Activation of NF-κB and p38 MAP Kinase Is Not Sufficient for Triggering Efficient HIV Gene Expression in Response to Stress†

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ABSTRACT: Recent studies have established an essential role for p38 MAP kinase in UV activation of human immunodeficiency virus (HIV) gene expression. However, p38 MAP kinase is not involved in activation of NF-κB, a key transcriptional activator of HIV gene expression, in response to UV, suggesting that NF-κB acts independently of p38 MAP kinase. In this study, we have investigated whether activation of HIV gene expression occurs when p38 MAP kinase and NF-κB are activated by separate stress-causing treatments, each relatively specific for activating only one of the factors. Treatment of cells with sorbitol (hyperosmotic shock) strongly activates p38 MAP kinase, whereas the cytokine TNF-α is a poor activator of p38 MAP kinase. On the other hand, TNF-α is a strong activator of NF-κB whereas sorbitol is not. Sorbitol, however, activates AP-1 DNA binding activity in a manner similar to that of UV. Most importantly, both sorbitol and TNF-α are poor activators of HIV gene expression in HeLa cells stably transfected with an HIVcat reporter gene, whereas UV elicits a strong response. The combined treatment with UV and hyperosmotic shock produces an additive effect on HIV gene expression, suggesting that these agents activate at least in part by different mechanisms. The combined treatment with sorbitol and TNF-α activates p38 and NF-κB to levels similar to those with UV, yet only results in 25−30% of the CAT levels elicited by UV. Inhibition of NF-κB activation by the protease inhibitor *N*-α-tosyl-L-phenylalanine chloromethyl ketone (TPCK) prevents UV activation of HIV gene expression, but does not inhibit p38 MAP kinase activation. We conclude that whereas both p38 MAP kinase and NF-κB are important for UV activation of HIV gene expression they act independently from each other and activation of both factors is not sufficient for triggering a full HIV gene expression response. Activation of HIV gene expression by UV must therefore involve additional cellular processes, such as those triggered by DNA damage, for generation of a full gene expression response.

Human immunodeficiency virus (HIV)[1] pathogenesis is characterized by a prolonged disease-free stage lasting over a period of years with little or no viral replication. This state of latency correlates with low transcriptional activity of the HIV proviral genome (*1*). Cytokines, such as TNF-α and IL-1, elicited in response to inflammation and stress stimulate HIV gene expression in a variety of cell types (*2*). Ultraviolet light (UV) and some other types of DNA damage also activate HIV gene expression efficiently (*3*, *4*). The mechanism by which UV activates signaling pathways and other cellular events necessary for the activation of HIV gene expression is not entirely clear (*5*).

We recently demonstrated that activation of p38 MAP kinase is essential, but not sufficient, for a full HIV gene expression response (*6*). Using the drug SB203580, which is highly specific for the p38 MAP kinase, and genetic experimentation as well, we were able to demonstrate that inhibition of p38 MAP kinase effectively prevents UV activation of HIV gene expression but does not affect HIV Tat-mediated stimulation. Furthermore, UV activation of the pleiotropic transcription factor NF-κB, important for HIV transcriptional activation, was not affected when p38 MAP kinase was inhibited, whereas at the same time, HIV gene expression was completely obliterated. This result is in line with our previous finding that NF-κB activation alone is not sufficient for generating a full HIV gene expression response (*5*, *7*).

Activation of NF-κB and p38 MAP kinase is mediated by distinct and separate stress pathways in response to arsenite, hydrogen peroxide, and TNF-α (*8*), although the response to TNF-α is controversial (*9*). TNF-α is a strong activator of NF-κB (*10*) but a relatively poor activator of p38 MAP kinase (*11*), whereas cell treatment with sorbitol (hyperosmotic shock) is a strong activator of p38 MAP kinase (*12*, *13*). Treatment of cells with UV activates both NF-κB and the p38 MAP kinase (*7*, *14*−*17*). Because NF-κB and p38 MAP kinase seem to play important roles in HIV gene regulation in response to UV, we investigated whether we could separately activate either one or both of

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[1] Abbreviations: CAT, chloramphenicol acetyltransferase; HIV, human immunodeficiency virus; JNK, c-Jun-NH_2-terminal kinase; MAP, mitogen-activated protein; PMA, phorbol-12-myristate 13-acetate; SAPK, stress-activated protein kinase; TNF-α, tumor necrosis factor α; TPCK, *N*-α-tosyl-L-phenylalanine chloromethyl ketone.

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these factors by agents that do not damage DNA to see whether this would be sufficient for a full HIV gene expression response. In the study presented here, we have examined whether p38 MAP kinase and NF-κB activation alone or in combination is sufficient for a full HIV gene expression response. We demonstrate that activation of p38 MAP kinase and NF-κB with agents that do not produce any DNA damage is not sufficient for eliciting a full HIV gene expression response comparable to that elicited by UV. We also demonstrate that activation of p38 MAP kinase by UV is not dependent on NF-κB.

MATERIALS AND METHODS

Reagents. TNF-α was obtained from GIBCO-BRL (Grand Island, NY), and sorbitol was from Sigma (St. Louis, MO). The CDP-Star system was from Tropix, Inc. (Bedford, MA). [1,2-^{14}C]Chloramphenicol was from Amersham (Arlington Heights, IL), and [γ-^{32}P]ATP was from New England Nuclear (Boston, MA). The p38 MAP kinase antibodies were purchased from New England Biolabs (Beverly, MA), and the anti-IκB-α antibody was from Santa Cruz Biotechnology (Santa Cruz, CA). The HIV NF-κB 33-mer (*7*) was purchased from Genosys Inc. (The Woodlands, TX), and the consensus AP-1 oligonucleotides were from Promega, Inc. (Madison, WI).

Cell Culture and Treatments. The HIVcat/HeLa (−485/+80) cells, carrying the HIVcat transcription unit, have been described previously (*3*). Cells were maintained in DMEM (high glucose) supplemented with 10% fetal bovine serum (Irvine Scientific, Irvine, CA), penicillin (100 units/mL), and streptomycin (100 μg/mL). UV irradiations were carried out at a dose rate of 1−2.5 W/m^2 with a calibrated (Spectronic model DM-254N) germicidal (254 nm) UV lamp. Treatment with sorbitol, SB203580, and CAT assays were carried out as described previously (*3*, *6*).

Gel-Shift Assay (EMSA) and Western Blotting. Nuclear extracts from HIVcat/HeLa cells were prepared from confluent dishes and used in EMSAs with NF-κB and AP-1 oligonucleotides as described previously (*7*). Proteins were separated by SDS−PAGE and transferred to PVDF membranes (Bio-Rad, Richmond, CA). The membranes were blocked with 5% dry milk in Tris-buffered saline containing 0.1% Tween 20 and probed with anti-phospho-p38 MAP kinase antibody or anti-IκB-α antibody. Blots were developed using the alkaline phosphatase/CDP-Star system followed by exposure to X-ray film. The same blot was used to probe for equal loading of protein using anti-p38 MAP kinase antibody.

RESULTS

UV Is More Potent Than Hyperosmotic Shock in Activating HIV Gene Expression, and the Treatment with Both Agents Produces an Additive Effect. One of the most thoroughly characterized stress response in yeast and mammalian cell culture systems is that inflicted by hyperosmotic shock using a high concentration of sorbitol or NaCl that activates the p38 MAP kinase (SAPK-2/RK) pathway (*12*, *13*, *18*). We have recently demonstrated that p38 MAP kinase is essential for UV activation of HIV gene expression, whereas activation by the HIV *trans*-activator Tat does not require this stress-activated protein kinase (*6*). Furthermore, treatment of

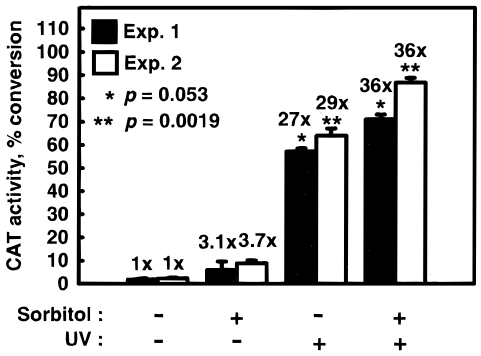

FIGURE 1: Additive effects of UV and hyperosmotic shock on HIVcat gene expression. HIVcat/HeLa cells were treated with UV (30 J/m^2), sorbitol (0.5 M for 30 min), or UV followed by sorbitol. After 20 h, cells were harvested and CAT activity was determined as described in Materials and Methods. The results of two independent experiments carried out in triplicate are shown (mean ± SEM). Significant differences are denoted by one and two asterisks.

HIVcat/HeLa cells with sorbitol (hyperosmotic shock) activates HIV gene expression one order of magnitude less efficiently than UV irradiation but activates p38 MAP kinase to levels similar to those with UV, suggesting that activation of p38 MAP kinase alone is not sufficient for a full HIV gene expression response. To investigate the reason for this differential effect on HIV gene expression, we first examined whether sorbitol treatment potentiated the UV effect. HIVcat/HeLa cells were irradiated with UV (30 J/m^2) and then treated with sorbitol for 30 min. We have previously determined that this sorbitol exposure time produced maximum HIV CAT activity of 3−4-fold increases above basal levels. After 20 h, the cells were harvested and CAT was activity determined. As demonstrated in Figure 1, sorbitol treatment only increased CAT activities between 3.1- and 3.7-fold. UV activated HIVcat gene expression 27−29-fold, whereas the combined effect of UV and sorbitol produced a significant ($0.0019 < p < 0.053$) synergistic increase (36-fold) in the level of HIVcat gene expression above the UV levels.

This result demonstrates that UV activates HIV gene expression one order of magnitude more effectively than hyperosmotic shock. Because UV and hyperosmotic shock produce an additive effect on HIVcat gene expression, these two agents act at least to some extent by different mechanisms to activate HIV gene expression. Furthermore, because sorbitol is a potent activator of p38 MAP kinase yet does not fully activate HIVcat gene expression, this result also suggests that p38 MAP kinase activation alone is not sufficient.

UV and TNF-α Are Potent Activators of NF-κB, whereas Sorbitol Is Not. To investigate further the reason for the additive effect of hyperosmotic shock and UV on HIV gene expression, we examined the effect of sorbitol treatment on activation of the transcription factor NF-κB. This transcription factor is activated in response to UV and ionizing radiation and to other stress-causing agents (*7*, *14*, *15*), and has been suggested as the major cause of increased levels of HIV gene expression in response to UV in transient transfection assays (*14*, *15*), but not when the UV response is studied in cells stably transfected with an HIV reporter gene (*7*, *19*). As demonstrated in Figure 2, hyperosmotic shock does not activate NF-κB in these cells between 1 and

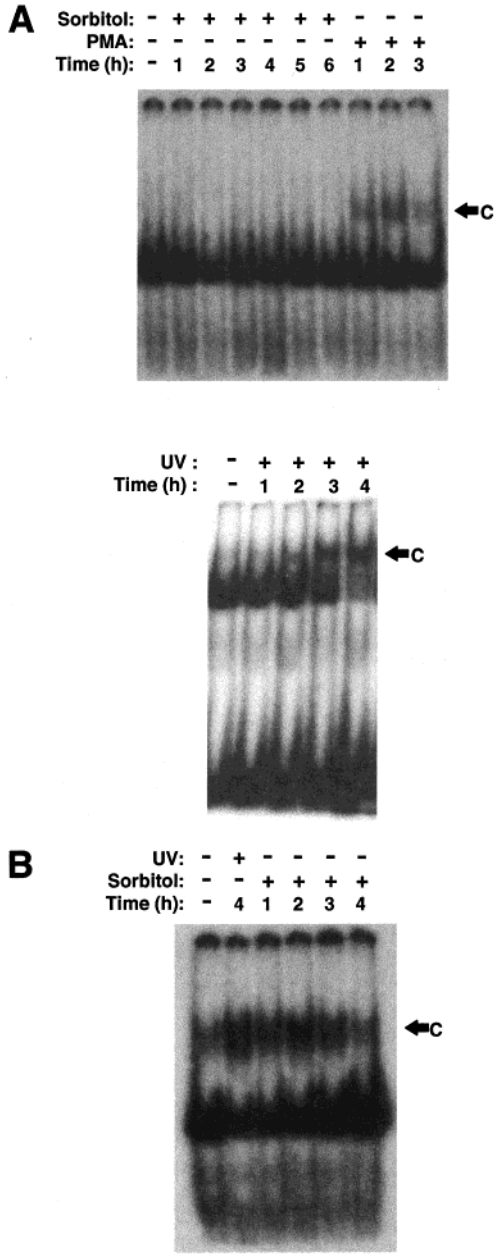

FIGURE 2: Time course of NF-κB and AP-1 activation in response to sorbitol, PMA, and UV. (A) HIVcat/HeLa cells were treated with sorbitol (0.5 M for 30 min), PMA (20 nM), or UV (30 J/m^2). After the indicated times, NF-κB DNA binding activity was measured by EMSA as described in Materials and Methods. (B) HIVcat/HeLa cells were treated with UV (30 J/m^2) or sorbitol (0.5 M for 30 min). After the indicated times, AP-1 DNA binding activity was measured by EMSA as described in Materials and Methods.

6 h, at times when UV and phorbol ester (PMA) are potent activators (Figure 2A). Because of this lack of an effect, we then used the same nuclear extract to see whether AP-1 activation could be detected in response to hyperosmotic shock. It was previously demonstrated that hyperosmotic shock activates *c-jun* transcription through an increased JNK activation level (*20*), and hence, it is expected that AP-1 would be activated by sorbitol treatment. Indeed, Figure 2B shows that AP-1 is activated equally well by sorbitol and UV. Peak AP-1 binding was observed 2−3 h after hyperosmotic shock. These results demonstrate that sorbitol is a poor activator of NF-κB but a relatively potent activator of

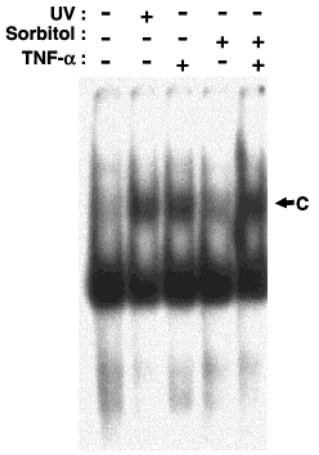

FIGURE 3: UV and TNF-α are potent activators of NF-κB, whereas sorbitol is not. HIVcat/HeLa cells were treated with UV (30 J/m^2), TNF-α (20 ng/mL), sorbitol (0.5 M for 30 min), or the combination of TNF-α and sorbitol. After 6 h, cells were harvested and NF-κB DNA binding activity was determined by EMSA as described in Materials and Methods.

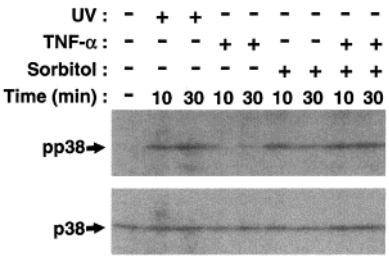

FIGURE 4: UV and hyperosmotic stress activate p38 MAP kinase, whereas TNF-α is a poor activator. HIVcat/HeLa cells were treated with UV (30 J/m^2), TNF-α (20 ng/mL), or sorbitol (0.5 M for 30 min). After the indicated times, Western blot analysis was carried out using phospho-specific p38 MAP kinase antibodies (pp38). The blot was reprobed with p38 MAP kinase antibody (p38) to normalize protein loading.

AP-1, suggesting that a reason for the one order of magnitude difference in the level of HIVcat gene expression seen between UV and sorbitol treatments may be the lack of an effect of sorbitol treatment on activation of NF-κB.

Like with UV, TNF-α treatment strongly activates NF-κB DNA binding activity in HeLa cells (Figure 3), in agreement with previous reports (*21*, *22*). We noticed that there was no further stimulation of NF-κB in response to TNF-α when cells were also treated with sorbitol (Figure 3). All together, sorbitol treatment is a poor activator of NF-κB whereas TNF-α, like UV, is a potent activator.

UV and Hyperosmotic Shock Activate p38 MAP Kinase to Similar Levels, whereas TNF-α Is a Relatively Poor Activator of p38 MAP Kinase. To investigate the effect of hyperosmotic shock on HIV gene expression and p38 MAP kinase activation and to directly compare the relative effect of hyperosmotic shock to those resulting from TNF-α and UV treatment, we examined the levels of p38 MAP kinase activation by Western blotting using phospho-specific p38 MAP kinase antibody. We found that treating HIVcat/HeLa cells with sorbitol for 10 or 30 min resulted in significant increases in the level of p38 MAP kinase activation (Figure 4). UV treatment (30 J/m^2) resulted in similar increases in the level of p38 MAP kinase phosphorylation, whereas treatment with TNF-α produced little to no increase. When cells were treated with both sorbitol and TNF-α, no change

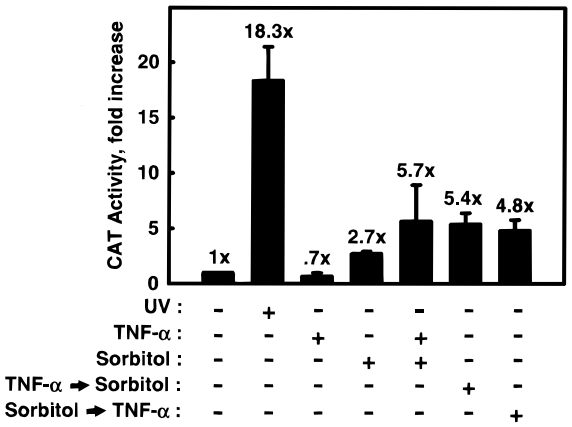

FIGURE 5: Treatment of cells with TNF-α and sorbitol does not produce a full HIV gene expression response. HIVcat/HeLa cells were treated with UV (30 J/m^2), TNF-α (20 ng/mL), sorbitol (0.5 M for 30 min), or the combination of TNF-α and sorbitol, and 20 h later, CAT assays were carried out. The TNF-α and sorbitol treatments were given either simultaneously or one before the other. Assays were carried out in triplicate (mean ± SEM).

in the level of p38 MAP kinase phosphorylation was observed over that seen after sorbitol treatment alone. This result demonstrates that in HeLa cells, UV and sorbitol are potent activators of p38 MAP kinase whereas TNF-α is a relatively poor activator.

Treatment of Cells with both TNF-α and Sorbitol Does Not Produce a Full HIV Gene Expression Response. UV activates both p38 MAP kinase and NF-*κ*B DNA binding activity. Sorbitol only activates p38 MAP kinase but not NF-*κ*B, and TNF-α only activates NF-*κ*B but not p38 MAP kinase, as we have demonstrated here. To test whether activation of p38 MAP kinase and NF-*κ*B would activate HIV gene expression to levels seen after UV irradiation, we treated HIVcat/HeLa cells with sorbitol and TNF-α to activate both p38 MAP kinase and NF-*κ*B. As demonstrated in Figure 5, UV treatment of HIVcat/HeLa cells resulted in a more than 18-fold increase in the level of HIVcat gene expression whereas sorbitol and TNF-α alone resulted in modest few-fold increases. When cells were treated with both TNF-α and sorbitol in any of three different ways (sorbitol and TNF-α together, sorbitol followed by TNF-α, or TNF-α followed by sorbitol), the level of HIVcat gene expression only increased ∼5-fold, or 25−30% of the levels elicited by UV.

Very recently, a report appeared that suggested that activation of p38 MAP kinase by sorbitol treatment results in the inhibition of I*κ*B-α degradation in response to TNF-α in human colon carcinoma cells and inhibition of NF-*κ*B-mediated gene expression (*9*). It was therefore important to examine whether sorbitol treatment also inhibits I*κ*B-α degradation in response to TNF-α in HIVcat/HeLa cells since no effect was seen on NF-*κ*B activation (Figure 3). Therefore, protein extracts isolated from HIVcat/HeLa cells that were either left alone or pretreated with sorbitol 30 min before being challenged with TNF-α were analyzed for I*κ*B-α and phospho-p38 MAP kinase levels by Western blotting at different times (Figure 6). In agreement with a previous report (*9*), TNF-α triggered the rapid degradation of I*κ*B-α also in these cells, but not completely, as approximately 50% remained intact. Pretreatment with sorbitol inhibited this degradation (Figure 6B). During the same time, an increased

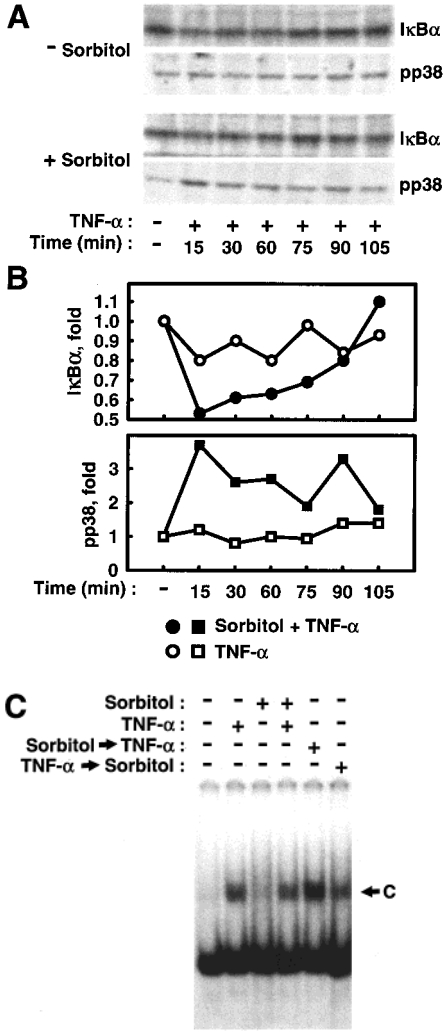

FIGURE 6: Sorbitol treatment inhibits degradation of I*κ*B-α but does not prevent NF-*κ*B activation by TNF-α. (A) Western blot analysis of I*κ*B-α and p38 MAP kinase phosphorylation after stimulation of HIVcat/HeLa cells with TNF-α (20 ng/mL). One set of dishes was left untreated, and a second set was pretreated with sorbitol (0.5 M) for 30 min prior to the addition of TNF-α. Lysates were prepared at the indicated times, separated by SDS−PAGE, transferred to PVDF membrane, and incubated with either anti-I*κ*B-α or anti-phospho-p38 antibodies. (B) Quantification of I*κ*B-α and p38 MAP kinase phosphorylation expressed as the *x*-fold value of untreated control. (C) NF-*κ*B EMSA with cell extracts isolated from HIVcat/HeLa cells treated the same as described for panel A. Different combinations of sorbitol and TNF-α treatments were carried out (see the legend of Figure 5). Cell extracts were prepared 5 h after treatment.

(∼3-fold) level of p38 MAP kinase phosphorylation was clearly evident. Furthermore, activation of NF-*κ*B by TNF-α was not affected by the sorbitol treatment irrespective of whether it was done immediately before, simultaneous with, or immediately after the stimulation with TNF-α (Figure 6C).

These results demonstrate that when HIVcat/HeLa cells are treated with both sorbitol and TNF-α, and NF-*κ*B and p38 MAP kinase are activated to levels seen after UV, only a partial HIVcat gene expression response is achieved. This partial response is not the result of any inhibitory effects of sorbitol on the activation of NF-*κ*B by TNF-α since no alteration in this response occurred despite an inhibition of I*κ*B-α degradation. These findings suggest that UV triggers other responses in addition to NF-*κ*B and p38 MAP kinase

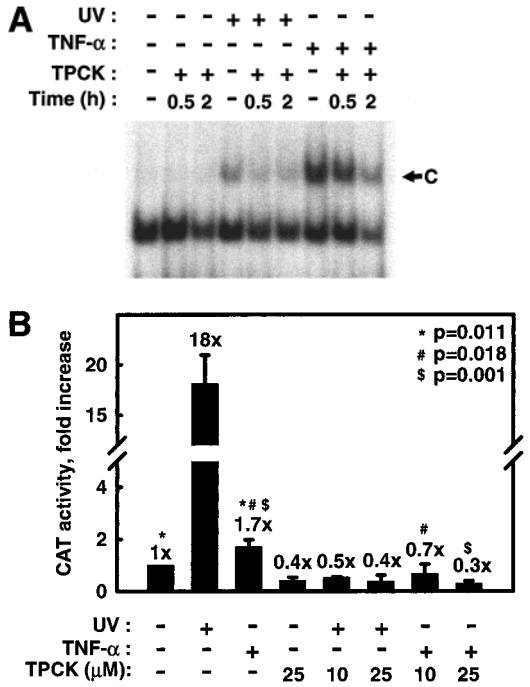

FIGURE 7: Protease inhibitor TPCK inhibits UV and TNF-α activation of NF-κB and HIVcat expression. HIVcat/HeLa cells were pretreated with TPCK (10 or 25 μg/mL) for either 0.5 or 2 h and then irradiated with UV (30 J/m^2) or treated with TNF-α (20 ng/mL). Cells were harvested after 5 h and EMSA (A) using NF-κB oligonucleotide as a probe, and CAT activities (B) were determined after 20 h as described in the legend of Figure 5.

activation that are important for a full HIV gene expression response.

UV Activation of NF-κB and HIV Gene Expression Is Inhibited by Treatment of Cells with the Protease Inhibitor TPCK without Affecting p38 MAP Kinase Activation. The activation of cytoplasmic NF-κB heterodimers consisting of p50 (NF-κB1) and p65 (RelA) subunits requires the degradation of an inhibitor, IκB-α, which sequesters NF-κB in the cytoplasm (*23*). Following phosphorylation of two conserved N-terminal serine residues and ubiquitination, IκB-α is degraded through the 26S proteasome pathway (*24*, *25*). Widely used serine protease inhibitors such as *N*-α-tosyl-L-phenylalanine chloromethyl ketone (TPCK) inhibit NF-κB activation by interfering with IκB-α proteolysis (*26*). To see whether inhibition of NF-κB nuclear translocation would affect signaling through the p38 MAP kinase pathway, we treated HIVcat/HeLa cells with TPCK prior to UV treatment and examined the effect on UV activation of NF-κB and p38 MAP kinase phosphorylation. We found that treatment with TPCK alone did not significantly increase the level of NF-κB activation, whereas both UV and TNF-α treatments significantly increased the level of NF-κB at 5 h as shown by EMSA (Figure 7A). Pretreatment of HIVcat/HeLa cells with TPCK for 0.5 and 2 h almost completely obliterated the increase in NF-κB DNA binding activity in response to either UV or TNF-α. The TPCK treatment also resulted in a reduction of CAT activity after UV from 18- to 0.4-fold, whereas the response to TNF-α was reduced from 1.7- to 0.3-fold above basal levels 20 h after treatment (Figure 7B). Five hours after treatment, a similar reduction in CAT activities was observed (data not shown). Most importantly, UV activation of p38 MAP kinase was not affected by the

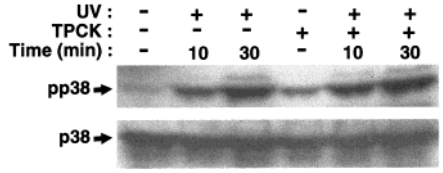

FIGURE 8: Protease inhibitor TPCK does not inhibit UV activation of p38 MAP kinase. HIVcat/HeLa cells were pretreated with TPCK (25 μg/mL) for 0.5 h or left untreated and then irradiated with UV (30 J/m^2). Cells were harvested 10 and 30 min after UV treatment and total cell lysates separated on SDS−PAGE, transferred to PVDF membrane, and sequentially immunoblotted with T^{p180}/Y^{p182}-p38-specific antibody (pp38) and anti-p38 (p38) antibody.

TPCK treatment (Figure 8), suggesting that p38 MAP kinase activation is not linked to NF-κB activation during the UV response.

These results demonstrate that inhibition of NF-κB activation and/or nuclear translocation using the protease inhibitor TPCK prevents UV activation of HIV gene expression without affecting p38 MAP kinase activation, suggesting that whereas both NF-κB and p38 MAP kinase activation are important for HIV gene expression, they act independently of each other.

DISCUSSION

Ultraviolet light is a very potent activator of HIV gene expression (*3*, *14*). UV produces numerous pleiotropic effects on the cell resulting from DNA damage, lipid peroxidation, and the formation of reactive oxygen intermediates that trigger signal transduction pathways, including the p42/p44 MAP kinase, JNK, and p38 MAP kinase pathways, which in turn activate certain transcription factors, such as NF-κB and AP-1 (*27*−*29*).

Two key events appear to be important in producing a full HIV gene expression response: activation of the p38 MAP kinase (*6*, *17*) and activation of the transcription factor NF-κB (*14*, *15*). However, whereas activation of p38 MAP kinase appears to be essential for the HIV UV response (*6*, *17*), NF-κB is not essential but may rather augment the response (*5*, *7*, *19*). As we have demonstrated here, other stress-causing agents such as sorbitol, which results in hyperosmotic shock, or the cytokine TNF-α are potent activators of p38 MAP kinase and NF-κB, respectively. However, activation of either factor alone only elicits a relatively minor HIV gene expression response, and even activation of both p38 MAP kinase and NF-κB results only in a partial HIVcat response compared to that with UV, suggesting that UV elicits additional responses that are important for stimulating HIV gene expression. Treatment of cells with agents such as ionizing radiation or TNF-α produces potent activation of NF-κB which in itself is insufficient for activating HIV gene expression when the transcription unit is integrated in the genome (*7*, *30*). Importantly, in addition of being a potent activator of both p38 MAP kinase and NF-κB, UV also generates DNA damage which most likely affects gene expression.

Our results and those of others have demonstrated strong activation of p38 MAP kinase in response to hyperosmotic shock (*6*, *12*). However, for the first time, we demonstrate that sorbitol is ineffective in activating NF-κB in HeLa cells. On the other hand, hyperosmotic shock increases the level of AP-1 DNA binding. In agreement with this finding,

hyperosmotic shock of hepatoma cells activates JNK and increases *c-jun* mRNA, suggesting an involvement of AP-1 and c-Jun phosphorylation in this response (*20*). Furthermore, sorbitol treatment activates luciferase expression in HeLa cells stably transfected with a plasmid having the human *c-jun* promoter controlling the expression of the luciferase gene (M. Taher et al., unpublished observations). Of particular interest is our finding that despite the apparent inhibitory effect of sorbitol on IκB-α degradation in response to TNF-α, NF-κB activation is not affected.

What is not clear at this point is whether NF-κB only plays a role at the HIV transcription level or is also necessary for some other process that is important for HIV gene expression in response to UV, such as DNA repair or apoptosis. Conflicting reports about the importance of NF-κB in the HIV UV response have appeared (*14*, *19*, *31*). These discrepancies may be related to the fact that some studies used transient transfections whereas others used stably transfected cell clones to assess the importance of specific transcription elements in HIV gene regulation in response to UV. However, it is clear that activation of NF-κB alone is not sufficient for increasing HIV gene expression in HeLa cells because phorbol ester and TNF-α are potent activators of NF-κB, whereas they are poor activators of HIV gene expression. This is also the case with ionizing radiation (*5*, *7*, *22*), which may be due to the relatively poor activation of p38 MAP kinase (M. Taher et al., in press). The lack of a correlation between the level of NF-κB activation and increased HIV transcriptional activation levels has been demonstrated in other cell types as well (*32*, *33*). It is possible that NF-κB is not directly needed in the HIV transcriptional process, but rather in other cellular processes associated with genotoxic stress, such as the apoptotic response (*34*), which then in turn activates HIV transcription. In fact, the possibility that NF-κB is not directly required for activation of HIV transcription was suggested for the regulation of HIV by p21/*WAF*1 and p300 in response to cell cycle arrest (*35*). However, triggering of apoptosis without DNA damage does not activate HIV gene expression (J. D. Oakley et al., manuscript in preparation), suggesting that the DNA damage itself is critical for UV activation of HIV gene expression.

As we have shown here, treatment of cells with the protease inhibitor TPCK, an inhibitor of NF-κB-mediated transcriptional activation of HIV (*36*, *37*), did not affect the phosphorylation of p38 MAP kinase after UV, but inhibited UV activation of NF-κB and HIV gene expression, suggesting that p65 (RelA) translocation to the nucleus is important for this response to occur. However, treatment with TPCK may also affect apoptosis through a mechanism not involving NF-κB but by interfering with non-caspase-mediated proteolysis (*38*). Nevertheless, the result suggests that NF-κB activation is independent of p38 MAP kinase in the response to UV. We recently reported that the reciprocal is also true; i.e., p38 MAP kinase does not act through NF-κB and the HIV enhancer because NF-κB EMSAs are not affected by SB203580 and UV activation of enhancer deletion constructs is still inhibited by SB203580 (*6*). Furthermore, engineered p38 MAP kinase-inactive HIVcat/HeLa cells that have the HIVcat UV response obliterated are still able to fully activate NF-κB.

The mechanism by which p38 MAP kinase regulates HIV gene expression in response to UV has not yet been established. p38 MAP kinase is required for IL-1β transcription (*39*), CD40-induced gene expression and proliferation of B lymphocytes (*40*), T-cell proliferation in response to IL-2 and -7 (*41*), and activation of *c-fos* and *c-jun* transcription by diverse stress-causing stimuli (*42*). A number of transcription factors, such as CREB, ATF-1, ATF-2, GADD153, and a protein with an unknown association with gene expression, small heat shock protein Hsp27, are downstream targets in the p38 MAP kinase pathway (*43*, *44*). However, the role of these proteins, if any, in the HIV gene expression response to UV is presently not known.

In summary, we have demonstrated that hyperosmotic shock and treatment with TNF-α strongly activate p38 MAP kinase and NF-κB, respectively, but only activate HIV gene expression at ∼10% of the level generated by UV. Treatment with both agents simultaneously results in CAT activity levels that are 25−30% of the levels generated by UV. Furthermore, UV activation of p38 MAP kinase occurs independently of NF-κB because inhibiting NF-κB nuclear translocation with the protease inhibitor TPCK did not prevent p38 MAP kinase from being activated by UV. Our results suggest that in addition to activating p38 MAP kinase and NF-κB, UV elicits other unknown cellular responses, perhaps associated with DNA damage and repair or with apoptosis, that are necessary for efficient HIV gene expression in response to stress.

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