Evidence for cytokine regulation of cholesterol metabolism in herpesvirus-infected arterial cells by the lipoxygenase pathway

Orli R. Etingin* **and David P. Hajjar**

Departments of Medicine,* Biochemistry, and Pathology, and the National Institutes of Health Specialized Center for Thrombosis Research, Cornell University Medical College, New York, NY 10021

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Abstract Cytokines such as tumor necrosis factor (TNF), interleukin-1 (IL-1), and γ-interferon (IF) are produced by activated hematopoietic cells. They possess antiviral activity and have other biological activities such as induction of cell proliferation and hemorrhagic necrosis of tumors. Since herpes simplex virus (HSV) infection of human vascular cells is known to produce a biochemical and cytopathological effect virtually indistinguishable from atherosclerosis, we hypothesized that these cytokines many prevent cholesteryl ester (CE) accumulation in arterial smooth muscle cells (SMC) that is seen with herpesvirus infection. We now report that TNF and IL-1 but not γ-IF prevent CE accumulation in HSV-infected arterial SMC by induction of cyclic AMP-dependent CE hydrolysis. This effect is mediated through the arachidonate 12-lipoxygenase pathway via 12-HETE since pretreatment of cells with several lipoxygenase inhibitors abolishes the antiviral effect and 12-HETE is the major (>99%) lipoxygenase metabolite produced by these cells. This conclusion is further based on our observations that TNF and IL-1 enhance 12-HETE production in SMC and that 12-HETE significantly increases both intracellular cyclic AMP and lysosomal CE hydrolysis. Moreover, dibutyryl cyclic AMP restored a normal phenotype in these virally infected cells. Collectively, these findings identify for the first time a biochemical mechanism involved in the reduction of lipid accumulation in virally infected arterial SMC by these potent cytokines.—**Etingin, O. R., and D. P. Hajjar.** Evidence for cytokine regulation of cholesterol metabolism in herpesvirus-infected arterial cells by the lipoxygenase pathway. *J. Lipid Res.* 1990. **31:** 299–305.

Supplementary key words tumor necrosis factor • interleukin-1 • γ-interferon

Tumor necrosis factor (TNF) is one of several biologically active cytokines with diverse functions which is produced primarily by activated macrophages and lymphocytes (1). TNF has been shown to mediate hemorrhagic necrosis of tumors (2), growth factor production by endothelial cells (3) and fibroblasts (4), and inhibition of viral replication in human tumor cell lines (5) by induction of interferon (IF)-β (6). TNF also modulates production of two potent cytokines, interleukin-1 (IL-1) (7) and IF-γ, both of which have similar cellular activities (8). In addition to mediating tumor necrosis, TNF can produce hypertriglyceridemia and cachexia during malignancy by inhibition of lipoprotein lipase in adipocytes (9, 10).

The role of TNF in the complex cytokine network in mammalian cells is not fully understood, particularly regarding its antiviral activity. While TNF is a known inducer of IF β_2 mRNA (5), others have shown that TNF's antiviral effect is not abolished in the presence of antiserum to IF-β-$_2$ (6). Wong and Goeddel (5) postulated that the antiviral activity of TNF is attributable to lysis of virally infected cells rather than induction of IF-β_2. Other possible mechanisms to explain the action of TNF on mammalian cells involve eicosanoid metabolism. Several lines of evidence support this conclusion: *1*) TNF's lytic effects in mice can be partially blocked by pretreatment with cyclooxygenase inhibitors (11); *2*) the hemodynamic and hematologic effects of IL-1 or TNF injection in mice can be reversed by pretreatment with cyclooxygenase inhibitors (12); and, *3*) cyclooxygenase inhibitors partially prevent the TNF-induced hemodynamic effects in humans previously exposed to endotoxin (13). These studies suggest that cytokines, such as TNF, can promote synthesis of eicosanoid product(s) which, in turn, may mediate its biological effects.

We have recently studied the mechanism of activitiy of these cytokines on cholesterol metabolism in a model of herpesvirus-induced atherosclerosis. Previously, we reported that arterial smooth muscle cells (SMC) demonstrate both the cytopathological and biochemical changes characteristic of atherosclerosis when infected with Marek's disease herpesvirus or herpes simplex virus (HSV) (14, 15). Cholesteryl ester (CE) accumulation, a

Abbreviations: TNF, tumor necrosis factor; IF, interferon; IL-1, interleukin-1; SMC, smooth muscle cells; CE, cholesteryl ester; NCEH, neutral cholesteryl ester hydrolase; HSV, herpes simplex virus; ACAT, acyl-CoA:cholesterol acyltransferase; ACEH, acid (lysosomal) cholesteryl ester hydrolase; CEH, cholesteryl ester hydrolase; MOI, multiplicity of infection.

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hallmark of human atherosclerosis (15), resulted from decreased lysosomal (acid) and cytoplasmic (neutral) CE hydrolytic activities (ACEH, NCEH) (14, 15) owing, in part, to reduced intracellular cyclic AMP levels or due to an inability to activate the cyclic AMP-dependent protein kinase (14–17). No changes were observed in CE synthetic activity (ACAT). Since lipid accumulation and decreased prostaglandin I_2 production occur in herpesvirus-infected arterial SMC (14, 15), and several cytokines have been shown to prevent viral replication (5), we hypothesized that TNF and other cytokines may reverse the viral-induced alterations in lipid metabolism by its actions on "secondary messengers" in the cell that may regulate lipolysis. In this study, we provide evidence for the first time that: *1*) cytokines regulate cholesterol metabolism in HSV-infected SMC, and *2*) that this regulation is mediated by the arachidonic acid lipoxygenase pathway.

MATERIALS AND METHODS

Materials

Human recombinant tumor necrosis factor (sp act 10^7 units/mg) was kindly provided by Dr. M. Shepard of Genentech; human recombinant IL-1 (sp act 10^8 units/mg) was purchased from Genzyme, Boston, MA; and γ-interferon was provided by Dr. H. Murray of this institution.

12-HETE was obtained from Cayman Labs, Ann Arbor, MI. Aspirin, ETYA (5,8,11,14 eicosatetraynoic acid), and 1-phenyl-3-pyrazolidone were also purchased from Cayman Labs or Sigma Co., St. Louis, MO.

Herpes simplex virus was obtained from the American Tissue Culture Collection, Rockville, MD (15).

Tissue Culture

Bovine smooth muscle cells were obtained from bovine thoracic arteries supplied by a local abattoir. Smooth muscle cells were cultured from arterial explants following the removal of adventitial tissue according to Hajjar et al. (15). Cells cultured from thoracic arteries were confirmed to be smooth muscle cells by their growth pattern as observed by phase contrast microscopy and ultrastructural characteristics by transmission electron microscopy.

Preparation of cell homogenates

For the assay of enzyme activities, cells were harvested by scraping, as previously described, after aspiration of the incubation medium and addition of 1.0 ml of ice-cold isotonic sucrose buffer (16). Cell preparations were homogenized over ice for 30 sec and aliquots were taken for the assays of lysosomal ACEH activity, cytoplasmic NCEH activity, CE synthetic (ACAT) activity, and protein content. Protein was determined by the method of Lowry et al. (18) with bovine serum albumin as standard.

Cholesteryl ester metabolism

To test the antiviral activity of the various cytokines or other agonists during subacute HSV-infection of SMC and its effects on cholesterol metabolism, we incubated these cells with TNF (0–500 ng/ml) or IL-1 (0–10 ng/ml) for 30 min prior to herpesviral exposure (15). When γ-interferon (0–1000 units/ml) was used to test for synergism between the cytokines, the incubation period was extended to 24 h because its biological activity requires longer exposure times (5). In some experiments, cells were pre-incubated with 0–10 pg/ml 12-HETE or 0.1 mM dibutyryl cyclic AMP 2 h prior to viral infection. Preliminary results indicated that a 30-min incubation time was optimal to induce the observed effects of TNF and IL-1, and 2 h was optimal time to induce the observed effects of cyclic AMP. The SMC were subsequently infected with HSV at a multiplicity of infection (MOI) of 0.1 (15). Replicate cells were mock-infected. Virus was removed after 2 h, and cells were washed to remove viruses remaining after inoculation (15). Cells were then re-fed with MEM. After 48 h, cells were harvested in sucrose buffer and the activities of the enzymes involved in the CE cycle were assessed. Lysosomal ACEH activity in arterial smooth muscle cells was assayed at pH 3.9 described previously (14). Cholesteryl [1-^{14}C]oleate was used as substrate at a final reaction concentration of 6.0 μM in a mixed micelle of egg lecithin-Na^+ taurocholate–cholesteryl oleate to assay NCEH activity in arterial SMC as previously described by Hajjar et al. (14).

Activity of microsomal ACAT was assayed by measuring the synthesis of cholesteryl oleate from radioactive oleoyl CoA and exogenous free cholesterol (14). Oleoyl CoA and cholesterol were prepared as unilamellar liposomes as described by Hajjar et al. (14).

Viral plaque assays

The effects of variable concentrations of TNF, IL-1, and IF on the number of herpesviral plaques were assessed by standard methods described elsewhere (19).

12-HETE and cyclic AMP assays

12-HETE and cyclic AMP levels from arterial SMC were assayed by radioimmunoassay (RIA) use RIA kits supplied from Advanced Magnetics Inc., Cambridge, MA and New England Nuclear Corp., Boston, MA, respectively (16, 20).

RESULTS

HSV-infected SMC demonstrated an approximate 70% decrease in lysosomal CE hydrolytic (ACEH) activity and a 65% decrease in cytoplasmic CE hydrolytic (NCEH) activity as compared to mock-infected SMC (**Fig. 1**). No

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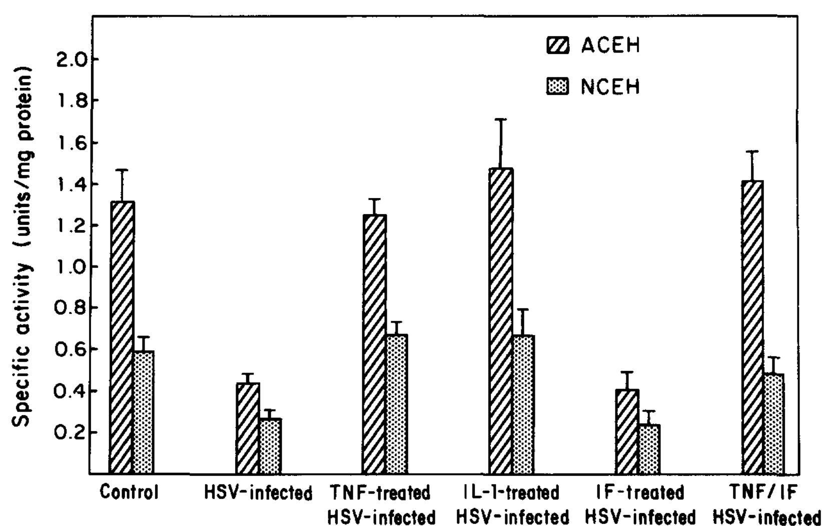

Fig. 1. Effect of cytokines on CE hydrolysis in HSV-infected arterial SMC. Bovine aortic SMC, subpassaged 3-6 times, were exposed to 50 ng/ml TNF or 10 ng/ml IL-1 for 30 min, or γ-interferon for 24 h in serum-free Dulbecco's MEM. Cells were infected with HSV at an MOI of 0.1 for 2 h (15). After 48 h, cells were harvested with a rubber policeman and CE hydrolytic activities were assayed as previously described (14, 15). Replicate cells were mock-infected as controls (15). One unit of specific activity of the enzyme corresponds to 1 nmol CE hydrolyzed per h per mg protein. Analysis of data (n = 5) was done by Student's t test ($P < 0.05$). Data are reported as mean ± SD. Each experiment was done in quadruplicate.

change in ACAT activity (CE synthetic activity) was observed between the HSV-infected and uninfected groups of cells (data not shown). In contrast, pretreatment with TNF or IL-1 prevented the decrease in ACEH and NCEH activities that accompanied HSV infection (Fig. 1). Mock-infected cells pretreated with TNF or IL-1 demonstrated no alterations in ACEH or NCEH activities (data not shown). Unlike previous reports of synergistic anti-viral activity between γ-IF and TNF (5), γ-IF did not prevent the HSV-induced decrease in ACEH or NCEH activities in TNF-treated SMC. To determine whether this TNF and IL-1 effect on lipid metabolism correlated with its antiviral activity, we performed viral plaque assays on cytokine-treated cells (**Table 1**). HSV-infected cells exposed to TNF, γ-IF, or IL-1 demonstrated fewer viral plaques than untreated, HSV-infected SMC. No decrease in either viral yield or in the prevention of altered cholesterol metabolic activity was observed in cells treated simultaneously with TNF or IL-1 at the time of HSV infection (data not shown). In addition, no alterations were observed when SMC were treated sequentially with HSV, and then with the cytokines. These findings suggest that pre-incubation with TNF or IL-1 prevents the cytopathological effect of viral infection on intracellular cholesterol metabolism (5, 15). Pretreatment with γ-IF, however, decreased viral plaque formation (Table 1) while it did not have any effect on CE hydrolytic activities, 12-HETE levels (see Table 3) or cyclic AMP levels (see Table 4). Since CE hydrolytic activity in arterial cells is known to be regulated by cyclic AMP and arachidonic acid metabolites (16, 20), γ-IF did not prevent the viral cytopathological effect on cholesterol metabolism because it did not alter levels of these "secondary messengers." Thus its antiviral effect was distinct from that of TNF or IL-1.

To test the hypothesis that these specific cytokines could oppose HSV effects on cholesterol metabolism by elaborating bioactive substances such as cyclooxygenase or lipoxygenase metabolites, we examined CE metabolic activity in

TABLE 1. Effects of cytokines on viral replication in arterial SMC

		Initial Viral Infection (MOI)	Pretreatment Conditions	Number of Vero Cell Plaques
1.	(n = 4)	0	0	0
2.	(n = 3)	0.1	0	83
3.	(n = 4)	0.1	50 ng/ml TNF	8
4.	(n = 2)	0.1	10 ng/ml IL-1	5
5.	(n = 3)	0.1	1000 units/ml γ-IF	8
6.	(n = 3)	0.1	10 pg/ml 12-HETE	86
7.	(n = 3)	0.1	0.1 mM cAMP	11

Bovine arterial SMC pretreated with 0-50 ng/ml of TNF, 0-10 ng/ml IL-1, 0-1000 U/ml IF, 10 pg/ml 12-HETE, or 0.1 mM dibutyryl cyclic AMP were infected with HSV at an MOI of 0.1 for 2 h. Uninfected cells served as a control. After 48 h, cells were harvested by scraping in 1 ml isotonic sucrose buffer (250 mM sucrose, 10 mM Tris, 50 mM EDTA, pH 7.3) (14) and rapidly freeze-thawed twice to produce lysis. Aliquots of cell lysates were added to confluent Vero cell monolayers in six-well plates. Two ml of 0.6% agarose was added to each well. Viral plaques were stained with Giemsa and counted 4-5 days later (19). We observed that 10 ng/ml IL-1, 50 ng/ml TNF, 1000 units/ml γ-IF, and 0.1 mM dibutyryl cyclic AMP were maximal concentrations that significantly ($P < 0.01$) reduced viral replication in these host cells. 12-HETE in the concentrations used (1-10 pg/ml) did not inhibit viral replication. Each experiment was done in quadruplicate.

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HSV-infected cells pretreated with either acetylsalicylic acid (aspirin; a potent cyclooxygenase inhibitor), ETYA (5,8,11,14 eicosatetraynoic acid, a cyclooxygenase and lipoxygenase inhibitor), or 1-phenyl-3-pyrazolidone (a potent lipoxygenase inhibitor) (**Table 2**) (20, 21). We observed that TNF and IL-1 prevented the alterations in CE hydrolysis seen in untreated, HSV-infected cells, while these cytokines had no effect on CE hydrolysis in virally infected cells that were pretreated with the two lipoxygenase inhibitors (ETYA and 1-Ph 3-P). Interestingly, HSV-infected cells treated with aspirin did demonstrate alterations in CE metabolic activity in response to TNF or IL-1 (Table 2). Neither aspirin nor lipoxygenase inhibitors used in this study induced alterations in cell viability as assayed by trypan blue exclusion. These data suggest that TNF and IL-1 may mediate their antiviral, cytoprotective effect on HSV-infected vascular cells through a lipoxygenase product, but not a cyclooxygenase metabolite.

Since TNF and IL-1 may mediate their antiviral effect on vascular cells through this lipoxygenase metabolite, we next determined the effect of TNF and IL-1 on 12-HETE production in HSV-infected cells. Two major observations emerged from these experiments (**Table 3**). First, HSV-infected cells demonstrated a 50% decrease in 12-HETE production. Second, TNF and IL-1 significantly increased

TABLE 2. Effect of cyclooxygenase and lipoxygenase inhibition on TNF and IL-1-mediated alterations of arterial CE hydrolysis

Cells	Lysosomal (Acid) CE Hydrolytic Activity
	units/mg protein
Control	1.4 ± 0.1^{a}
HSV-infected	$0.7 \pm 0.2^{a,b,e}$
TNF/HSV-infected	$1.3 \pm 0.1^{b,c,d}$
ASA/TNF/HSV-infected	1.2 ± 0.1
ETYA/TNF/HSV-infected	0.8 ± 0.2^{c}
1-Ph 3-P/TNF/HSV-infected	0.7 ± 0.2^{d}
IL-1/HSV-infected	$1.3 \pm 0.2^{e,f,g}$
ETYA/IL-1/HSV-infected	0.8 ± 0.3^{f}
1-Ph 3-P/IL-1/HSV-infected	0.6 ± 0.2^{g}
TNF/DDA/HSV-infected	0.6 ± 0.1
12-HETE	3.3 ± 0.4^{a}

Data, reported as mean ± SD for three separate experiments, were examined by analysis of variance. Each experiment was done in quadruplicate. Values with the same superscript are significantly different ($P < 0.05$). Cells were pretreated with 0.1 mM aspirin for 30 min, or 1 mM ETYA, Ph-3-P or 10 μM DDA for 90 min in Dulbecco's MEM without serum (20). Cells were washed twice with phosphate-buffered saline to remove the inhibitors. They were then treated with TNF (0–500 ng/ml) or IL-1 (0–10 ng/ml) prior to HSV infection as described. Ten ng/ml IL-1 or 50 ng/ml TNF was the maximal concentration to maintain lysosomal CE hydrolytic activity at control levels. Optimal condition for 12-HETE's effect on lysosomal hydrolytic activity was found to be 100 ng/ml. Separate control experiments confirmed inhibition of PGI_2 and/or 12-HETE production by the inhibitor concentrations used in the experiments. The addition of these eicosanoid inhibitors did not alter baseline (control) levels of CE hydrolytic activity.

TABLE 3. Effect of cytokines on 12-HETE production by arterial SMC

Cells	12-HETE
	pg/mg protein
Control	$130 \pm 21^{a,c}$
HSV-infected	$61 \pm 5^{a,b,d}$
TNF-treated	314 ± 11^{a}
TNF/HSV-infected	205 ± 63^{b}
IL-1-treated	288 ± 39^{c}
IL-1/HSV-infected	150 ± 19^{d}
γ-IF/HSV-infected	70 ± 14^{e}
γ-IF-treated	138 ± 20^{e}

Values represent mean ± SD (n = 3). Data were analyzed by analysis of variance. Each experiment was done in triplicate. Values with the same superscript are significantly different ($P < 0.05$). 12-HETE production was measured by RIA (20). Eight treatment groups of cells were examined for 12-HETE production: cells treated with *1)* PBS (control); *2)* HSV-infected cells (MOI = 0.1); *3)* TNF (500 ng/ml); *4)* TNF (500 ng/ml) + HSV infection (MOI = 0.1); *5)* IL-1 (10 ng/ml); *6)* IL-1 (10 ng/ml) + HSV infection (MOI = 0.1); *7)* γ-IF (1000 U/ml) + HSV infection (MOI = 0.1); and *8)* γ-IF (1000 U/ml). Cells were harvested by scraping, followed by brief sonication. Aliquots of cell lysates were removed for 12-HETE assay.

12-HETE production in both normal and HSV-infected cells as compared to untreated cells. These findings support the hypothesis that cytokines such as TNF are involved in elaboration of inflammatory mediators such as the hydroxy acids. In addition, SMC treated with TNF or IL-1 (but not IF) prior to HSV infection produced 12-HETE in quantities equivalent to that synthesized by untreated cells (205 ± 63 vs 150 ± 19 vs 130 ± 21 pg/mg protein) (Table 3). Thus, the antiviral properties of TNF and IL-1 in HSV-infected cells may be mediated, in part, by 12-HETE. In our arterial cell system, γ-IF did not induce measurable alterations in either CE hydrolysis, 12-HETE production, or cyclic AMP levels, suggesting that its effect on HSV-infected cells is mediated by another mechanism. Additionally, SMC simultaneously treated with HSV infection and the cytokines did not significantly elevate 12-HETE or cyclic AMP levels compared to those cells pretreated with TNF or IL-1 and then infected with HSV. These findings suggest that these mesenchymal cells require time to elaborate secondary messengers such as 12-HETE and cyclic AMP prior to infection, substances that are needed as agonists of lipolytic activity. Furthermore, 12-HETE itself did not inhibit viral replication in SMC at concentrations tolerated by the cells in vitro (Table 1). However, preincubation with 0.1 mM dibutyryl cyclic AMP, a target intracellular messenger for 12-HETE, significantly inhibited the cytopathological effects of HSV infection in SMC over a 24–48-h period (Table 1).

Data presented in Table 2 indicate that the antiviral effects of TNF and IL-1 are blocked by lipoxygenase inhibitors and not by cyclooxygenase inhibitors. Therefore, we studied whether cyclic AMP, an activator of CE catabo-

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lism in the vascular wall (16, 22–24), also plays a role in this pathway with respect to antiviral activity of cytokines. Cells were incubated with either 10 μM 2'5'-dideoxyadenosine (DDA) to inhibit adenylate cyclase activity (25) or buffer alone for 1 h prior to TNF or IL-1 treatment and HSV infection. In HSV-infected cells pretreated with DDA, TNF did not prevent the virus-induced reduction in lysosomal CE hydrolytic (ACEH) activity as compared to cells in which cyclic AMP production was normal (Table 2). Furthermore, 12-HETE increased lysosomal CE hydrolytic activity in these arterial cells (Table 2) (31). Similarly, 12-HETE actually enhanced intracellular cyclic AMP levels in a dose-dependent manner (**Table 4**). We also examined the effect of TNF, γ-IF, and IL-1 on intracellular levels of cyclic AMP in HSV-infected SMC since it was previously reported that eicosanoids can increase cyclic AMP in uninfected SMC (16, 23, 24). Virally infected cells demonstrated a 60% decrease in cyclic AMP levels (Table 4), consistent with similar decreases in CE hydrolytic activities. However, TNF- or IL-1-treated, HSV-infected cells demonstrated basal intracellular levels of cyclic AMP, consistent with the observed levels of enzyme activities. Interferon did not act similarly with respect to restoring basal levels of intracellular cyclic AMP or enzyme activities. This is the first time that cytokine induction of CE hydrolysis has been shown to be linked to the lipoxygenase pathway in vascular cells, by increasing intracellular cyclic AMP.

TABLE 4. Effect of 12-HETE, TNF, and IL-1 on cyclic AMP levels

Cells	Cyclic AMP
	pmol/mg protein
Control	$0.95 \pm 0.05^{a,b,c,d,e}$
12-HETE (0.1 μg/ml)	0.95 ± 0.15
12-HETE (0.5 μg/ml)	1.40 ± 0.17^{a}
12-HETE (2.0 μg/ml)	1.55 ± 0.05^{b}
HSV-infected	$0.40 \pm 0.01^{c,f}$
TNF (50 ng/ml)	$2.40 \pm 0.36^{d,h}$
TNF/HSV-infected	$1.02 \pm 0.08^{h,f}$
IL-1 (10 ng/ml)	$3.60 \pm 0.90^{e,g}$
IL-1/HSV-infected	1.10 ± 0.10^{g}
γ-IF (1000 U/ml)	0.90 ± 0.10^{h}
γ-IF/HSV-infected	0.35 ± 0.08^{h}

Values represent mean ± SD for four separate experiments. Data were analyzed by analysis of variance. Each experiment was done in triplicate. Values with the same superscript are significantly different ($P < 0.05$). Bovine aortic SMC were grown in Dulbecco's MEM with 10% fetal calf serum, 2% penicillin–streptomycin, 2% fungizone. Cultures were at 37°C in 95% air, 5% CO_2 (15). Cells were plated into six-well plates 36–48 h prior to an experiment. Cells were treated with 12-HETE (0.1 μg–2.0 μg/ml or TNF (50–500 ng/ml), or IL-1 (10 ng/ml) for 20 min in Dulbecco's MEM. The cells treated with γ-IF (1000 U/ml) were pretreated for 24 h. Cells were harvested by gentle scraping after the addition of 0.5 ml of isotonic sucrose buffer. They were sonicated for 15 sec and 50 μl of 100% trichloroacetic acid was added to the lysates followed by storage at −70°C. At the time of cyclic AMP assay, cells were thawed and centrifuged at 2000 rpm at 5°C for 10 min to remove proteinaceous material. Aliquots of supernatant were assayed for cyclic AMP (16, 23).

DISCUSSION

The interaction of TNF with other cytokines in mammalian cells is complex and not fully understood. While TNF is a known inducer of IF-β_2 mRNA (5), its antiviral effect is not abolished in the presence of antiserum to IF-β_2 (6). Wong and Goeddel (5) postulated that the antiviral activity of TNF is attributable to lysis of virally infected cells rather than induction of IF-β_2. Another possible mechanism to explain the action of TNF on mammalian cells may involve eicosanoid metabolism (11–13). Our studies suggest that cytokines such as TNF may promote synthesis of eicosanoid metabolites which, in turn, may mediate other biological effects, such as in the regulation of cholesterol metabolism. Previous research has shown that the enzymes involved in CE hydrolysis are modulated by intracellular signals such as growth factors (PDGF) (20), prostaglandin I_2 (16, 23, 24), cyclic AMP (16, 17, 23, 24), and the cyclic AMP-dependent protein kinase (24).

Products of the lipoxygenase pathway, such as 12-HETE, the major lipoxygenase product of activated platelets (26) and SMC (20), are known to possess varied biological activities that are not susceptible to cyclooxygenase inhibition. A role for 12-HETE has been described in epidermal cell proliferation (27), chemotactic activity (28), procoagulant activity (29), and aldosterone secretion (30). In previous co-culture studies (20, 31) and studies reported herein with cultured SMC alone, we observed that the monohydroxy acid 12-HETE directly enhances lysosomal CE hydrolytic activity (Table 2) and cyclic AMP levels in the cell (Table 4). The activation of this enzyme may assist in the mobilization of cholesterol by increasing lysosomal CE hydrolysis. Since 12-HETE does not alter ACAT activity in the cell (31), it may be possible that these monohydroxy acids can potentiate cholesterol efflux directly from lysosomal compartments of these cells. Since lipoxygenase products survive longer in the circulation than cyclooxygenase metabolites (notably PGI_2), they would be more likely to mediate cell–cell interactions within the vessel wall over a longer period of time, particularly since they can penetrate cells.

Herpesvirus infection of SMC is known to decrease prostacyclin production (15). In this report, we document a decrease in SMC 12-HETE production after HSV infection as well. Since both of these eicosanoids increase cAMP levels (Table 4, refs. 16, 31) as well as lysosomal CE hydrolytic activity (Table 2, refs. 20, 31), the combined effect of a decrease in PGI_2 and 12-HETE by HSV infection may be synergistic in decreasing CEH activities.

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Nevertheless, TNF treatment of aspirin-treated, HSV-infected cells did not restore CEH activities to baseline, suggesting that 12-HETE regulation of CEH activities may be a more potent regulator of enzyme activation than PGI_2.

The antiviral effect of TNF on cholesterol metabolism in HSV-infected arterial SMC is mediated by the elaboration of a major lipoxygenase product in these cells. This metabolite enhances intracellular cyclic AMP and CE hydrolytic activity, thus protecting or priming the CEH enzyme from down-regulation by HSV. Recently, other data have emerged showing that increased intracellular cyclic AMP can enhance IL-6 synthesis (32), a cytokine primarily associated with antiviral and hepatic stimulatory activities. It is possible that the antiviral properties of TNF and IL-1 may also be mediated by other cytokines in a network of cytokine communication in mesenchymal cells. By increasing cyclic AMP and 12-HETE, TNF and IL-1 may participate jointly in the prevention of cytopathological effects of HSV since it has been shown that TNF can stimulate IL-1 production in vascular cells (33, 34). It remains to be determined what other agonists of antiviral activity exist in the cell following TNF or IL-1 activation.

Finally, it has been documented that both lipoxygenase and cyclooxygenase serve to catalyze the oxygenation of arachidonic acid to induce intermediate and biologically active eicosanoids. Both in vivo and in vitro studies have demonstrated that TNF-induced effects can be mediated through cyclooxygenase-derived eicosanoids (11-13). Notwithstanding this association with cyclooxygenase metabolites, we have demonstrated herein for the first time that lipoxygenase metabolites mediate the antiviral properties of TNF and IL-1 in a cell culture system that mimics viral-induced atherosclerosis, an arteriopathy where inflammatory-type lesions develop (35). While inflammatory mediators such as lipoxygenase products (12-HETE) are known to be involved in cell-cell signaling under normal physiologic conditions (20, 31), they may also comprise a unique class of antiviral or antitumor agonists that contribute to enzyme activation through cyclic AMP-dependent mechanisms.

We wish to thank Ms. Barbara Summers for excellent technical assistance and Dr. Mitchell Gaynor for assisting with the Vero plaque assays in our preliminary studies. This research was supported by the NIH Specialized Center of Research in Thrombosis, HL-18828, an NIH Clinical Investigator Award to O.R.E. HL-01687, and a Grant-in-Aid from the American Heart Association (New York Affiliate) to D. P. H. Dr. Hajjar is an Established Investigator of the American Heart Association (New York Affiliate).

Manuscript received 16 June 1989, in revised form 31 August 1989, and in re-revised form 27 September 1989.

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