Differential Regulation of Interleukin-8 and Intercellular Adhesion Molecule-1 by H$_2$O$_2$ and Tumor Necrosis Factor-$\alpha$ in Endothelial and Epithelial Cells

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The reactive oxygen intermediate H$_2$O$_2$ can function as a signaling molecule to activate gene expression. In this study, we demonstrate that oxidant stress induced by tumor necrosis factor $\alpha$ (TNF$\alpha$) or H$_2$O$_2$ differentially regulates intercellular adhesion molecule-1 (ICAM-1) and interleukin-8 (IL-8) gene expression in endothelial and epithelial cells. Northern blot analysis revealed that TNF$\alpha$ induced both ICAM-1 and IL-8 expression in either the A549 lung epithelial cell line or the human microvessel endothelial cell line (HMEC-1). In contrast, H$_2$O$_2$ selectively induced only ICAM-1 in HMEC-1 and only IL-8 in A549. This cell type-specific pattern of IL-8 expression was also observed in several other endothelial and epithelial cells. TNF$\alpha$ induced greater IL-8 gene expression as compared with H$_2$O$_2$, but the kinetics of induction were similar. The induction of epithelial IL-8 message was accompanied by a corresponding increase in functional IL-8 protein secretion as determined by a neutrophil motility assay. The increased neutrophil motility stimulated by conditioned media from H$_2$O$_2$- or TNF$\alpha$-exposed A549 cells was completely inhibited by an anti-IL-8 antibody. TNF$\alpha$ and H$_2$O$_2$ also induced a differential pattern of CC chemokine expression in A549. While TNF$\alpha$ induced both RANTES and MCP-1, H$_2$O$_2$ induced only MCP-1. These data suggest that epithelial cells under oxidant stress contribute to the inflammatory cytokine network by selective production of IL-8, MCP-1, and RANTES, which may critically influence the site-specific recruitment of leukocyte subsets.

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§The abbreviations used are: IL-8, interleukin-8; ICAM-1, intercellular adhesion molecule-1; TNF$\alpha$, tumor necrosis factor-$\alpha$; FMN, pyromorphonuclear leukocytes; AP-1, activator protein-1; NF-$\kappa$B, nuclear factor $\kappa$B; ARE, anti-oxidant responsive element; HUVEC, human umbilical vein endothelial cell; HMEC-1, human microvessel endothelial cell line; C/EBP, CAAT enhancer-binding protein; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; MOPS, 4-morpholinepropanesulfonic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
been shown to induce IL-8 (37). As with ICAM-1, the IL-8 promoter contains a functional AP-1 site (38), and a potential ARE (39). However, the mechanism by which oxidants induce IL-8 expression is unknown and the regulation of oxidant-induced IL-8 and ICAM-1 in epithelial and endothelial cells has not been investigated.

TNFα has also been shown to generate oxidant stress (40, 41). Therefore to determine whether IL-8 and ICAM-1 are induced by a common oxidant stress-mediated mechanism, we compared the effects of oxidant stress generated by H_2O_2 and TNFα on the expression of IL-8 and ICAM-1 in either a human microvascular endothelial cell line (HMEC-1) or a human lung type II epithelial cell line (A549). The results herein demonstrate that, although TNFα and H_2O_2 both generate oxidant stress, they differentially regulate the expression of the ICAM-1 and IL-8 genes in endothelial and epithelial cells. We further show that TNFα and H_2O_2 can differentially induce other chemokine genes. We propose that oxidant stress constitutes cell type- and gene type-specific activation signals in epithelial and endothelial cells that may critically influence the site-specific recruitment of leukocyte subsets in inflammatory reactions.

**EXPERIMENTAL PROCEDURES**

Materials—40% H_2O_2, MOPS, fibronectin, fetal bovine serum, actinomycin D, and 3-[4,5-dimethyl thiazol-2-yl]-2,5-diphenyl tetrazolium bromide tetrazolium salt were purchased from Sigma. Dulbecco’s modified Eagle’s medium, antimycotics, 10% phosphate-buffered saline (PBS), F-12K media, MCDB 131 media, and 1% trypsin-EDTA were purchased from Life Technologies, Inc. (Grand Island, NY). Restriction enzymes, Dulron-UV, QuickHyb™, Prime It II, random labeling kit, and Posiblot and UV cross-linker (Stratagene) were purchased from Life Technologies, Inc. (Culver City, CA). The A549 cell line was obtained from American Type Culture Collection (Rockville, MD). The human microvascular endothelial cell line (HMEC-1) was obtained from the Center for Disease Control (Atlanta, GA). Human epidermal growth factor was purchased from Becton Dickinson (San Jose, CA). IL-8 cDNA was obtained from Dr. H. Ari Jaffe, University of Illinois, Chicago, IL. Human glyceraldehydrase (GAPDH) cDNA was provided by Dr. A. Finnegan, Rush Medical College, Chicago, IL, and the ICAM-1 cDNA was provided by Dr. T. Springer, Harvard Medical School, Boston MA.

**Cell Culture, Treatments, and IL-8 Secretion**—The A549 human lung adenocarcinoma cell line representative of distal respiratory epithelium was grown in F-12 media with 10% fetal calf serum, 1% penicillin/streptomycin, and 10% phosphate-buffered saline (PBS), F-12K media, MCDB 131 media, and 1% trypsin-EDTA. HMEC-1, a simian virus (SV)-40 transformed human dermal microvascular endothelial cell line (42), was cultured in MCDB 131 media. HMEC-1, a simian virus (SV)-40 transformed human dermal growth factor to 90% confluency in 24-well dishes. HMEC-1, a simian virus (SV)-40 transformed human dermal growth factor to 90% confluency in 24-well dishes. HMEC-1, a simian virus (SV)-40 transformed human dermal growth factor to 90% confluency in 24-well dishes. HMEC-1, a simian virus (SV)-40 transformed human dermal growth factor to 90% confluency in 24-well dishes. HMEC-1, a simian virus (SV)-40 transformed human dermal growth factor to 90% confluency in 24-well dishes. HMEC-1, a simian virus (SV)-40 transformed human dermal growth factor to 90% confluency in 24-well dishes. HMEC-1, a simian virus (SV)-40 transformed human dermal growth factor to 90% confluency in 24-well dishes.
compared the induction of the IL-8 and ICAM-1 genes in the two cell types. We examined first the effect of H$_2$O$_2$ on epithelial IL-8 and ICAM-1 expression by Northern analysis. Total RNA was isolated from A549 cells exposed for 3 h to H$_2$O$_2$ (100–800 μM). As shown in Fig. 1, H$_2$O$_2$ markedly increased IL-8 mRNA expression at 400 and 800 μM H$_2$O$_2$ (compare lane 1 with lanes 4 and 5). In contrast, steady-state ICAM-1 mRNA, although induced by TNFα (see Fig. 2), was only weakly induced by 400 μM H$_2$O$_2$. This is considerably less than the 2.5–3-fold induction we reported for ICAM-1 in human umbilical vein endothelial cells (22). We previously reported that actinomycin D, a potent transcriptional inhibitor, prevented H$_2$O$_2$ induction of endothelial ICAM-1 gene expression (22). Similarly, actinomycin D prevented the H$_2$O$_2$ induction of IL-8 (Fig. 1, lane 6), suggesting H$_2$O$_2$ activates IL-8 gene transcription. Because the greatest IL-8 induction was achieved with 800 μM H$_2$O$_2$, this concentration was used in all subsequent experiments.

H$_2$O$_2$ and TNFα Induce Different Patterns of IL-8 and ICAM-1 mRNA Expression in Epithelial Cells—To directly compare the effects of H$_2$O$_2$ and TNFα on IL-8 and ICAM-1 expression, we examined the kinetics of steady-state mRNA induction in A549 cells by Northern analysis. Total RNA was isolated from A549 cells treated with H$_2$O$_2$ (800 μM) (lanes 2, 5, and 8) or TNFα (100 units/ml) (lanes 3, 6, and 9) for 1, 3, or 6 h. Control cells received no treatment (lanes 1, 4, and 7). Total RNA was isolated and analyzed by Northern blot. A, autoradiogram; B, bar graph of densitometry of bands from IL-8 and ICAM-1 normalized to GAPDH signal and expressed as fold increase over spontaneous mRNA expression (lanes 1). Data are representative of two separate experiments.

H$_2$O$_2$ and TNFα Induce IL-8 Secretion in Epithelial Cells—To determine whether induction of the IL-8 gene results in increased IL-8 release, we compared the kinetics of H$_2$O$_2$ and TNFα induction of IL-8 production in A549 cells.

FIG. 1. H$_2$O$_2$ induction of IL-8 mRNA expression in A549 cells. A549 cells were treated with 100, 200, 400, and 800 μM H$_2$O$_2$, or 800 μM H$_2$O$_2$ in the presence of actinomycin D (1 h pretreatment) (lane 6). Total RNA was extracted after 3 h and analyzed by Northern blot. A, autoradiogram; B, bar graph of densitometry of bands from IL-8 and ICAM-1 normalized to GAPDH signal and expressed as fold increase over spontaneous mRNA expression (lane 1). Data are representative of two separate experiments.

FIG. 2. Kinetics of IL-8 and ICAM-1 mRNA induction by H$_2$O$_2$ and TNFα in A549 cells. A549 cells were treated with H$_2$O$_2$ (800 μM) (lanes 2, 5, and 8) or TNFα (100 units/ml) (lanes 3, 6, and 9) for 1, 3, or 6 h. Control cells received no treatment (lanes 1, 4, and 7). Total RNA was isolated and analyzed by Northern blot. A, autoradiogram; B, bar graph of densitometry of bands from IL-8 and ICAM-1 normalized to GAPDH signal and expressed as fold increase over spontaneous mRNA expression (lanes 3, 6, and 9). Data are representative of three separate experiments.
were treated with H$_2$O$_2$ (800 µM) or TNFα (100 units/ml) and the medium was monitored over time for IL-8 accumulation by ELISA. As shown in Fig. 3, H$_2$O$_2$ and TNFα markedly increased IL-8 secretion over that spontaneously released from unstimulated control cells. The kinetics of H$_2$O$_2$ (Fig. 3A) and TNFα (Fig. 3B) induction of IL-8 were similar, although the magnitude of TNFα induction was significantly greater correlating with their mRNA expression. H$_2$O$_2$-induced IL-8 secretion was detected as early as 4 h, peaked at 24 h, and remained at this level at 36 h. In contrast, TNFα-induced a 10-fold greater level of IL-8 secretion that continued to increase between 24 and 36 h. These data suggest that TNFα and H$_2$O$_2$ activate IL-8 expression through different mechanisms.

**H$_2$O$_2$ and TNFα Induce Different Patterns of IL-8 and ICAM-1 mRNA Expression in Endothelial Cells**—To determine whether H$_2$O$_2$ can induce IL-8 expression in endothelial cells, we compared the effect of H$_2$O$_2$ on the induction of IL-8 and ICAM-1 in HMEC-1 cells by Northern analysis. As shown in Fig. 4, H$_2$O$_2$ increased ICAM-1 mRNA expression, consistent with our previous data demonstrating H$_2$O$_2$ induction of ICAM-1 mRNA in HUVEC (22). Maximal ICAM-1 was induced by 50 µM H$_2$O$_2$, whereas in A549 cells, 50 µM H$_2$O$_2$ produced no detectable induction of ICAM-1 (data not shown). In contrast to ICAM-1, H$_2$O$_2$ did not increase IL-8 mRNA in HMEC-1 even at the highest concentrations (Fig. 4, A and C). As in A549 cells, TNFα induced both ICAM-1 and IL-8 in HMEC-1 (Fig. 4, B and C). These data demonstrate that H$_2$O$_2$ induces the inverse pattern of ICAM-1 and IL-8 gene expression in endothelial cells.

**H$_2$O$_2$ and TNFα Induce IL-8 Secretion in Endothelial Cells**—
To determine the effect of H\textsubscript{2}O\textsubscript{2} and TNF\textalpha on endothe-
lial IL-8 protein secretion, we monitored IL-8 release into the
medium 24 h after treatment with increasing concentrations of
H\textsubscript{2}O\textsubscript{2}. As shown in Fig. 5, H\textsubscript{2}O\textsubscript{2} increased IL-8 secretion by less
than 50% over that spontaneously secreted by HMEC-1 even at
the highest concentrations of H\textsubscript{2}O\textsubscript{2} examined (400 \textmu M). In
contrast, TNF\textalpha increased IL-8 secretion more than 3-fold.
These data demonstrate a close association between IL-8 gene
induction and IL-8 protein secretion in HMEC-1.

Because A549 and HMEC-1 are grown in very different me-
dia, it is conceivable that the different components in their
media might influence the pattern of gene expression in the
two cell types. For example, in contrast to A549 cells, HMEC-1
are grown in the presence of hydrocortisone and epidermal
growth factor. To determine whether these agents affect the
induction of IL-8, A549 cells were cultured on the same growth
media as the HMEC-1 cells for 72 h prior to stimulation under
serum-free conditions. Analysis of the medium 24 h after stim-
ulation by TNF\textalpha showed in three independent experiments no
statistical difference in IL-8 induction (data not shown).

H\textsubscript{2}O\textsubscript{2} and TNF\textalpha Differentially Induce IL-8 Secretion in En-
dothelial and Epithelial Cells—To further demonstrate that
the cell type-specific induction of IL-8 is not the result of factors
in the growth media but a general property of epithelial and
endothelial cells, we examined the induction of IL-8 in several
other epithelial and endothelial cell types. As shown in Table I,
H\textsubscript{2}O\textsubscript{2} induced IL-8 secretion in BEAS-2B, a bronchial epithelial
cell line, but not in HUVEC or HPAEC (human pulmonary
artery endothelial cell), two primary endothelial cell types. In
contrast, TNF\textalpha induced IL-8 secretion in both the epithelial
and endothelial cell types. Thus, the differential pattern of IL-8
expression appears to be a general feature of these cell types.

We previously demonstrated that the ICAM-1 promoter is
induced by H\textsubscript{2}O\textsubscript{2} in EAhy926 cells (22), a epithelial/endothelial
hybrid cell line generated from fusion of the A549 cell line with
HUVEC (48). As shown in Table I, IL-8 secretion was induced
in the EAhy926 cells by both H\textsubscript{2}O\textsubscript{2} and TNF\textalpha, the same pattern
observed in A549 cells. Thus, a endothelial/epithelial hybrid

cell can mediate H\textsubscript{2}O\textsubscript{2} induction of both IL-8 and ICAM-1,
indicating that this hybrid cell line has lost the ability to
differentially regulate the two genes. The dominance of the
H\textsubscript{2}O\textsubscript{2} gene induction implicates the involvement of positive
trans-acting factors in the discordant oxidant regulation of
ICAM-1 and IL-8 in epithelial and endothelial cells.

**FIG. 4.** H\textsubscript{2}O\textsubscript{2} and TNF\textalpha induction of IL-8 and ICAM-1 mRNA expression in HMEC-1. HMEC-1 were treated with 10–500 \textmu M H\textsubscript{2}O\textsubscript{2} or
TNF\textalpha (100 units/ml) for 1 h. Total RNA was isolated and analyzed by Northern blot. A, H\textsubscript{2}O\textsubscript{2} induction of IL-8 and ICAM-1 mRNA expression. B,
TNF\textalpha induction of IL-8 and ICAM-1 mRNA expression. C, bar graph of densitometry of band for IL-8 and ICAM-1 normalized to GAPDH signal
and expressed a fold increase over spontaneous mRNA expression. Data are representative of two separate experiments.

**FIG. 5.** H\textsubscript{2}O\textsubscript{2} and TNF\textalpha induction of IL-8 secretion in HMEC-1.
HMEC-1 cells were treated with H\textsubscript{2}O\textsubscript{2} (10–400 \textmu M) or TNF\textalpha (100
units/ml) for 24 h. IL-8 protein secretion was measured by ELISA. The
bars represent the mean release of IL-8 (ng/ml) from triplicate cultures
plus the standard error. Asterisks indicate significant increase over
unstimulated control cells using ANOVA (p < 0.05).
H2O2 selectively induces the release of a chemotactic factor both stimulated neutrophil motility. These data suggest that H2O2 also differentially induce other chemokines in A549 cells.

**DISCUSSION**

Chemokines and cell adhesion molecules are critical protein factors in the recruitment of leukocytes to sites of inflammation and injury, and oxidant stress is an important regulator of their expression (30, 50). In this study, we demonstrate that TNFα and H2O2 induce different patterns of IL-8 and ICAM-1 gene expression in epithelial and endothelial cells. TNFα induced both ICAM-1 and IL-8 gene expression in epithelial and endothelial cells, whereas H2O2 selectively induced only ICAM-1 in endothelial cells and only IL-8 in epithelial cells. These results are consistent with our previous data demonstrating H2O2 induction of ICAM-1 in HUVEC (22) and the report by DeForge et al. (30) demonstrating H2O2 induction of IL-8 protein secretion in A549 cells. The discordant expression of ICAM-1 and IL-8 induced by oxidant stress may critically influence neutrophil extravasation.

ICAM-1 and IL-8 orchestrate the transendothelial migration of neutrophils to sites of inflammation and injury (7). The up-regulation of ICAM-1 on the surface of the endothelium is required for the firm adhesion of rolling neutrophils (1) and a chemotactic gradient of IL-8 is critical for the adherent neutrophils to migrate across the alveolar-capillary membrane during lung inflammation and injury (8, 9). H2O2 produced at sites of inflammation could constitute cell type- and gene type-specific activation signals capable of inducing different patterns of ICAM-1 and IL-8 expression in pulmonary cells of the alveolar-capillary membrane. Bradley et al. (51) reported that H2O2 can also interfere with cytokine signaling through the impairment of their cell surface receptors. The oxidant regulation of the ICAM-1 and IL-8 genes may have evolved to promote their discordant expression during the inflammatory response.

In addition to the differential regulation of the CXC chemokine IL-8, we found that TNFα and H2O2 can also selectively regulate CC chemokine expression. In A549 epithelial cells, TNFα induced both RANTES and MCP-1, whereas H2O2 selectively induced only MCP-1. In contrast to IL-8, H2O2 induced MCP-1 in HUVEC (52). The differential induction of RANTES, MCP-1, and IL-8 in epithelial and endothelial cells by H2O2 suggests that oxidant stress can regulate the expression of both the CC and CXC chemokines. Although we were unable to detect any significant induction of IL-8 expression in any of the endothelial cells we examined, it has been reported that oxidant stress generated by hypoxia followed by reoxygenation can induce IL-8 expression in human endothelial cells (34) and...
H$_2$O$_2$ can induce IL-8 expression in human microvessel endothelial cells (53). Thus, oxidant induced IL-8 in endothelial cells may be dependent on both the type of endothelial cell and the form of oxidant stress.

With regard to IL-8 functional activity, the cell type-specific IL-8 mRNA expression and protein secretion was associated with increased neutrophil motility, demonstrating that oxidant activation of the IL-8 gene leads to the secretion of biologically active IL-8. These data suggest oxidant-exposed epithelial cells can contribute to the inflammatory cytokine network through the selective production of CC and CXC chemokines such as IL-8, RANTES, and MCP-1, which could critically influence the recruitment of leukocyte subsets to sites of inflammation and injury. Indeed, the differential expression of chemokines in epithelial and endothelial cells may contribute to the immuno-
Pathophysiology of several inflammatory diseases. Differential expression of GROα, ENA-78, and IL-8 has been reported in a model of psoriasis (54). TNFα has also been shown to differentially regulate RANTES and IL-8 in rheumatoid synovial fibroblasts (55). Respiratory syncytial virus also selectively induces RANTES and IL-8 in upper airway epithelial cells (56, 57).

In addition to their cell type- and gene type-specific regulation, chemokines are also regulated in a stimulus-dependent manner. Transforming growth factor β1 induces IL-8 expression in epithelial cells (58), but inhibits IL-8 expression in endothelium (59). TNFα induces RANTES and MCP-1 in human corneal keratocytes but not in corneal epithelial cells (60). TNFα induces IL-8 and MCP-1, but not RANTES in HMEC-1 (61). In contrast, we found TNFα induces all three chemokines in A549 cells, while H2O2 induces IL-8 and MCP-1, but not RANTES. This differential expression of chemokines suggests TNFα and H2O2 activate distinct signaling pathways in epithelial and endothelial cells. TNFα and H2O2 also activate T-cells through distinct signaling pathways that are thought to converge to activate NF-κB (62). Consistent with H2O2 and TNFα activation signals stimulating different second messenger pathways, we found that the anti-inflammatory cytokine IL-10 can differentially inhibit H2O2 and TNFα induction of IL-8. Thus, in A549 cells IL-10 inhibited H2O2 but not TNFα induction of IL-8.

With regard to the transcriptional mechanism, oxidant stress has been shown to modulate the DNA binding activities of the transcription factors AP-1 and NF-κB (63–65). Both the IL-8 and ICAM-1 promoters contain binding sites for AP-1 and NF-κB. Moreover, these transcription factors have been demonstrated to be involved in IL-8 and ICAM-1 expression (29, 66–69). NF-κB is critical for the TNFα response of both ICAM-1 and IL-8. The TNFα response is mediated by the cooperative binding of NF-κB and C/EBP to adjacent binding sites in the proximal regions of the IL-8 and ICAM-1 promoters (16, 25–27). This cooperative binding mechanism appears to function in both epithelial and endothelial cells, since TNFα activated the expression of IL-8 and ICAM-1 in both cell types, although the TNFα induction of IL-8 was substantially greater in A549 cells than in any of the endothelial cells examined. We have recently shown that the antioxidant pyrrolidine dithiocarbamate, a potent inhibitor of NF-κB, can abrogate the H2O2 induction of IL-8 in A549 cells.2 In contrast, pyrrolidine dithiocarbamate itself induces ICAM-1 expression (29) and does not inhibit H2O2 induction of ICAM-1 in endothelial cells (22), suggesting a role for NF-κB in the cell type-specific induction of these genes.

Das et al. (70) have demonstrated that AP-1 and NF-κB are differentially regulated by oxidant and antioxidants in A549 cells, providing a potential mechanism by which genes containing AP-1 and NF-κB-binding sites could be selectively activated by oxidant stress. They found that thiols induced NF-κB but not AP-1 in A549 cells, while oxidants like H2O2 induced AP-1 but not NF-κB. Differential activation of AP-1 and NF-κB has also been shown in other systems (63, 71, 72). The differential activation of these transcription factors has been shown to be regulated by the intracellular levels of glutathione disulfide (GSSG) (73). In this regard it is interesting to note that the cellular glutathione (GSH) levels are markedly higher in A549 epithelial cells than in fibroblasts (74). Indeed, high levels of GSH could account for the higher concentrations of H2O2 (100–800 μM) required to activate IL-8 gene expression in A549 cells compared with the low concentrations of H2O2 (10–400 μM) needed to activate ICAM-1 in endothelial cells. Thus, different GSSH/GSH ratios in epithelial and endothelial cells could lead to cell type-specific gene expression via differential activation of redox-sensitive transcription factors like AP-1 and NF-κB.

With regard to the oxidant regulation of ICAM-1 expression, we showed that the NF-κB- and C/EBP-binding sites are not sufficient to mediate the H2O2 response (22). This is in agreement with the present study suggesting TNFα and H2O2 activate gene expression through distinct mechanisms. The H2O2 response is mediated by a region of the ICAM-1 promoter containing two 16-base pair direct repeats, binding sites for the transcription factors AP-1 and Ets (22). AP-1/Ets composite elements have been found in promoters of the macrophage scavenger receptor gene (75) as well as the mouse glutathione S-transferase Ya subunit gene (76). The macrophage scavenger receptor AP-1/Ets composite element is nearly identical to the ICAM-1-AP-1/Ets elements and can mediate H2O2 activation signals (22). The AP-1/Ets element from the glutathione S-transferase promoter can also mediate an H2O2 response (76). The AP-1/Ets element is also similar to the ARE found in several anti-oxidant response genes (65, 77–79). Indeed, the ARE binds AP-1 and has been shown to mediate H2O2 transcriptional responses (80, 81). In contrast to ICAM-1, the IL-8 promoter does not appear to contain an AP-1/Ets composite element. The IL-8 promoter, however, does possess separate consensus AP-1 and ARE sites. Although the role these elements play in mediating oxidant stress induction of the IL-8 gene is presently not known, we have recently shown that binding activity on the IL-8 ARE is induced by H2O2.2 Thus, sequence and positional differences between the ICAM-1 and IL-8 ARE sites may contribute to the cell type-specific induction of these genes.

In addition to AP-1 and NF-κB, H2O2 may induce cell type-specific transcription factors, which in turn could induce the different patterns of IL-8 and ICAM-1 expression in epithelial and endothelial cells. Recently, we demonstrated that oxidant stress induced by H2O2 also activates HFH-11, a winged helix transcription factor that is expressed in embryonic epithelial cells and whose expression is reactivated in adult cells by proliferative signals (82). As with ICAM-1, HFH-11 is induced by H2O2 in HMEC-1 (82), but not A549 cells.3 However, since the kinetics of H2O2 induction of HFH-11 was identical to that of ICAM-1, we do not believe HFH-11 mediates the H2O2 induction of ICAM-1.

In summary, H2O2 and TNFα can induce different patterns of chemokine gene expression in endothelial and epithelial cells. We believe this differential expression induced by oxidant stress could critically influence the site-specific recruitment of leukocyte subsets during inflammatory reactions when epithelial and endothelial cells are under oxidant stress.

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