

Induction of NF- κ B during monocyte differentiation is associated with activation of HIV-gene expression

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SUMMARY

Cells of the monocyte-macrophage lineage are important targets of HIV infection. We report here that the phenotypic differentiation of monocyte cell lines induced by phorbol esters or tumour necrosis factor α (TNF α) is associated with expression of nuclear factor kappa B (NF- κ B). In parallel with such differentiation, HIV transcription, monitored using an HIV long terminal repeat reporter gene construct, is activated in such cells under the influence of enhanced NF- κ B expression. Also, in a promonocyte cell line chronically infected with HIV, NF- κ B expression and HIV transcription were enhanced on stimulation with phorbol ester or TNF α . Thus, stimulation of monocyte cell lines by phorbol esters or TNF α induces cell differentiation and activates HIV transcription. Such a process may have fundamental implications in AIDS pathogenesis *in vivo* and may be important in disease progression induced by opportunistic infections directly or indirectly involving macrophages.

Key-words: HIV, Monocyte, Macrophage, AIDS, NF- κ B; Phorbol ester, TNF α , Cell maturation, Transcription.

INTRODUCTION

Monocytes provide an important cellular reservoir for HIV (Ho *et al.*, 1986; Nabel and Baltimore, 1987; Meltzer *et al.*, 1990). The mobility of monocyte macrophages throughout the body into both organs of the reticulo-endothelial system, *e.g.* lymph nodes and spleen and virtually all other organ systems, may provide an important vector for the virus thus facilitating its widespread dis-

semination. Macrophages are derived from stem cells in the bone marrow under appropriate stimuli, such as cytokines *e.g.* granulocyte-macrophage/colony-stimulating factor (GM-CSF) and it is thought that such marrow stem cells may be chronically infected *per se* (Donahue *et al.*, 1987; Folks *et al.*, 1988). In addition, monocytes and macrophages may become infected directly by CD4-receptor-mediated mechanisms. Terminal differentiation into

the macrophage phenotype may be induced artificially *in vitro* using phorbol esters or by more physiological stimuli such as tumour necrosis factor (TNF α). We report, in this paper, a series of experiments in which we investigated the role of nuclear transcription factor NF- κ B in this differentiation process. In addition, we examined the role of this factor in the activation of HIV transcription in such cells induced to differentiate (Griffin *et al.*, 1989).

MATERIALS AND METHODS

Cell lines and cells were maintained in RPMI medium enriched with 7.5 % foetal calf serum, glutamine and supplemented with penicillin and streptomycin. Cell lines of promonocytic phenotype HL60 and U937 (non-adherent, absent Mo1 expression, non-phagocytic, non-specific-esterase-negative) were of human origin. Cells of mature macrophage were of phenotype THP-1, PU5-1.8 and P388D1. Some experiments were carried out on promonocytic cell line U1, chronically infected with HIV, which expresses very low levels of HIV transcription when unstimulated. In some experiments, monocytes were prepared from human blood of normal volunteers by dextran sedimentation and centrifugation through a discontinuous Ficoll-Hypaque gradient. Monocytes of intermediate homogeneity (80 %) were prepared by one cycle of adherence onto plastic and further purification (95 %) was achieved by repeated cycles of washing using RPMI medium over a 3-day period to remove contaminating non-adherent cells. In another series of experiments, mouse peritoneal macrophages were prepared from ascitic fluid induced by intraperitoneal injection of mineral oil or complete Freund's adjuvant and purified by adherence on plastic and repeated cycles of washing.

Cell transfection

Cells were transfected either in suspension or adherent using the conventional DEAE-dextran technique (Nabel and Baltimore, 1987) with the exception that 10 μ M chloroquine was used instead of the higher concentrations of this agent originally described. Cells

(10^7) were transfected with 10 μ g of a plasmid containing the HIV enhancer linked to the chloramphenicol acetyl transferase (CAT) gene. Control experiments were carried out in which cells were transfected with a mutant plasmid (10 μ g) altered in both κ B-binding sites using DEAE-dextran. Then, 24 h after transfection, cells were washed with RPMI and incubated with medium alone, or in the presence of 40 nM 12-O-tetradecanoyl-phorbol-13-acetate (TPA) or the water-soluble phorbol-12, 13-dibutyrate (PDB) at a concentration of 20 nM which is more easily removed from cells by cycles of washing. Twenty hours after stimulation, cells were harvested and cytoplasmic (Nabel and Baltimore, 1987) and nuclear extracts prepared (Osborn *et al.*, 1989). Protein concentration in the extracts was measured using the Bradford method (Biorad).

Chemical assays

CAT was assayed by standard methods (Nabel and Baltimore, 1987) on cytoplasmic extracts. NF- κ B-binding activity was determined on nuclear extracts containing 10 μ g protein using conventional electrophoretic mobility shift assay (Leung and Nabel, 1988) in the presence of 1 μ g dIdC. Extracts were incubated with a κ B probe alone or in the presence of a double-stranded oligonucleotide competitor containing the κ B site or an unrelated interleukin-2 (IL-2) promoter fragment in order to determine the specificity of binding.

RESULTS

Nuclear NF- κ B-binding activity

NF- κ B-binding activity in nuclear extracts from the cells is

shown in figure 1. No NF- κ B-binding activity was detected in the promonocytic cell lines in the unstimulated state (fig. 1A; HL-60 and U937). However, in these cells, stimulation with TPA resulted in phenotypic differentiation and the appearance of NF- κ B-binding activity. In contrast, constitutive NF- κ B-binding activity was demonstrated in the mature cell lines (fig. 1A; THP-1, PU5-1.8 and P388DI). In addition, NF- κ B-binding activity was detected in the unstimulated state. Since the immature cell lines were both of human origin and the mature cell lines were derived from mouse cells, we therefore wished to demonstrate NF- κ B-binding activity in cells harvested directly *in vivo*. NF- κ B-binding activity was detected in human monocytes (fig. 1B) and in mouse peritoneal macrophages. TPA stimulation of cells is known to be irreversible but TPA is lipid soluble and cannot be removed from cells. We therefore used PDB, a water-soluble phorbol ester which can easily be removed from cells by washing, in an effort to see if short term stimulation (2 h) followed by washing resulted in long-term expression of NF- κ B-binding activity. PDB treatment of HL-60 and U937 cells resulted in expression of NF- κ B after 2 h of treatment which was still present 48 h after the phorbol ester was washed away from cells.

HIV transcription

HIV CAT

Transcription of transfected HIV-CAT did not occur in immature monocytes, however on stimulation with phorbol ester, transcription was enhanced 17-fold (fig. 2A). In contrast, the mature cell lines constitutively expressed

CAT = chloramphenicol acetyl transferase.
GM-CSF = granulocyte-macrophage/colony-stimulating factor.
IL = interleukin.
LPS = lipopolysaccharide.

NF- κ B = nuclear factor κ B.
PDB = phorbol-12,13-dibutyrate.
TNF = tumour necrosis factor.
TPA = 12-O-tetradecanoyl-phorbol-13-acetate.

CAT and such expression was not enhanced by phorbol ester stimulation. No CAT activity was detected in any of the cells transfected with the plasmid containing mutant κ B.

HIV production

To determine whether monocyte differentiation and NF- κ B induction affected production of intact virus, the latently infected U1-cell line (Folks, 1988) was incubated with PDB or TNF α . Under these conditions, phenotypic differentiation of the cells occurred as monitored using change in cell surface characteristics (adhesion, Mo1 expression). In addition, HIV production was enhanced in these cells as shown by increased reverse transcriptase activity and p24 detected in the incubating medium (fig. 3). Both NF- κ B activity and HIV production persisted 24-48 h after the stimulus was removed.

DISCUSSION

In these experiments, we have demonstrated that NF- κ B-binding activity was induced in nuclear extracts of immature cells of monocyte lineage, in response to stimulation by phorbol ester or cytokine. Such cells assumed and retained a macrophage phenotype as a result of such stimulation. Transient exposure to PDB (< 2 h) did not lead to constitute expression of NF- κ B binding and had no effect on cell morphology (data not shown). Once monocyte differentiation occurred, however, NF- κ B-binding activity remained, as seen constitutively expressed in normal monocytes prepared from human blood and mouse peritoneal exudate (fig. 1).

In the process of cell differentiation, we have demonstrated that the appearance of NF- κ B contributes to the activation of HIV in monocytes. This has great clinical implications in that the presence of an opportunistic infection, or HIV

infection of monocyte/macrophages *per se*, is likely to activate HIV transcription and therefore presumably enhance disease progression. It is known, however, that NF- κ B is not the sole activator of HIV in macrophages. For example GM-CSF, which does not induce NF- κ B binding, activates HIV expression in mature macrophages (Koyanagi *et al.*, 1988) which already contain NF- κ B, and therefore alternate non-NF- κ B activation mechanisms are likely to exist *in vivo*.

Phorbol esters provide a potent reproducible stimulus *in vitro* for monocyte differentiation and HIV

activation; however, such a stimulus is *artificial*. It is now becoming apparent that, in clinical HIV infection, the physiological stimuli most likely to induce HIV activation are the cytokines or some bacterial products, for example endotoxin (LPS). HIV infection of monocyte/macrophages is clearly an important cell-activating event *per se* and several groups have reported stimulation of cytokine transcription and release in response to this event.

For example, the production of TNF α , β and interferon has been demonstrated in monocytes (Vyakarnam *et al.*, 1990). In addi-

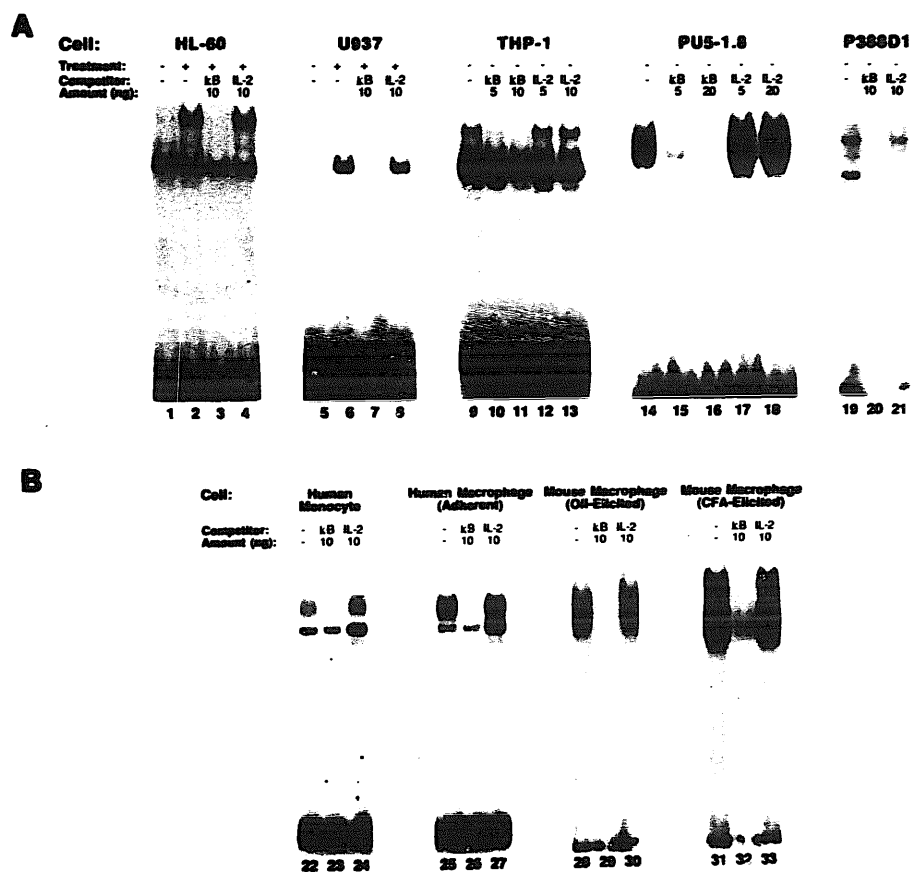


Fig. 1A. NF- κ B-binding activity in nuclear extracts of immature monocyte cell lines is induced by exposure to 40 nM TPA (lanes 1-8) and is found constitutively in mature macrophage cell lines (lanes 9-21).

Arrows denote specific inducible complexes completed by double-stranded κ B oligonucleotide.

Fig. 1B. NF- κ B-binding activity is demonstrated in human peripheral blood monocytes (lanes 22-27) and elicited mouse peritoneal macrophages (lanes 28-33).

tion, it has been shown that THP-1 cells acutely infected with HIV demonstrate phenotypic changes associated with maturation, and released significantly higher levels of TNF α and IL-1 β compared to chronically infected cells (Molina *et al.*, 1989).

However, the same group reported that *in vitro* HIV infection of human peripheral blood monocyte/macrophages did not result in cytokine production but that these cells retained the capac-

ity to produce cytokines after LPS stimulation (Molina *et al.*, 1990). These findings were supported by a similar study using human monocyte/macrophages infected *in vitro* (Munis *et al.*, 1990). LPS has been shown to be a potent stimulus of HIV1 expression in monocyte/macrophage cell lines, but not surprisingly LPS has no similar activity towards on lymphocytes (Pomerantz *et al.*, 1990). Some evidence is also accumulating that TNF α released from

monocyte/macrophages may be acting in an autocrine manner in HIV-infected cells to promote HIV activation (Poli *et al.*, 1990).

Macrophages are known to be crucial cells in host defence against infection. Many stimuli are known to activate such cells via different mechanisms and receptors. However, a final common end point of terminal differentiation of monocyte into macrophage appears to be the constitutive expression of NF- κ B. We have demonstrated that such differentiation is associated with activation of HIV transcription and virus production in these cells. The relationship of other receptor-mediated activation processes of macrophages, for example phagocytosis, which are relevant to the *in vivo* situation clearly deserve investigation.

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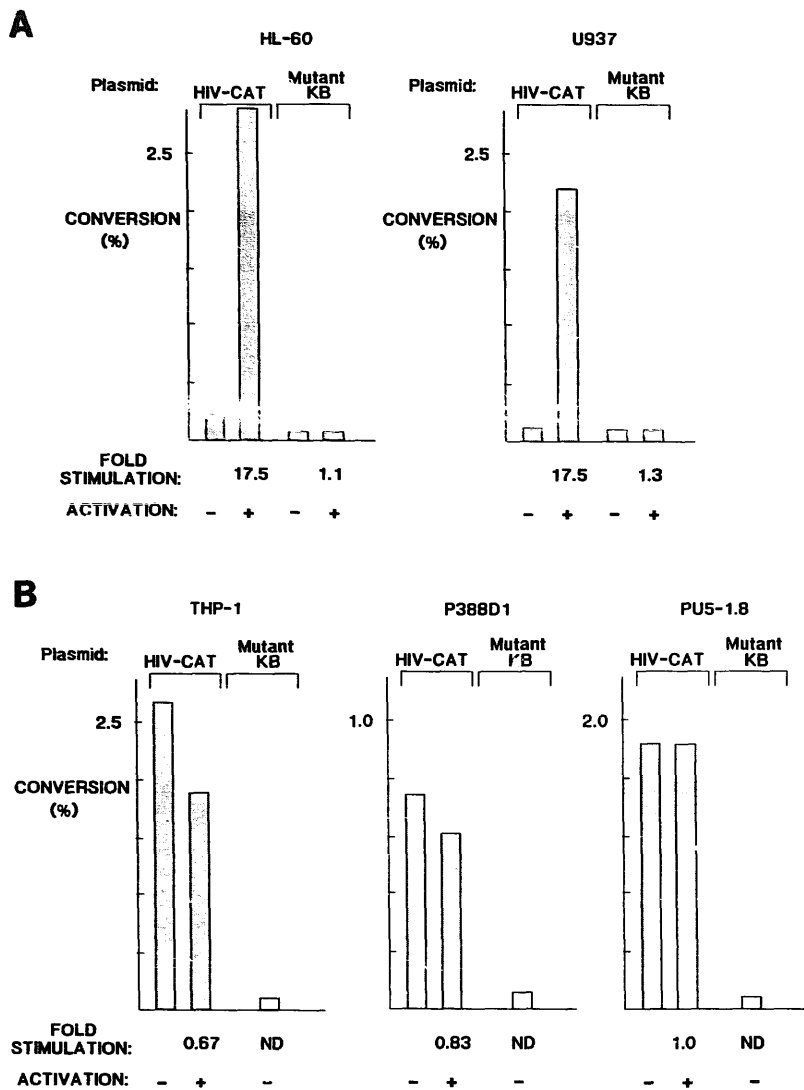


Fig. 2. Activation of HIV-CAT expression in immature monocyte cells (A) is elicited by exposure to 40 nM TPA. In contrast constitutive CAT activity is seen in unstimulated mature cells (B).

L'induction de NF- κ B au cours de la différenciation des monocytes est associée à l'activation de l'expression des gènes du VIH

Les cellules de la lignée monocyte-macrophage sont des cibles importantes du VIH (virus de l'immunodéficience humaine). Nous rapportons ici que la différenciation phénotypique de lignées de cellules monocytaires induite par des esters de phorbol ou par le TNF α (tumeur necrosis factor) est associée à l'expression du facteur nucléaire kappa B (NF- κ B). Parallèlement à une telle différenciation, la transcription du VIH, contrôlée par la construction d'un gène reportant le LTR (long terminal

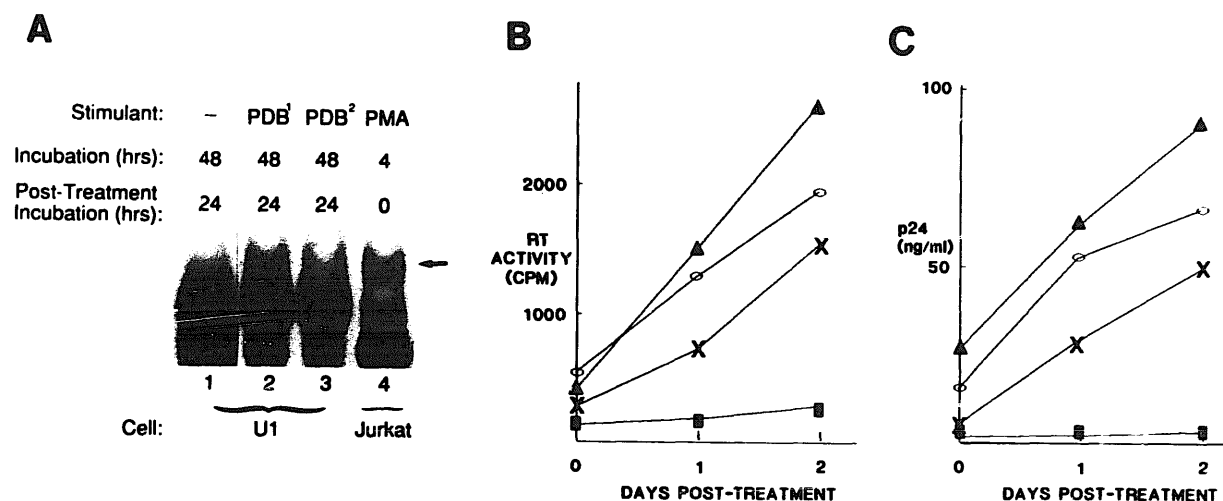


Fig. 3. Increased constitutive expression of NF- κ B (A) and HIV (B and C) in an HIV chronically infected promonocytic cell line, U1, following cellular differentiation induced by PDB or TPA.

Reverse transcriptase (RT) activity and p24-antigen activity were determined in tissue culture supernatants (B) as indices of HIV production.

U1 cells were incubated in medium alone (■) or incubated with 2 nM PDB (▲), 20 nM PDB (●) or 10^3 units/ml TNF α for 48 h followed by washing and incubation for a further 24 or 48 h.

repeat) du VIH, est activée dans de telles cellules sous l'influence de l'augmentation de l'expression NF- κ B. De même, dans une lignée cellulaire promonocytaire infectée chroniquement par le VIH, l'expression du NF- κ B et la transcription du VIH sont stimulées par les esters de phorbol ou le TNF α . Ainsi, la stimulation de lignées de cellules monocytaires par les esters de phorbol ou par le TNF α induit la différenciation cellulaire et active la transcription du VIH. Un tel processus peut avoir des implications fondamentales dans la pathogenèse du VIH *in vitro*, et peut être important dans la progression de la maladie induite par des infections opportunistes impliquant directement ou indirectement les macrophages.

Mots-clés: VIH, Monocyte, Macrophage, SIDA, Facteur nucléaire kappa B; Esters de phorbol, TNF α , Maturation cellulaire, Transcription.

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