Cell Type–Specific Mechanisms of Interleukin-8 Induction by Dengue Virus and Differential Response to Drug Treatment

Carey L. Medin and Alan L. Rothman
Center for Infectious Disease and Vaccine Research and Department of Medicine, University of Massachusetts Medical School, Worcester

In vitro infection with dengue virus induces interleukin (IL)–8 secretion, which increases endothelial cell permeability; this has been proposed as a mechanism for plasma leakage in dengue hemorrhagic fever. We studied the mechanisms of IL-8 induction, using luciferase reporter constructs, and the effect of pharmacological inhibitors of either IL-8 secretion or nuclear factor–κB (NF-κB) activation on IL-8 induction by dengue 2 virus (DEN2V) infection. IL-8 induction by DEN2V infection was associated with activation of NF-κB and activator protein–1 (AP-1) in HEK293A cells but only with activation of AP-1 in HepG2 cells. Treatment with SB203580, a mitogen-activated protein kinase inhibitor, and rolipram, a phosphodiesterase IV inhibitor, partially inhibited DEN2V-induced IL-8 secretion in HEK293A cells but increased DEN2V-induced IL-8 secretion in HepG2 cells. In contrast, treatment with dexamethasone increased DEN2V-induced IL-8 secretion in HEK293A cells but had no effect on DEN2V-induced IL-8 secretion in HepG2 cells. These results demonstrate that anti-inflammatory drugs have variable effects on IL-8 secretion in different cell types during DEN2V infection.

Dengue hemorrhagic fever (DHF) is characterized by increased capillary permeability resulting in decreased plasma volume, which may be accompanied by hemorrhagic manifestations. The pathogenesis of dengue virus (DENV) infection is thought to involve dysregulation of the immune response. Chemokines have been proposed to contribute to inflammation and disease pathogenesis. In vitro studies have shown an increase in chemokine induction by DENV infection in primary monocyte/macrophages, endothelial cells, peripheral blood mononuclear cells, liver cells, and mast cells [1–4]. Increased levels of chemokines have been reported in DENV-infected patients and have been associated with more-severe disease [1, 2, 5–7]. Interleukin (IL)–8 has been shown to modulate endothelial cell permeability [8–11]. Chemokines have been proposed to contribute to inflammation and disease pathogenesis by attracting memory T cells to the sites of viral replication during secondary DENV infection [12]. DENV-specific memory T cells have a rapid response to DENV antigen and can increase the inflammatory response and plasma leakage during DENV infection. Recent studies have also shown that there is expression of the IL-8 receptor, CXCR1, on terminally differentiated memory CD8+ T cells [11, 13]. These results suggest a role for IL-8 in plasma leakage, which is the hallmark of DHF.

NF-κB plays a predominant role in chemokine and cytokine induction in many experimental systems [14]. Similarly, studies have reported that NF-κB plays a major role in DENV-induced IL-8 secretion [3, 15], and our previous experiments with HEK293A cells yielded similar conclusions [16]. However, the lack of NF-κB activation has been reported by other researchers [17].

Cytokines play an important role in inflammatory diseases, and much effort has been made to identify the
pharmacological inhibitors of proinflammatory cytokine production. The benefit of these small-molecule inhibitors is that they can access signaling pathways within a cell to inhibit a wide range of cytokines and chemokines and, thus, have anti-inflammatory therapeutic potential [18]. We were interested in the effects of the drugs SB203580, a p38 mitogen–activated protein kinase (MAPK) inhibitor [19–21]; rolipram, a phosphodiesterase IV inhibitor [22, 23]; and dexamethasone, a glucocorticoid [24], on IL-8 induction during dengue 2 virus (DENV2) infection. These drugs have been used previously to inhibit cytokine and chemokine production. We found that SB203580 and rolipram increased chemokine production in a DENV2-infected human hepatocarcinoma cell line (HepG2) [25], whereas dexamethasone had the same effect in a human embryonic kidney cell line (HEK293A) [26]. We conclude that drugs that inhibit signaling pathways involved in cytokine production in other experimental systems can have variable effects on chemokine induction in different cell types during DENV2 infection.

MATERIALS AND METHODS

Infection with DENV2. HepG2 (American Type Culture Collection) and HEK293A (Invitrogen) cells were maintained as described elsewhere [16]. Cells were infected with DENV2 strain New Guinea C, as described elsewhere [27]. The titers of the virus pools were determined by fluorescent focus assay in CV1 cells [28]. Cells were infected with DENV2 at an MOI of 5 and incubated at 37°C for 2 h. For assays assessing chemokine protein levels by ELISA, supernatants were harvested at each time point, and fresh medium was added to the cell cultures. Cells were fixed with 1% paraformaldehyde and stained with DENV antigen with a DENV complex–fluorescein isothiocyanate conjugate antibody (Fitzgerald Industries International) or an isotype control. The percentage of infected cells was assessed from 20,000 events by flow cytometry.

ELISA. IL-8 protein concentrations were determined in cell culture supernatants by use of commercially available ELISA kits, in accordance with the manufacturer’s instructions (R&D Systems).

Pharmacological inhibitors. SB203580—4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)imidazole—rolipram, and dexamethasone were purchased from Sigma-Aldrich. All inhibitors were diluted in dimethylsulfoxide (DMSO) to a stock solution (10 mmol/L rolipram, 1 mmol/L dexamethasone, and 1 mmol/L SB203580), as suggested by the manufacturer. The final concentration of DMSO in cell cultures was <1%. Cells were infected with DENV2 at an MOI of 5 and incubated at 37°C for 2 h (as above) or incubated in the presence of 10 μmol/L SB203580, 100 μmol/L rolipram, or 250 nmol/L dexamethasone. These drug concentrations were selected on the basis of their ability to inhibit cytokine production in previous studies. At specified time points, the medium was replaced with fresh medium containing the same drug.

Plasmids. Four luciferase reporter constructs containing the IL-8 promoter were provided by N. Mukaida (Kanazawa University, Kanazawa, Japan): 1 with wild-type (wt) binding sites for NF-κB, activator protein (AP)–1, and CCAAT/enhancer-binding protein (c/EBP) and 3 with a mutant binding site for either NF-κB, AP-1, or c/EBP [29]. Luciferase reporter constructs containing a tandem repeat of the consensus binding site for the transcription factor c/EBP, AP-1, or NF-κB were obtained from Stratagene.

Luciferase reporter gene assay. HEK293A and HepG2 cells were transfected with reporter plasmids, as described elsewhere [16]. After 24 h, cells were infected with DENV2; 72 or 96 h later, luciferase activity was determined. A Renilla reniformis luciferase reporter, pRl-TK, was used as an internal control to normalize luciferase reporter gene activity. Luciferase activity was determined with a luminometer, using the dual luciferase reporter gene assay in accordance with the manufacturer’s instructions (Promega). All conditions were tested in triplicate. At least 2 independent experiments were performed for each assay. Two-tailed Student’s t test was used to compare the mean normalized luciferase activity between replicate wells of experimental groups.

RESULTS

Induction of IL-8 and activation of transcription factors in HEK293A and HepG2 cells. Previously, we reported that HepG2 and HEK293A cells secrete IL-8 after DENV2 infection. In addition, we showed that HEK293A cells induce transcription from the IL-8 promoter and activate predominantly NF-κB and AP-1 after DENV2 infection [16]. We wanted to further assess transcription factors involved in the induction of IL-8 in DENV2-infected HepG2 cells and compare the results in DENV2-infected HEK293A cells. We transfected HEK293A and HepG2 cells with luciferase reporter constructs containing a tandem repeat of the consensus binding site for the transcription factor c/EBP, AP-1, or NF-κB or a construct containing the wt IL-8 promoter. DENV2 infection induced transcription from the wt IL-8 promoter in both HEK293A and HepG2 cells (figure 1A and 1B). Consistent with previous reports, DENV2 infection of HEK293A cells activated NF-κB to a greater extent (6-fold increase vs. activity in medium-treated cells) than it did AP-1 (3-fold increase) or c/EBP (2-fold increase) (figure 1A). In contrast, DENV2 infection of HepG2 cells activated AP-1 (~3-fold increase vs. activity in medium-treated cells), whereas there was no detectable activation of the c/EBP or NF-κB reporter (figure 1B). These results suggest that NF-κB, AP-1, and c/EBP are involved in IL-8 induction during DENV2 infection of HEK293A cells, whereas AP-1 may play the predominant role in IL-8 induction in DENV2-infected HepG2 cells.
vation of transcription factors by DEN2V, therefore, appears to be dependent on the cell type that is infected.

To verify that our luciferase reporter constructs were able to be induced in HepG2 cells, we treated DEN2V-infected cells with PMA and ionomycin. We found that there was induction of transcription from the NF-κB luciferase reporter construct after treatment with PMA and ionomycin (~17-fold increase vs. activity in medium-treated cells; data not shown), suggesting that the failure of DEN2V infection to activate NF-κB in HepG2 cells does not reflect a general defect in NF-κB activation in this cell line.

To determine the importance of these transcription factors within the context of the IL-8 promoter, we transfected HepG2 and HEK293A cells with luciferase reporter constructs containing a mutation (mt) in the binding site for c/EBP (mtc/EBP), AP-1 (mtAP-1), or NF-κB (mtNF-κB), as described elsewhere [29]. We analyzed whether DEN2V infection would differentially activate these promoters, depending on the cell type that was infected. HEK293A cells infected with DEN2V showed ≤30% reduction in activation of the mtAP-1 and mtNF-κB promoters, compared with that of the wt IL-8 promoter (figure 2A). In contrast, none of the mts in the transcription binding sites within the IL-8 promoter significantly inhibited IL-8 induction after DEN2V infection of HepG2 cells (figure 2B). These results suggest that activation of both AP-1 and NF-κB are needed for full IL-8 induction in DEN2V-infected HEK293A cells. However, the results from DEN2V-infected HepG2 cells suggest that, although AP-1 is activated, binding to its site is not necessary for IL-8 induction during DEN2V infection of HepG2 cells.

**Effects of pharmacological inhibitors.** We wanted to examine IL-8 expression in DEN2V-infected cells and analyze the effects of pharmacological inhibitors that were previously reported to inhibit NF-κB and cytokine expression in other systems. Because the studies described above showed differences in activation of transcription factors by DEN2V infection of HEK293A and HepG2 cells, we also wanted to determine if the effects of the pharmacological inhibitors on the induction of chemokines would differ between HEK293A and HepG2 cells. HEK293A and HepG2 cells were infected with DEN2V at an MOI of 5 in the presence of the pharmacological inhibitors. The inhibitors were present in the culture medium throughout the incubation. Supernatants were collected on several different days after infection for chemokine analysis by ELISA (figure 3). In addition, cells treated with each of the inhibitors were stained with a DEN2V-specific antibody and analyzed by flow cytometry for infection rates. There were no significant differences in infection rates in DEN2V-infected HEK293A (mean ± SD infection rate, 88.25% ± 4.9% on day 2 after infection) and DEN2V-infected HepG2 (mean ± SD infection rate, 40.5% ± 5.0% on day 6 after infection) cells treated with inhibitors, compared with those in medium-treated cells.

To characterize the effects that these inhibitors had on transcription from the IL-8 promoter, we transfected HEK293A and HepG2 cells with a luciferase reporter construct containing the wt IL-8 promoter (table 1 and figure 4). In addition, we transfected cells with luciferase reporter constructs containing a tandem repeat of the consensus binding site for the transcription factor CCAAT/enhancer-binding protein (c/EBP), activator protein–1 (AP-1), or NF-κB or a construct containing the wild-type IL-8 promoter and were infected 24 h later with DEN2V at an MOI of 5. At 72 h after infection, luciferase reporter gene activity was measured. For each construct, data were normalized for transfection efficiency on the basis of Renilla reniformis luciferase reporter gene activity and expressed as fold induction relative to the activity of medium-treated cells. Data are expressed as means ± SDs of triplicate wells from a representative experiment of a minimum of 2 separate experiments. *p < .05, vs. medium-treated wells (Student’s t test).

**Figure 1.** Activation of interleukin (IL)–8 promoter and transcription factors by dengue 2 virus (DEN2V) infection of HEK293A and HepG2 cells. HEK293A (A) and HepG2 (B) cells were transfected with luciferase reporter constructs containing a tandem repeat of the consensus binding site for the transcription factor CCAAT/enhancer-binding protein (c/EBP), activator protein–1 (AP-1), or NF-κB or a construct containing the wild-type IL-8 promoter and were infected 24 h later with DEN2V at an MOI of 5. At 72 h after infection, luciferase reporter gene activity was measured. For each construct, data were normalized for transfection efficiency on the basis of Renilla reniformis luciferase reporter gene activity and expressed as fold induction relative to the activity of medium-treated cells. Data are expressed as means ± SDs of triplicate wells from a representative experiment of a minimum of 2 separate experiments. *p < .05, vs. medium-treated wells (Student’s t test).

**Treatment of DEN2V-infected cells with SB203580.** After DEN2V infection, the secretion of IL-8 by HEK293A cells was ~50% lower in the presence of SB203580, compared with that in the presence of medium only (figure 3A). There was a similar degree of inhibition of transcription from the wt IL-8 promoter in DEN2V-infected HEK293A cells in the presence of SB203580.
Figure 2. Effect of dengue 2 virus (DEN2V) infection on transcription from mutated interleukin (IL)–8 promoters in HEK293A and HepG2 cells. HEK293A (A) and HepG2 (B) cells were transfected with luciferase reporter constructs containing a mutation (mt) in the binding site for CCAAT/enhancer-binding protein (mtc/EBP), activator protein–1 (mtAP-1), or NF-κB (mtNF-κB) or a construct containing the wild-type IL-8 promoter and were infected after 24 h with DEN2V at an MOI of 5. At 72 h after infection, luciferase reporter gene activity was measured. For each construct, data were normalized for transfection efficiency on the basis of Renilla reniformis luciferase reporter gene activity and expressed as fold induction relative to the activity in medium-treated cells. Data are expressed as means ± SDs of triplicate wells from a representative experiment of a minimum of 2 separate experiments. *P = .05, vs. medium-treated wells (Student’s t test).

on day 3 after infection, whereas there was a ~2-fold increase in activation of AP-1 (figure 4A). However, these differences were no longer seen on day 4 after infection (data not shown). In contrast, SB203580 treatment of DEN2V-infected HepG2 cells did not inhibit IL-8 secretion. In fact, a significant increase in IL-8 secretion occurred in SB203580-treated cells by day 6 after infection, compared with that in medium-treated cells (figure 3B). Furthermore, there was an increase in transcription from the wt IL-8 promoter in SB203580-treated cells, compared with that in medium-treated cells, by day 3 after infection (figure 4B). Activation of transcription from the AP-1 reporter was increased in SB203580-treated HepG2 cells, compared with that in medium-treated HepG2 cells, whereas there was no significant change in activation of transcription from the c/EBP and NF-κB reporters (figure 4B). These results indicate that inhibition of the p38 MAPK pathway reduces the induction of transcription from the IL-8 promoter and IL-8 secretion by DEN2V infection of HEK293A cells, whereas increased IL-8 transcription and IL-8 secretion occurs in DEN2V-infected HepG2 cells, potentially through an increase in AP-1 activation.

Treatment of DEN2V-infected cells with rolipram. After DEN2V infection, treatment of HEK293A cells with rolipram decreased IL-8 secretion by 34%, compared with that in medium-treated HEK293A cells, on days 3 and 4 after infection (figure 3A and data not shown). Induction of transcription from the wt IL-8 promoter was also reduced in HEK293A cells (figure 4A). After DEN2V infection, transcription from promoters containing a tandem repeat of the consensus binding site for the transcription factor c/EBP, AP-1, or NF-κB in rolipram-treated HEK293A cells was not significantly different from that in medium-treated HEK293A cells (figure 4A). Rolipram treatment of HepG2 cells did not inhibit IL-8 secretion on day 3 after infection, compared with that in medium-treated HepG2 cells (figure 3B). As was seen with SB203580 treatment, rolipram treatment increased IL-8 secretion in HepG2 cells by day 6 after infection, compared with that in medium-treated cells. In contrast, rolipram treatment inhibited transcription from the wt IL-8 promoter in DEN2V-infected HepG2 cells (figure 4B). In HepG2 cells, activation of the AP-1 reporter was increased with rolipram.

Figure 3. Interleukin (IL)–8 secretion from dengue 2 virus (DEN2V)–infected HEK293A and HepG2 cells treated with pharmacological inhibitors. HEK293A (A) and HepG2 (B) cells were infected with DEN2V at an MOI of 5 and incubated at 37°C for 2 h with or without inhibitors. Supernatants were sampled at the indicated time points for detection of IL-8 protein by ELISA. Data are representative results from 1 of 2 independent experiments. Dex, 250 μmol/L dexamethasone; RP, 100 μmol/L rolipram; SB, 10 μmol/L SB203580.
pram treatment on day 3 after infection, compared with that in medium-treated cells (figure 4B). However, there was no significant difference in activation of the AP-1 promoter on day 4 after infection (data not shown). Furthermore, there was no significant difference in activation of the NF-kB and c/EBP reporters in DEN2V-infected HepG2 cells treated with rolipram, compared with that in DEN2V-infected HepG2 cells treated with medium only.

**Treatment of DEN2V-infected cells with dexamethasone.**

After DEN2V infection, IL-8 levels in the culture supernatants were increased (by ~25% on day 3 and by ~35% on day 4 after infection) in dexamethasone-treated HEK293A cells, compared with those in medium-treated HEK293A cells (figure 3A). In contrast, dexamethasone treatment reduced transcription of the wt IL-8 promoter in HEK293A cells by ~25% by day 3 after infection and by ~60% by day 4 after infection (P ≤ .02) (figure 4A and data not shown). In dexamethasone-treated HEK293A cells, there was a 50% increase in activation of the AP-1 promoter on day 3 after infection, but there was no significant difference in activation of the c/EBP or AP-1 promoter on day 4 after infection (figure 4A and data not shown). However, there was an ~80% decrease in activation of NF-kB on days 3 and 4 after infection (figure 4A and data not shown). Dexamethasone treatment of DEN2V-infected HEK293A cells failed to inhibit IL-8 secretion (figure 3B). In addition, there was no significant difference in activation of the IL-8 promoter in dexamethasone-treated HepG2 cells, compared with that in medium-treated HepG2 cells (figure 4B). Analysis of transcription from the c/EBP, AP-1, and NF-kB reporters also showed that dexamethasone treatment of DEN2V-infected HepG2 cells had a minimal effect on transcription, compared with that in DEN2V-infected HepG2 cells treated with medium only (figure 4B). These results showed that dexamethasone treatment increased IL-8 secretion in DEN2V-infected HEK293A cells but did not increase transcription, whereas there was no significant effect on IL-8 secretion or activation of c/EBP, AP-1, and NF-kB in DEN2V-infected HepG2 cells.

**DISCUSSION**

The present study, which compared the cellular response to DENV infection in different cell types, has demonstrated the diversity of mechanisms by which DENV infection induces IL-8 secretion and provided insight into the potential effects of pharmacological inhibitors in this model. We found that DENV infection of HEK293A cells induced NF-kB activation (figure 1A), as was reported by others [3, 15, 30]. However, we found that NF-kB was not activated in HepG2 cells during DENV infection, which was contrary to the findings of Marianneau et al. [30]. Using PMA and ionomycin treatment, we were able to induce NF-kB production in HepG2 cells, which suggests that these cells are not deficient in NF-kB. Our findings may differ from those of Marianneau et al. because of our use of a lower MOI (MOI of 5 vs. MOI of 100), which may be more physiologically relevant. Another research group recently found that there was no inhibitory-NF-kB phosphorylation in another hepatic cell line, Huh-7, at 48 h after DENV infection, which correlates with our findings [17]. NF-kB–independent induction of IL-8 secretion by hepatitis C virus E2, HIV gp120, or tumor necrosis factor (TNF)-α has also been reported in hepatic cell lines [31, 32].

Other researchers also found increased AP-1 binding to DNA that correlated with an increase in IL-8 secretion [31, 32]. In addition, AP-1 activation was seen in DENV infection of Huh-7 cells [17]. Similarly, we found that transcription from the AP-1 reporter was induced in DEN2V-infected HepG2 cells (figure 1B). Other studies have shown that AP-1 can interact with NF-kB or c/EBP to increase transcription of TNF-α and IL-8 [33, 34]. Thus, AP-1 could cooperatively interact with other transcription factors, such as c/EBP, to induce chemokine expression.

---

**Table 1. Effects of pharmacological inhibitors on dengue 2 virus (DEN2V)-infected HepG2 and HEK293A cells.**

<table>
<thead>
<tr>
<th>Cell line, inhibitor</th>
<th>IL-8 secretion</th>
<th>IL-8 transcription</th>
<th>c/EBP activation</th>
<th>AP-1 activation</th>
<th>NF-kB activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEK293A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB203580</td>
<td>‡</td>
<td>‡</td>
<td>†</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>Rolipram</td>
<td>‡</td>
<td>‡</td>
<td>†</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>‡</td>
<td>‡</td>
<td>†</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td>HepG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB203580</td>
<td>‡‡</td>
<td>‡‡</td>
<td>††</td>
<td>††</td>
<td></td>
</tr>
<tr>
<td>Rolipram</td>
<td>‡‡</td>
<td>‡‡</td>
<td>††</td>
<td>††</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>‡‡</td>
<td>‡‡</td>
<td>††</td>
<td>††</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** The response in inhibitor-treated DEN2V-infected cells was compared with that in medium-treated DEN2V-infected cells. **AP-1, activator protein-1; c/EBP, CCAAT/enhancer-binding protein; IL-8, interleukin-8; 1, 1.5–2-fold increase; ‡, 1.5–2-fold decrease; ‡‡, >2-fold increase; ‡‡‡, >2-fold decrease; †, no difference.**
Figure 4. Activation of transcription from the interleukin (IL–8) promoter and transcription factors CCAAT/enhancer-binding protein (c/EBP), activator protein–1 (AP-1), and NF-κB in dengue 2 virus (DEN2V)–infected HEK293A and HepG2 cells treated with pharmacological inhibitors. HEK293A (A) and HepG2 (B) cells were transfected with luciferase reporter constructs containing a tandem repeat binding site for the transcription factor c/EBP, AP-1, or NF-κB or a construct containing the wild-type IL–8 promoter. After 24 h, cells were left uninfected (white bars) or were infected with DEN2V at an MOI of 5 and incubated at 37°C for 2 h (black bars) with or without inhibitors. Cell lysates were harvested for analysis of luciferase activity at 72 h after infection. For each condition, data were normalized for transfection efficiency on the basis of Renilla reniformis luciferase reporter gene activity and expressed as fold induction relative to the activity in media-treated cells. Data are expressed as means ± SDs of triplicate wells from a representative experiment of a minimum of 2 separate experiments. *P ≤ .05, vs. medium-treated wells (Student’s t test). **P ≤ .05, for comparison of medium-treated and inhibitor-treated DEN2V-infected cells. Dex, 250 μmol/L dexamethasone; RP, 100 μmol/L rolipram; SB, 10 μmol/L SB203580.
in DEN2V-infected HepG2 cells, and this induction could be independent of NF-κB activation.

The use of immunomodulatory drugs has been proposed in the treatment of DENV infection. Therefore, we wanted to assess drugs that have been reported to inhibit NF-κB activation and cytokine production for their effects on DENV-induced IL-8 secretion. Our findings are summarized in table 1. Surprisingly, the inhibitors we tested were not consistently effective as inhibitors of DENV-induced IL-8 secretion, and, in some cases, IL-8 secretion was actually increased.

Inhibition of the p38 MAPK pathway by SB203580 inhibited IL-8 secretion in HEK293A cells but increased IL-8 secretion in HepG2 cells. SB203580 treatment of HEK293A and HepG2 cells activated transcription from the AP-1 reporter. However, there was no difference in activation of transcription from the c/EBP and NF-κB reporters in SB203580-treated cells, and transcription from the IL-8 promoter was increased by SB203580 treatment only in HepG2 cells. AP-1 activation can occur through extracellular regulated kinase (ERK) 1, ERK2, and c-Jun N-terminal kinase (JNK), in addition to p38 [35]. These results suggest that AP-1 activation during DEN2V infection involves 1 or more of these p38 MAPK–independent pathways.

A previous study showed that treatment of HIV-infected T cells with rolipram decreased TNF-α and IL-10 secretion [22]. The authors attributed the cytokine suppression to the inhibition of NF-κB and nuclear factor of activated T cells. The results of the present study showed that treatment with rolipram increased IL-8 protein expression in DEN2V-infected HepG2 cells but not in DEN2V-infected HEK293A cells. In contrast, transcription from the IL-8 promoter was inhibited by rolipram in both HEK293A and HepG2 cells infected with DEN2V (table 1). There was little difference in activation of c/EBP, AP-1, or NF-κB between rolipram-treated cells and medium-treated cells (figure 4). These results indicate that the effects of phosphodiesterase IV inhibition on chemokine secretion from DEN2V-infected cells are not due to an increase in transcription from the IL-8 promoter and suggest that post-transcriptional mechanisms—for example, mRNA stability or translation—are responsible for the increase of chemokine protein levels in rolipram-treated DEN2V-infected HepG2 cells. Increased intracellular levels of cAMP have been shown to be correlated with increased stability of mRNA for a fructose transporter [36], and inhibition of NF-κB has been shown to increase the stability of mRNA for the cytokine granulocyte-macrophage colony-stimulating factor [37].

Dexamethasone has been reported to inhibit both the JNK pathway and NF-κB production [38, 39]. Our results also showed that there was inhibition of NF-κB activation in dexamethasone-treated HEK293A cells infected with DEN2V. Previous reports have shown that dexamethasone can increase production of proteins such as tissue factor in human monocytes and cyclooxygenase-1 in neuronal cells by increasing mRNA stability [40, 41]. Treatment with the glucocorticoid dexamethasone increased secretion of IL-8 in DEN2V-infected HEK293A cells, whereas transcription from the IL-8 promoter was inhibited. These results suggest that the increase in IL-8 secretion in DEN2V-infected HEK293A cells treated with dexamethasone is due to a posttranscriptional mechanism, such as an increase in mRNA stability.

One way in which our model differed from most of the models used in previous studies of these inhibitors was the longer duration of culture (>3 days vs. ≤24 h) [19–24]. The results of our experiments raise questions about how these drugs would potentially affect proinflammatory chemokines over an extended period. Fever typically lasts 4–7 days in symptomatic DENV infection. Although these drugs may inhibit certain pathways initially, prolonged use during DENV infection may lead to activation of pathways that could increase the inflammatory response via IL-8 induction in certain tissues, such as those in the liver.

IL-8 has been shown to directly increase the permeability of an endothelial monolayer [11]. IL-8 is also a chemoattractant for cells expressing CXCR1, such as neutrophils and cytotoxic memory CD8+ T cells [13, 42, 43]. Both of these mechanisms could potentially play a role in plasma leakage that occurs during DHE. Furthermore, IL-8 can subvert the innate immune defense by inhibiting the antiviral effects of interferon-α, thereby potentially enhancing viral dissemination [44]. Through a combination of these effects, increased IL-8 secretion by DENV may increase the likelihood of more-severe disease, particularly during secondary DENV infection. In the absence of an animal model for DENV infection, it is difficult to determine if these effects occur in vivo. However, if suppression of DENV-induced IL-8 secretion is a desirable goal of therapy for DENV infection and if our results with continuous cell lines are representative of the effects that could be expected in vivo, then treatment of DENV-infected patients with the drugs tested in our study could have unintended consequences.

Acknowledgments

We thank Jurand Janus, for technical support; Kate Fitzgerald, for the donation of materials for and technical assistance with the luciferase assays; and N. Mukaido, for the donation of the luciferase reporter constructs.

References


Drug Effect on DENV-Induced IL-8 • JID 2006:193 (15 April) • 1077