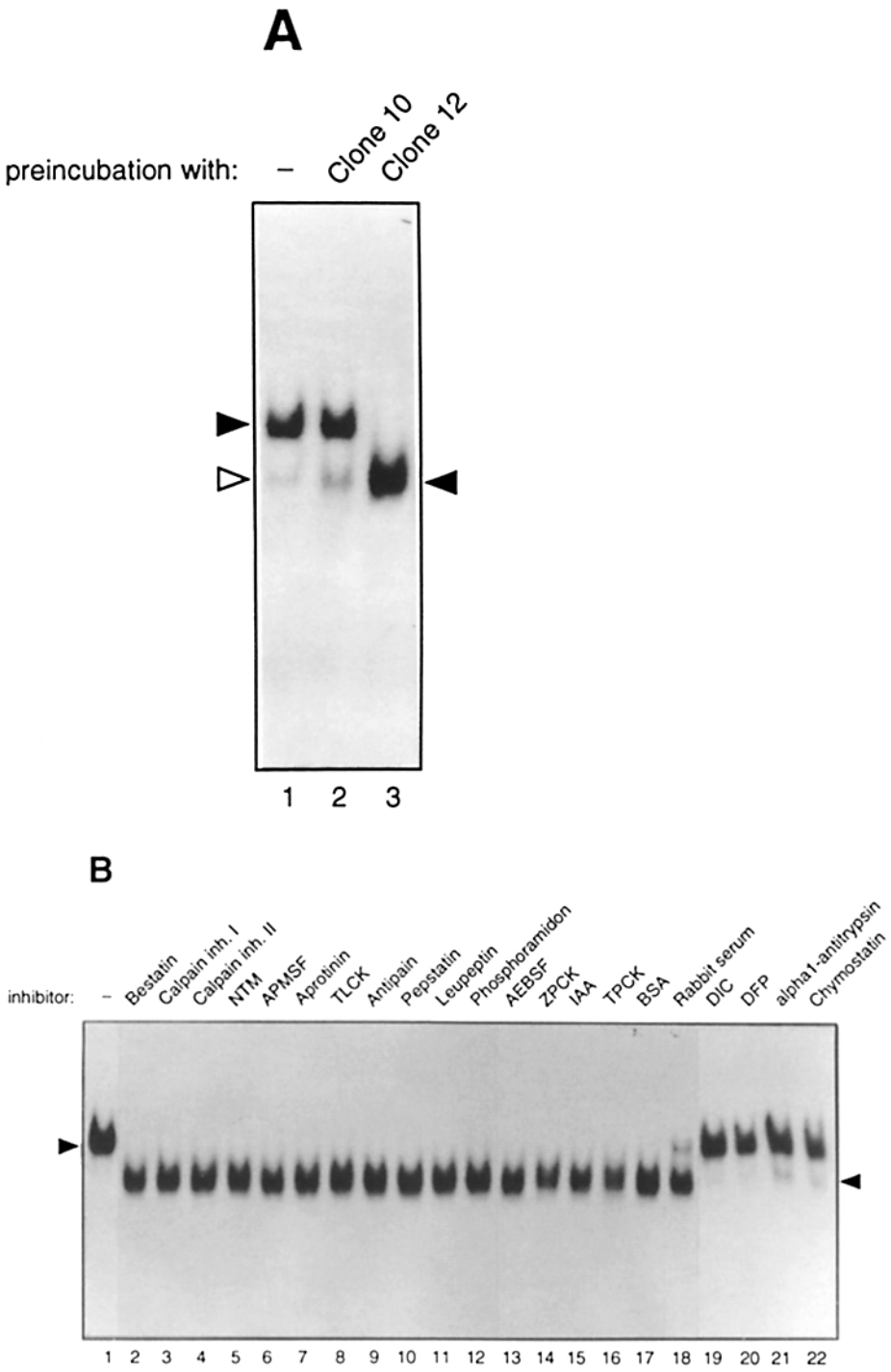
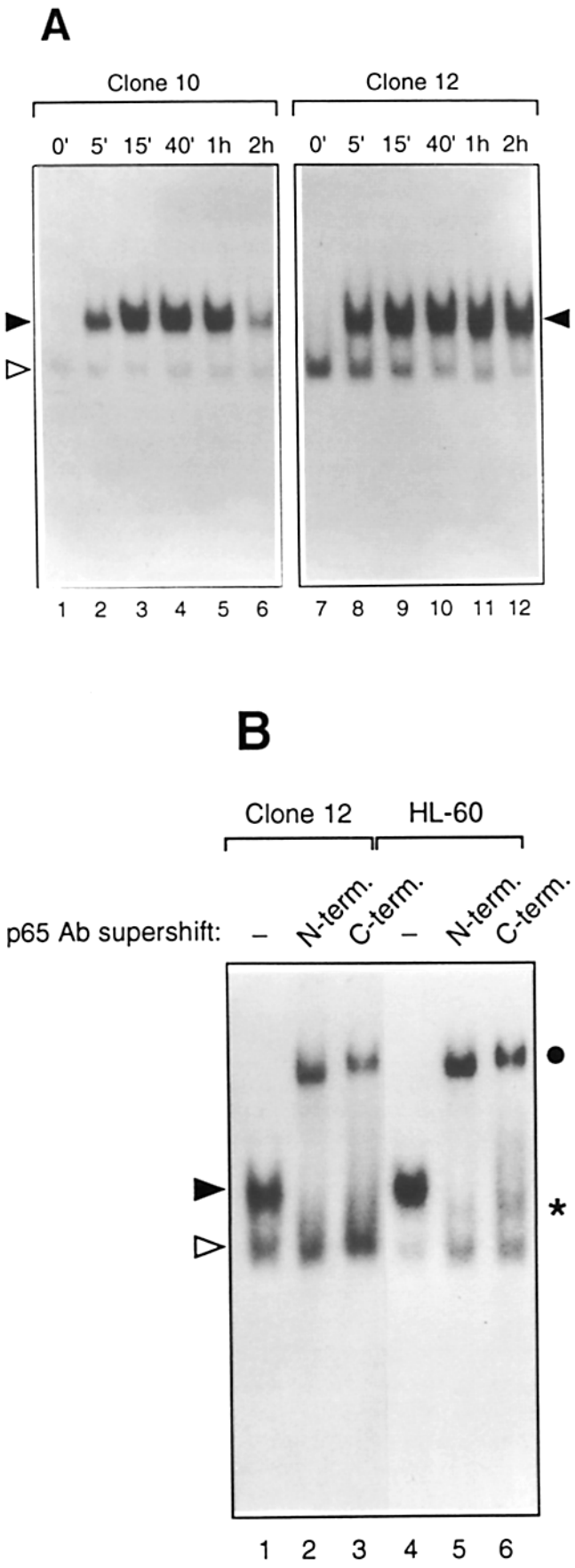


Figure 4. Extracts from certain U937 clones contain a protease activity which specifically affects the electrophoretic mobility of exogenously added p50-p65 heterodimers. (*A*) EMSA performed with a nuclear extract obtained from transfected NTera-2 cells (5, 6; for details see Materials and Methods). NTera-2 (▷) p50-p50 and (►) p50-p65 complexes are indicated on the left; (◄) modified heterodimers are on the right. (*B*) In vitro cleavage of the p50-p65 complex is selectively blocked by serine protease inhibitors. NTera-2 and clone 12 extracts were premixed as described in *A*, except that clone 12 extract was pretreated with the indicated protease inhibitor (for concentrations see Materials and Methods). (►) Unmodified and (◄) cleaved NTera-2 heterodimers. The probe was the PD-κB probe.

(37, 38), cathepsin G (36), and PR-3 (39), are highly and coordinately expressed in myelo-monocytic cells only (50–52) and that their expression is strongly downregulated during terminal differentiation into macrophages or after TPA treatment of HL-60, U937, and primary monocytes (50–52). We detected by Western analysis high levels of cathepsin G in our HL-60 and clone 12 cells (minus) and to a lesser extent in our THP-1 cells, whereas no such protein was detected in clone 10 cells (plus) (Fig. 6 *B*). Furthermore, cathepsin G protein was not seen in HL-60 cells and clone 12 cells after a 5-d treatment with the differentiating agent TPA. Thus, the expression of cathepsin G, and presumably of the other coordinately expressed proteases (52), correlated with the presence in these extracts of truncated p65 forms (Fig. 6 *A*). To more directly demonstrate a potential role of these proteases in the cleavage of p65, we also tested the ability of purified elastase and cathepsin G enzymes to cleave p65 in vitro (Fig. 7). Incubation of p50-p65 heterodimers derived from trans-



fected NTera-2 cells with these enzymes generated a complex still able to bind DNA but with a mobility essentially indistinguishable from that observed in minus U937 cell clones and in our HL-60 cell line, indicating that these proteases are likely candidates for the digestion of p65 in vitro.

Discussion

This study demonstrates that a group of U937 subclones, so-called minus clones, do not support rapid growth of HIV-1. In contrast to plus clones, which allow efficient HIV replication, minus clones contain members of a specific family of lysosomal serine proteases, including cathepsin G (36; Fig. 6 *B*), elastase (37, 38), and PR-3 (39). These particular proteases, also termed serprocidins (53), are released during cell extraction generating an apparently novel, faster-migrating NF-κB complex that is characterized by a specific truncation of the COOH-terminal part of the p65 subunit. We demonstrate directly that these proteases can generate the modified, truncated NF-κB complex in vitro. Expression of serprocidins is restricted to the neutrophil and monocytic lineages (50, 52, 54) and to certain sublines of the pro-monocytic leukemia cells U937 (minus clones), the pro-myelocytic cells HL-60 (50, 51, 55, 56) and, to some extent, THP-1 cells (Fig. 6 *B*). Whereas standard cell extraction procedures do not interfere with the activity of the released proteases, inclusion of specific protease inhibitors does, thus allowing the detection of normal NF-κB even in the minus clones and in the other myelomonocytic cells. It is curious that the specific truncation of p65 removes all transactivation functions but none of the DNA binding functions (Fig. 2; 44, 45). Whereas the existence of a nontransactivating NF-κB complex might have offered an explanation for why the NF-κB–dependent HIV-1 virus did not grow well in these cells, our data indicate otherwise, since the truncation appeared to occur during extraction only. Rather, the proteases themselves may contribute to the observed growth phenotype of HIV-1 in these cells.

Many studies have investigated NF-κB activity in stimulated myelo-monocytic cells, including U937, THP-1, HL-60, and primary monocytes. Revisiting these studies in light

Figure 5. Inclusion of DFP in the extraction buffer allows the recovery of the full-length p65 subunit. (*A*) EMSA performed with extracts of U937 clone 10 (lanes *1–6*) and clone 12 (lanes *7–12*) cells collected at the indicated time points after TNF-α stimulation. For this experiment, cells were extracted in a modified lysis buffer containing the protease inhibitor DFP (30 mM; for details see Materials and Methods). (▷) p50 and (►, ◄) p50-p65 heterodimers. An equivalent amount of extract was loaded in each lane. (*B*) Supershift analysis performed on extracts made (as described above) from clone 12 and HL-60 cells (lanes *1–3* and *4–6*, respectively) treated for 1 h with TNF-α, by using antibodies directed to an NH_2-terminal (lanes *2* and *5*) as well as to a COOH-terminal p65 peptide sequence (lanes *3* and *6*). (▷) p50-p50 and (►) p50-p65 band shift; and (●) supershifts. The residual binding activity seen with both anti-p65 antibodies was due either to p50 homodimers or to c-Rel-p50 heterodimers (*), as assessed by use of specific antibodies (data not shown). The probe was the PD-κB probe.

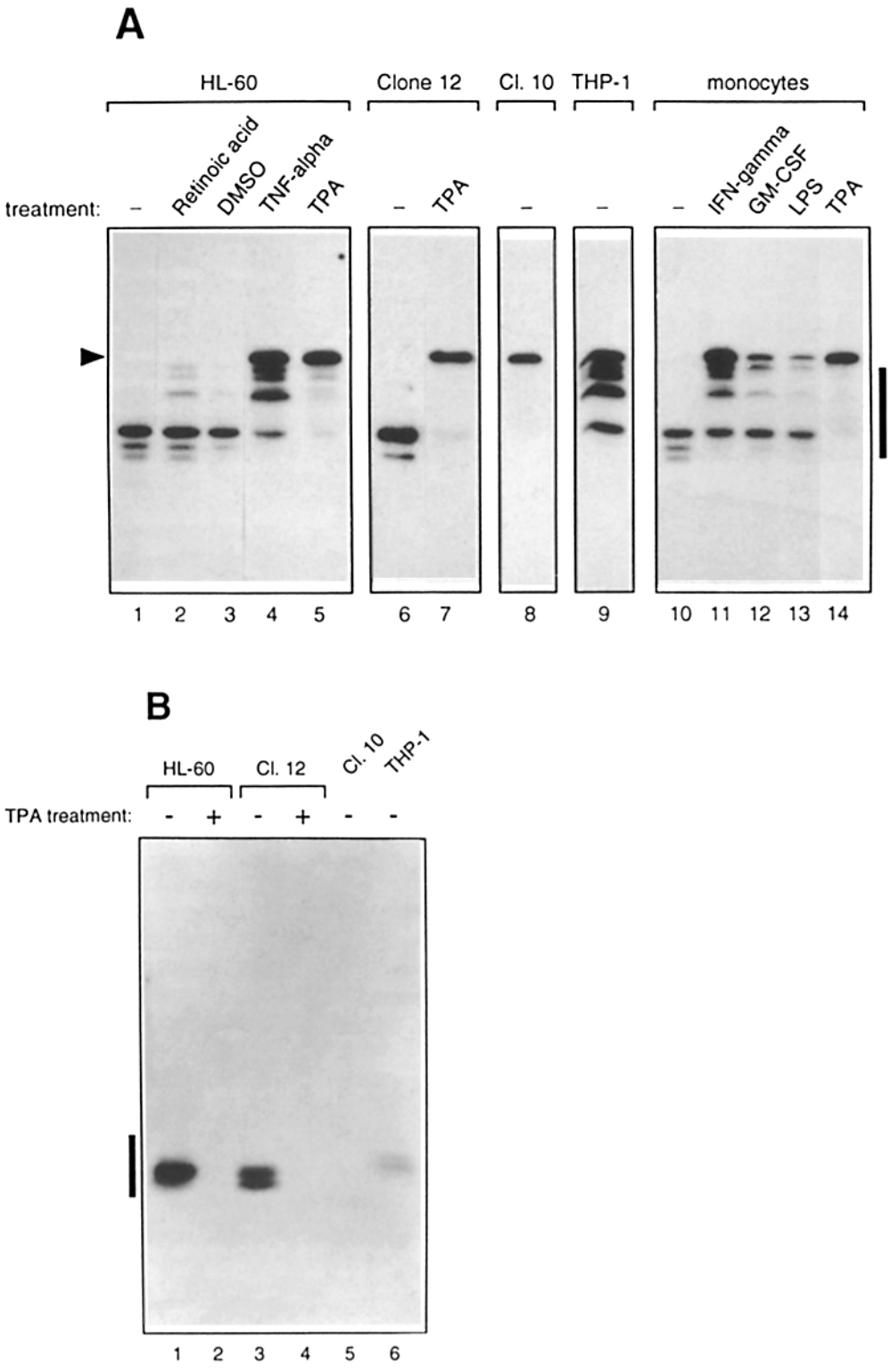


Figure 6. Expression of cathepsin G, and presumably of related serine proteases, correlates with the appearance of truncated forms of p65. (*A*) Truncated p65 forms can be detected in extracts made from a variety of myelo-monocytic cells and the full-length p65 subunit is restored after treatment with differentiating agents. Whole cell extracts, obtained (in absence of DFP) from HL-60 (lanes *1–5*), U937 clone 12 and 10 (lanes *6–7* and *8*, respectively), THP-1 (lane *9*) cells and primary monocytes (lanes *10–14*), were subjected to immunoprecipitation followed by Western blot performed with a p65 mouse antibody, as in Fig. 2. Cells were either untreated (lanes *1, 6, 8–10*), treated with TPA (HL-60, lane *5*; *Clone 12*, lane *7*; primary monocytes, lane *14*) or other differentiating agents, as indicated (for details see Materials and Methods). (►) Full-length and (┃) truncated p65 species. (*B*) The presence of cathepsin G correlates with the appearance of the truncated p65 species. Western blot performed on the same extracts used in Fig. 5 *A*, with an anti-cathepsin G polyclonal antibody (Calbiochem-Novabiochem Corp.). Extracts were used as indicated and normalized for protein concentration. The detection of multiple cathepsin G specific bands (┃) is probably due to glycosylation of the protein.

of our data suggests that in at least several cases the abnormal, faster-migrating NF-κB complexes may have been present, rather than the normal one (9, 10, 34, 57–63). This includes the case of the chronically HIV-infected U1 cells, a derivative of U937 cells (64, 65). In addition, one report (34) noted the absence of normal NF-κB in those THP-1 cells that were restricted in supporting the growth of HIV. The authors concluded that NF-κB activity was somehow inhibited in these cells. Finally, another laboratory has noted an unusual, faster-migrating NF-κB complex upon induction of primary monocytic cells (Mondal, K., and S. Haskill, personal communication). An unusual, faster-migrating NF-κB complex has

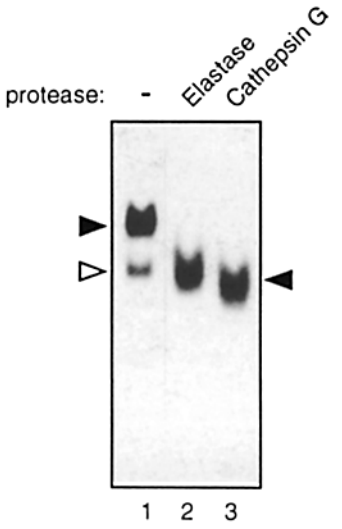


Figure 7. Cathepsin G and neutrophil elastase convert a normally migrating p50-p65 heterodimer into a faster-migrating one, in vitro. EMSA performed with extracts obtained from NTera-2 cells transfected with p50 and p65 vectors (see Materials and Methods) and preincubated with 30 ng of purified elastase (lane *2*) or cathepsin G (lane *3*) before adding the PD-κB probe. (▷) p50 homodimer and (►) p50-p65 heterodimer bands; (◄) migration of the digested heterodimeric complex.

also been detected in regenerating liver cells (66). Since liver contains cells of myelo-monocytic origin as well as high levels of various serine proteases (67–69) and since liver can be rapidly infiltrated with circulating neutrophils (70, 71) this complex may also have been generated by proteolytic digestion in vitro. However, this has not been experimentally tested by us. Nevertheless, the extraction procedures employed in each of the instances listed above would not be expected to inhibit the specific proteases that process p65. We conclude that special precautions must be taken when preparing extracts from any cells of the myelo-monocytic lineage, because of the presence of this class of proteases in certain cell sublines or primary cell mixtures.

In monocytes and neutrophils, serprocidins are contained within specialized lysosomes, termed azurophil granules (72) and perform important antimicrobial functions, such as the killing of phagocytosed microorganisms (53, 73). In addition, to this described role, our data suggest the intriguing possibility that serprocidins (present only in the nonpermissive minus clones) may negatively affect the life cycle of HIV-1 in U937, as well as in other monocytic cells such as THP-1 (34). The presence of such proteases in circulating monocytes could also explain the low viral burden associated with these cells in HIV-infected individuals (13, 14, 24, 25, 28, 32). Treatment of monocytes in vitro with differentiating agents, such as GM-CSF and IFN-γ, however, greatly enhance the ability of these cells to support HIV replication in vitro (26–31). This is of interest since expression of elastase, cathepsin G, and PR-3 genes is coordinately downmodulated by TPA (50, 52, 55, 56), GM-CSF, IFN-γ, or other agents that promote differentiation of monocytic and HL-60 cells into macrophages (See Fig. 6). In contrast, their expression increases in HL-60 after treatment with DMSO, an agent that differentiates these cells into granulocytes (49, 50, 74). In the case of our U937 plus clones, the lack of expression of serprocidins seems not to be strictly correlated with terminal differentiation, as demonstrated by the absence of a differentiated phenotype or a decrease in the proliferation rate (data not shown). Another possible explanation of the poor growth of HIV in minus clones is that whereas serprocidins may not themselves be directly retarding HIV-1 replication, they are induced coincidently with some other protein which is the effector. Alternatively, the process which positively regulates the expression of serprocidins may also negatively regulate HIV-1 expression. However, this latter possibility seems unlikely. As shown, both minus and plus clones contain normal p50-p65 heterodimers that are essential to the transcription of the HIV-LTR. These complexes are functional in both minus and plus cell types, as shown by the strong induction of IκB-α synthesis after TNF-α stimulation (data not shown; see Results). Finally, a transcriptional defect affecting the HIV-LTR in minus clones is inconsistent with previous data on the chronically infected U937 cell line U1 (28). Although the U1 cells have a minus phenotype and their extracts contain the truncated NF-κB complex (64, 65; G. Poli and A. S. Fauci, unpublished observations), stimulation of these cells causes rapid production of HIV-RNA and virions (75–77). Therefore, impaired HIV-1 replication in the U937 minus clones is likely to be due to events preceding or coincident with HIV integration. Whether early events during HIV replication are affected directly or indirectly by the specific serine proteases discussed here is under investigation.

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References

1. Baeuerle, P.A., and T. Henkel. 1994. Function and activation of NF-κB in the immune system. *Annu. Rev. Immunol.* 12:141.
2. Grilli, M., J.J.S. Chiu, and M.J. Lenardo. 1993. NF-κB and Rel: participants in a multiform transcriptional regulatory system. *Int. Rev. Cytol.* 148:1.
3. Brown, K., S. Park, T. Kanno, G. Franzoso, and U. Siebenlist. 1993. Mutual regulation of the transcriptional activator NF-κB and its inhibitor, IκB-α. *Proc. Natl. Acad. Sci. USA.* 90:2532.
4. Sun, S.-C., P.A. Ganchi, D.W. Ballard, and W.C. Greene. 1993. NF-κB controls expression of inhibitor IκB-α: evidence for an inducible autoregulatory pathway. *Science (Wash. DC).* 259:1912.
5. Franzoso, G., V. Bours, S. Park, M. Tomita-Yamaguchi, K. Kelly, and U. Siebenlist. 1992. The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-κB–mediated inhibition. *Nature (Lond.).* 359:339.
6. Franzoso, G., V. Bours, V. Azarenko, S. Park, M. Tomita-Yamaguchi, T. Kanno, K. Brown, and U. Siebenlist. 1993. The oncoprotein Bcl-3 can facilitate NF-κB–mediated transactivation by removing inhibiting p50 homodimers from select κB sites. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:3893.
7. Bours, V., P.R. Burd, K. Brown, J. Villalobos, S. Park, R. Ryseck, R. Bravo, K. Kelly, and U. Siebenlist. 1992. A novel mitogen-inducible gene product related to p50-p105-NF-κB participates in transactivation through a κB site. *Mol. Cell. Biol.* 12:685.
8. Bours, V., G. Franzoso, V. Azarenko, S. Park, T. Kanno, K. Brown, and U. Siebenlist. 1993. The oncoprotein Bcl-3 directly transactivates through κB motifs via association with DNA-binding p50B homodimers. *Cell.* 72:729.
9. Griffin, G.E., K. Leung, T.M. Folks, S. Kunkel, and G.J. Nabel. 1989. Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. *Nature (Lond.).* 339:70.
10. Bachelerie, F., J. Alcami, F. Arenzana-Seisdedos, and J.-L. Virelizier. 1991. HIV enhancer activity perpetuated by NF-κB induction on infection of monocytes. *Nature (Lond.).* 350:709.
11. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (Lond.).* 335:783.
12. Bellas, R.E., N. Hopkins, and Y. Li. 1993. The NF-κB binding site is necessary for efficient replication of simian immunodeficiency virus of macaques in primary macrophages but not in T cells in vitro. *J. Virol.* 67:2908.
13. Laurence, J. 1993. Reservoirs of HIV infection or carriage: monocytic, dendritic, follicular dendritic, and B cells. *Ann. N.Y. Acad. Sci.* 693:52.
14. Poli, G., and A.S. Fauci. 1992. The role of monocyte/macrophages and cytokines in the pathogenesis of HIV infection. *Pathobiology.* 60:246.
15. Kalter, D.C., H.E. Gendelman, and M.S. Meltzer. 1991. Monocytes, dendritic cells, and langerhans cells in human immunodeficiency virus infection. *Dermatologic Clinics.* 9:415.
16. Rappersberger, K., S. Gartner, P. Schenk, G. Stingl, V. Groh, E. Tschachler, D.L. Mann, K. Wolff, K. Donrad, and M. Popovic. 1988. Langerhans cells are an actual site of HIV-1 replication. *Intervirology.* 29:185.
17. Embretson, J., M. Zupancic, J.L. Ribas, A. Burke, P. Racz, K. Tenenr-Racz, and A. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature (Lond.).* 362:359.
18. Koenig, S., H.E. Gendelman, J.M. Orenstein, M.C. DalCanto, G.H. Pezeshkpour, P. Yungbluth, F. Jannotta, A. Aksamit, M.A. Martin, and A.S. Fauci. 1986. Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science (Wash. DC).* 233:1089.
19. Stoler, M.H., T.A. Eskin, and S. Benn. 1986. Human T-cell lymphotrophic virus type III infection of the central nervous system: preliminary *in situ* analysis. *JAMA (J. Am. Med. Assoc.).* 256:2360.
20. Gartner, S., P. Markovits, D.M. Markovitz, M.H. Kaplan, R.C. Gallo, and M. Popovic. 1986. The role of mononuclear phagocytes in HTLV/LAV infection. *Science (Wash. DC).* 233:215.
21. Salahuddin, S.Z., R.M. Rose, J.E. Groopman, P.D. Markham, and R.C. Gallo. 1986. Human T lymphotropic virus type III infection of human alveolar macrophages. *Blood.* 68:281.
22. McElrath, M.J., J.E. Pruett, and Z.A. Cohn. 1989. Mononuclear phagocytes of blood and bone marrow: comparative roles as viral reservoirs in human immunodeficiency virus type 1 infections. *Proc. Natl. Acad. Sci. USA.* 86:675.
23. Innocenti, P., M. Ottman, O. Morand, P. Leclercq, and J. Seigneurin. 1992. HIV-1 in blood monocytes: frequency of detection of proviral DNA using PCR and comparison with the total CD4 count. *AIDS Res. Hum. Retroviruses.* 8:261.
24. Schnittman, S.M., M.C. Psalidopoulos, H.C. Lane, L. Thompson, M. Baseler, F. Massari, C.H. Fox, N.P. Salzman, and A.S. Fauci. 1989. The reservoir for HIV-1 in human peripheral blood is a T cell that maintains expression of CD4. *Science (Wash. DC).* 245:305.
25. Spear, G.T., O. Chin-Yih, H.A. Kessler, J.C. Moore, G. Schochetman, and A.L. Landay. 1990. Analysis of lymphocytes, monocytes, and neutrophils from human immunodeficiency virus (HIV) infected persons for HIV DNA. *J. Infect. Dis.* 162:1239.
26. Gendelman, H.E., J.M. Orenstein, M.A. Martin, C. Ferrua, R. Mitra, T. Phipps, L.A. Wahl, H.C. Lane, A.S. Fauci, D.S. Burke, et al. 1988. Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1–treated monocytes. *J. Exp. Med.* 167:1428.
27. Perno, C.-F., R. Yarchoan, D.A. Cooney, N.R. Hartman, D.S.A. Webb, Z. Hao, H. Mitsuya, D.G. Johns, and S. Broder. 1989. Replication of human immunodeficiency virus in monocytes. *J. Exp. Med.* 169:933.
28. Folks, T.M., J. Justement, A. Kinter, C.A. Dinarello, and A.S. Fauci. 1987. Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. *Science (Wash. DC).* 238:800.
29. Koyanagi, Y., W.A. O'Brien, J.Q. Zhao, D.W. Golde, J.C. Gasson, and I.S. Chen. 1988. Cytokines alter production of HIV-1 from primary mononuclear phagocytes. *Science (Wash. DC).* 241:1673.
30. Kitano, K., G.C. Baldwin, M.A. Raines, and D.W. Golde. 1990. Differentiating agents facilitate infection of myeloid leukemia cell lines by monocytotropic HIV-1 strains. *Blood.* 76:1980.
31. Schuitemaker, H., N.A. Kootstra, M.H.G.M. Koppelman, S.M. Bruisten, H.G. Husiman, M. Tersmette, and F. Miedema. 1992. Proliferation-dependent HIV-1 infection of monocytes occurs during differentiation into macrophages. *J. Clin. Invest.* 89:1154.
32. Meltzer, M.S., D.R. Skillman, D.L. Hoover, B.D. Hanson, J.A. Turpin, D.C. Kalter, and H.E. Gendelman. 1990. Macrophages and the human immunodeficiency virus. *Immunol. Today.* 11:217.
33. Boulerice, F., R. Geleziunas, S. Bour, H. Li, M. D'Addario, A. Roulston, J. Hiscott, and M.A. Wainberg. 1992. Differential susceptibilities of U-937 cell clones to infection by human immunodeficiency virus type 1. *J. Virol.* 66:1183.

34. Raziuddin, J.A. Mikovits, I. Calvert, S. Ghosh, H.-F. King, and F.W. Ruscetti. 1991. Negative regulation of human immunodeficiency virus type 1 expression in monocytes: Role of the 65-kDa plus 50-kDa NF-κB dimer. *Proc. Natl. Acad. Sci. USA.* 88:9426.
35. Mikovits, J.A., Raziuddin, M. Gonda, M. Ruta, N.C. Lohrey, H.-F. Kung, and F.W. Ruscetti. 1990. Negative regulation of human immune deficiency virus replication in monocytes. Distinctions between restricted and latent expression in THP-1 cells. *J. Exp. Med.* 171:1705.
36. Salvesen, G., D. Farley, J. Shuman, A. Przybyla, C. Reilly, and J. Travis. 1987. Molecular cloning of human cathepsin G: structural similarity to mast cell and cytotoxic T lymphocyte proteinases. *Biochemistry.* 26:2289.
37. Farley, D., G. Salvesen, and J. Travis. 1988. Molecular cloning of human neutrophil elastase. *Biol. Chem. Hoppe-Seyler.* 369(Suppl): 3.
38. Takahashi, H., T. Nukiwa, K. Yoshimura, C.D. Quick, D.J. States, M.D. Holmes, J. Whang-Peng, T. Knutsen, and R.G. Crystal. 1988. Structure of the human neutrophil elastase gene. *J. Biol. Chem.* 263:14739.
39. Rao, N.V., N.G. Wehner, B.C. Marshall, W.R. Gray, B.H. Gray, and J.H. Hoidal. 1991. Characterization of proteinase-3 (PR-3), a neutrophil serine proteinase. *J. Biol. Chem.* 266:9540.
40. Biswas, P., G. Poli, J.M. Orenstein, and A.S. Fauci. 1994. Cytokine-mediated induction of HIV expression and cell death in chronically infected U1 cells. Do tumor necrosis factor-alpha and interferon-gamma selectively kill HIV infected cells? *J. Virol.* 68:2598.
41. Figdor, C.G., J.M.M. Leemans, W.S. Bont, and J.E. deVries. 1983. Theory and practice of centrifugal elutriation (CE). Factors influencing the separation of human blood cells. *Cell Biophys.* 5:105.
42. Willey, R.L. 1988. In vitro mutagenesis identifies a region within the envelope gene of the human immunodeficiency virus that is critical for infectivity. *J. Virol.* 62:139.
43. Rice, N.R., M.L. MacKichan, and A. Israel. 1992. The precursor of NF-κB p50 has IκB-like functions. *Cell.* 71:243.
44. Schmitz, M.L., and P.A. Baeuerle. 1991. The p65 subunit is responsible for the strong transcription activation potential of NF-κB. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3805.
45. Ballard, D.W., E.P. Dixon, N.J. Peffer, H. Bogerd, S. Doerre, B. Stein, and W.C. Greene. 1992. The 65-kDa subunit of human NF-κb functions as a potent transcriptional activator and a target for v-Rel-mediated repression. *Proc. Natl. Acad. Sci. USA.* 89:1875.
46. Crystal, R.G. 1990. α1-antitrypsin deficiency, emphysema, and liver disease. Genetic basis and strategies for therapy. *J. Clin. Invest.* 85:1343.
47. Chiao, P.J., S. Miyamoto, and I.M. Verma. 1994. Autoregulation of IκBa activity. *Proc. Natl. Acad. Sci. USA.* 91:28.
48. deMartin, R., B. Vanhove, Q. Cheng, E. Hofer, V. Csizmadia, H. Winkler, and F.H. Bach. 1993. Cytokine-inducible expression in endothelial cells of an IκBα-like gene is regulated by NF-κB. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:2773.
49. Olsson, I., U. Gullberg, and M. Lantz. 1990. Cytokine and non-cytokine differentiating agents for myeloid leukemic cells. *Leukemia Res.* 14:711.
50. Takahashi, H., T. Nukiwa, P. Basset, and R.G. Crystal. 1988. Myelomonocytic cell lineage expression of the neutrophil elastase gene. *J. Biol. Chem.* 263:2543.
51. Hanson, R.D., N.L. Connolly, D. Burnett, E.J. Campbell, R.M. Senior, and T.J. Ley. 1990. Developmental regulation of the human cathepsin G gene in myelomonocytic cells. *J. Biol. Chem.* 265:1524.
52. Zimmer, M., R.L. Medcalf, T.M. Fink, C. Mattmann, P. Lichter, and D.E. Jenne. 1992. Three human elastase-like genes coordinately expressed in the myelomonocyte lineage are organized as a single genetic locus on 19pter. *Proc. Natl. Acad. Sci. USA.* 89:8215.
53. Gabay, J.E., and R.P. Almeida. 1993. Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. *Curr. Opin. Immunol.* 5:97.
54. Campbell, E.J., E.K. Silverman, and M.A. Campbell. 1990. Elastase and cathepsin G of human monocytes. *J. Immunol.* 143:2961.
55. Harris, P., and P. Ralph. 1985. Human leukemic models of myelomonocytic development: a review of the HL-60 and U937 cell lines. *J. Leuk. Biol.* 37:407.
56. Welgus, H.G., N.L. Connolly, and R.M. Senior. 1986. 12-*o*-tetradecanoyl-phorbol-13-acetate–differentiated U937 cells express a macrophage-like profile of neutral proteinases. High levels of secreted collagenase and collagenase inhibitor accompany low levels of intracellular elastase and cathepsin G. *J. Clin. Invest.* 77:1675.
57. Arenzana-Seisdedos, F., B. Fernandez, I. Dominguez, J.M. Jacque, D. Thomas, M.T. Diaz-Meco, J. Moscat, and J.L. Virelizier. 1993. Phosphatidylcholine hydrolysis activates NF-κB and increases human immunodeficiency virus replication in human monocytes and T lymphocytes. *J. Virol.* 67:6596.
58. Roulston, A., P. Beauparlant, N. Rice, and J. Hiscott. 1993. Chronic human immunodeficiency virus type 1 infection stimulates distinct NF-κB/Rel DNA binding activities in myelomonoblastic cells. *J. Virol.* 67:5235.
59. Suzan, M., D. Salaun, C. Neuveut, B. Spire, I. Hirsch, P. LeBouteiller, G. Querat, and J. Sire. 1991. Induction of NF-κB during monocyte-differentiation by HIV type 1 infection. *J. Immunol.* 146:377.
60. Hohmann, H.-P., M. Brockhaus, P.A. Baeuerle, R. Remy, R. Kolbeck, and A.P.G.M. vanLoon. 1990. Expression of the types A and B tumor necrosis factor (TNF) receptors is independently regulated, and both receptors mediate activation of the transcription factor NF-κB. *J. Biol. Chem.* 265:22409.
61. Hohmann, H.-P., R. Remy, C. Scheidereit, and A.P.G.M.v. Loon. 1991. Maintenance of NF-κB activity is dependent on protein synthesis and the continuous presence of external stimuli. *Mol. Cell. Biol.* 11:259.
62. Israel, N., M.-A. Gougerot-Pocidalo, F. Aillet, and J.-L. Virelizier. 1992. Redox status of cells influences constitutive or induced NF-κB translocation and HIV long terminal repeat activity in human T and monocytic cell lines. *J. Immunol.* 149:3385.
63. Brach, M.A., S. deVos, C. Arnold, H.-J. Grub, R. Mertelsmann, and F. Herrmann. 1992. Leukotriene B4 transcriptionally activates interleukin-6 expression involving NK-κB and NF-IL-6. *Eur. J. Immunol.* 22:2705.
64. Pomerantz, R.J., M.B. Feinberg, D. Trono, and D. Baltimore. 1990. Lipopolysaccharide is a potent monocyte/macrophage-specific stimulatory of human immunodeficiency virus type 1 expression. *J. Exp. Med.* 172:253.
65. Poli, G., A.L. Kinter, and A.S. Fauci. 1994. Interleukin 1 induces expression of the human immunodeficiency virus alone and in synergy with interleukin 6 in chronically infected U1 cells: inhibition of inductive effects by the interleukin 1 receptor antagonist. *Proc. Natl. Acad. Sci. USA.* 91:108.
66. Tewari, M., P. Dobrzanski, K.L. Mohn, D.E. Cressman,

J.-C. Hsu, R. Bravo, and R. Taub. 1992. Rapid induction in regenerating liver of RL/IF-1 (an IκB that inhibits NF-κB, RelB-p50, and c-Rel-p50) and PHF, a novel κB site-binding complex. *Mol. Cell Biol.* 12:289.
67. Cappelluti, E., S.C. Strom, and R.B. Harris. 1992. Potential role of two novel elastase-like enzymes in processing pro-transforming growth factor-alpha. *Biochemistry.* 32:551.
68. Barrett, A.J. 1984. The lysosome and its membrane. *Biochem. Soc. Trans.* 12:899.
69. Jones, E.A., and J.A. Summerfield. 1988. Kupffer cells. *In* The Liver: Biology and Pathobiology. I.M. Arias, W.B. Jakoby, H. Popper, D. Schacter, and D.A. Shafritz, editors. Raven Press, Ltd. New York. 683–704.
70. Riess, H., M. Jochum, W. Machleidt, G. Himmelreich, G. Blumhardt, R. Roissanit, and P. Neuhaus. 1991. Possible role of extracellularly released phagocyte proteinases in coagulation disorder during liver transplantation. *Transplantation (Baltimore).* 52:482.
71. Hamazaki, K., H. Minura, M. Kato, K. Okamoto, M. Yunoki, M. Mori, K. Orita, and N.J. Lygidakis. 1993. The significance of post-hepatectomy changes in polymorphonuclear elastase and endotoxin levels. *Hepato-Gastroenterology.* 40:352.
72. Bainton, D.F., J.L. Ullyot, and M.G. Farquhar. 1971. The development of neutrophilic polymorphonuclear leukocytes in human bone marrow: origin and content of azurophil and specific granules. *J. Exp. Med.* 134:907.
73. Spitznagel, J.K. 1990. Antibiotic proteins of human neutrophils. *J. Clin. Invest.* 86:1381.
74. Yoshimura, K., and R.G. Crystal. 1992. Transcriptional and posttranscriptional modulation of human neutrophil elastase gene expression. *Blood.* 79:2733.
75. Poli, G., A.L. Kinter, J.S. Justement, P. Biswas, D. Weissman, L.M. Fox, D. Goletti, P. Bressler, S.K. Stanley, and A.S. Fauci. 1993. The chronically infected promonocytic cell lie U1: a model of HIV expression regulated by cytokines. *Immunomethods.* 3:50.
76. Biswas, P., G. Poli, A.L. Kinter, J.S. Justement, S.K. Stanley, W.J. Maury, P. Bressler, J.M. Orenstein, and A.S. Fauci. 1992. Interferon-γ induces the expression of human immunodeficiency virus in persistently infected promonocytic cells (U1) and redirects the production of virons to intracytoplasmic vacuoles in phorbol myristate acetate–differentiated U1 cells. *J. Exp. Med.* 176:739.
77. Bressler, P., G. Poli, J.S. Justement, P. Biswas, and A.S. Fauci. 1993. Glucocorticoids synergize with tumor necrosis factor alpha in the induction of HIV expression from a chronically infected promonocytic cell line. *AIDS Res. Hum. Retro.* 9:547.