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Influenza virus A infection induces interleukin-8 gene expression in human airway epithelial cells

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To determine the role of the airway epithelial cell in mediating virus-induced inflammation, we infected primary cultures of human airway epithelial cells with human influenza type A/Port Chalmers/72 (H3N2). After two days, the medium was collected for measurement of the chemotactic cytokine interleukin-8 by enzyme-linked immunosorbent assay. The RNA was extracted from the cells for analysis of interleukin-8 mRNA by Northern blot analysis. Interleukin-8 production was more than doubled by viral infection, while interleukin-8 mRNA was increased four-fold. Thus induction of interleukin-8 gene expression in virus-infected airway epithelium may be an important early step leading to virus-induced airway inflammation.

Interleukin-8; Airway epithelial cell; Virus; Gene expression; Inflammation; Cytokine

1. INTRODUCTION

Airway inflammation is characteristic of influenza infection in humans [1], the most intense infiltration of inflammatory cells being in the epithelial layer. This epithelial inflammation, along with the fact that the epithelial cell is the primary cell infected by influenza, suggests that a chemotactic factor may be released by virus-infected airway epithelial cells. Previous studies using mumps-infected African green monkey kidney cells [2] and herpes simplex-infected rabbit kidney cells [3] showed that these cells released chemotactic substances in response to viral infection.

Interleukin-8, a strongly chemotactic cytokine [4], can be produced by A549 cells [5], which are of pulmonary epithelial origin. It has recently been shown that several human airway epithelial cell lines can also produce interleukin-8 [6]. Expression of the interleukin-8 gene in these cells is inducible by tumor necrosis factor, interleukin-1, and phorbol myristate acetate [6]. We carried out the present study to determine whether infection with influenza virus induced expression of the interleukin-8 gene, and production of interleukin-8, by human airway epithelial cells.

2. MATERIALS AND METHODS

2.1. *Isolation and culture of airway epithelial cells*

Cartilagenous airways were obtained from surgical lung resections. The airways used were 8–15 mm in diameter, and represented main bronchi and lobar or segmental bronchi. Epithelium was cultured using a modification of the technique devised for dog airway epithelium by Coleman et al. [7]. Airways were placed in a solution of 0.5% pronase in Hank's buffered salt solution (HBSS) and were left overnight at 4°C after which the epithelial cells were removed from the underlying tissues by jets of 10% fetal bovine serum in HBSS. The isolated epithelial cells were resuspended in serum-free LHC 8e culture medium (Biofluids) [8] and were plated on rat-tail collagen-coated six-well plates at a density of 1.2×10^5 cells/cm^2. Cultures were incubated at 34°C in 6% CO_2/air, and the cells grew to confluence in about one week.

2.2. *Virus stock*

Human influenza type A/Port Chalmers/72 (H3N2) (American Type Culture Collection) was grown in Rhesus monkey kidney cells (Viromed). Cultures were frozen and thawed to disrupt cells, and the infected culture fluid was cleared by centrifugation and stored in aliquots at −70°C. Uninfected monolayers were treated in a similar way to produce control medium.

To determine the viral content of these fluids, fresh kidney cell monolayers were exposed to serial ten-fold dilutions. Viral infection of these cells was demonstrated one week later by hemadsorption, as previously described [9]. The virus stock was found to contain 10^7 times the amount of virus required to infect 50% of kidney cell monolayers ($TCID_{50}$) per ml. No virus was detected in the control medium.

2.3. *Infection of airway epithelial cells*

Confluent epithelial cell monolayers were exposed to either virus stock diluted in LHC 8e medium to a concentration of 10^5 $TCID_{50}$/ml or control medium similarly diluted. After 1 h, the medium was replaced with fresh LHC 8e. The medium was again changed 24 h later, and was collected 48 h after infection for interleukin-8 assay. Virus infection of airway epithelium was demonstrated by recovering virus from the culture medium into fresh monolayers of Rhesus monkey kidney cells as described above. A hemadsorption assay was also performed in several of the monolayers to demonstrate expression of the viral hemagglutinin on the infected cells' membranes.

2.4. *Interleukin-8 assay*

Interleukin-8 concentration in the culture medium collected 48 h after infection was determined using a commercially available enzyme-

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linked immunosorbent assay (Research and Development). This concentration reflects the release of interleukin-8 by epithelial cells between 24 and 48 h after infection into a 2 ml volume of culture medium.

2.5. *Northern blot analysis*

Total RNA was isolated by the guanidine isothiocyanate/cesium chloride method [10]. Quantitation of RNA was determined by absorbance at 260 nm. Aliquots containing 5 μg of total RNA was denatured in 20 μl loading buffer (50% formamide, 6.5% formaldehyde, 10 mM sodium acetate, 10 mM EDTA, and 40 mM 3-(*N*-morpholinopropanesulfonic acid, pH 7.5), and electrophoresed in 1.0% agarose gel in denaturing 1.1% formaldehyde. RNA was then transferred to Gene Screen Plus (Dupont, Boston, MA) membrane filters using 10 × SSC (1.5 M sodium chloride, 0.15 M sodium citrate) [11]. Blots were then exposed to Kodak X-O mat X-ray film at −70°C, using Kodak intensifying screens. Autoradiograms were quantitated by densitometric scanning. To control for variation in either the amount of RNA in different samples or loading errors, all blots were rehybridized with an oligonucleotide probe corresponding to 18S RNA after stripping of the original probe. Ethidium bromide staining of RNA samples was also performed to assess integrity of RNA and equal loading of RNA. The densitometric value for interleukin-8 was normalized to values obtained for 18 S RNA obtained on the same blot.

2.6. *Probes*

A 40 bp synthetic oligonucleotide for human interleukin-8 was obtained from Oncogene Science (#ON413). This sequence is of the antisense orientation and is derived from 5'-untranslated sequences of exon 1 [12]. Human interleukin-1α (#ON123), granulocyte macrophage colony-stimulating factor (#ON116, GM-CSF) and collagenase (#ON344) were also obtained from Oncogene Science. A 24 base pair oligonucleotide (5'-ACG GTA TCT GAT CGT CTT CGA ACC-3') complementary to 18 S RNA was synthesized (391 DNA Synthesizer, Applied Biosystems). All oligonucleotide probes were 3' end-labeled using terminal deoxynucleotidyl transferase (Bethesda Research Laboratories, Gaithersburg, MD).

3. RESULTS

3.1. *Infection of airway epithelial cells*

As previously described [9,13], all monolayers of airway epithelium that were exposed to influenza virus stock solution became infected, as determined by recovery of virus from the supernatant culture medium into fresh monolayers of Rhesus monkey kidney cells. Hemadsorption was also detected in the virus-exposed airway epithelial monolayers in which it was tested.

Fig. 2. Northern blot analysis of interleukin-8 mRNA expression by virus-infected human airway epithelial cells. The blot was probed sequentially with oligonucleotide for human interleukin-8 and 18 S RNA. The data shown are representative of three separate experiments.

3.2. *Interleukin-8 production*

Interleukin-8 was detectable in the supernatants of control airway epithelial cell monolayers, and was more than doubled by viral infection (Fig. 1).

3.3. *Interleukin-8 mRNA*

A low basal level of interleukin-8 mRNA expression was detected in uninfected epithelial cells whereas a 3.5–4.0-fold induction of interleukin-8 mRNA was observed in the virus-infected cells (Fig. 2). The effect of virus on interleukin-8 expression was specific, and was not due to a general effect on transcription as the expression of interleukin-1α, GM-CSF, and collagenase were not affected by viral treatment (Fig. 3). In the case of GM-CSF, we found no expression in either control or virus-infected cells (data not shown).

4. DISCUSSION

These data provide the first evidence that viral infection stimulates expression of the gene for interleukin-8 in human airway epithelial cells, and that this leads to increased production of interleukin-8 by the cells. The

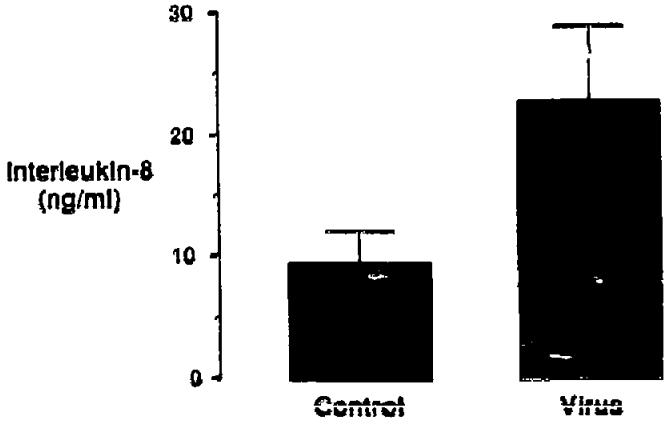

Fig. 1. Concentration of interleukin-8 in supernatants (2 ml volume overlying a 10 cm² monolayer) from control and virus-infected epithelial cells. n=8, mean ± S.E.M., P<0.05, unpaired t-test.

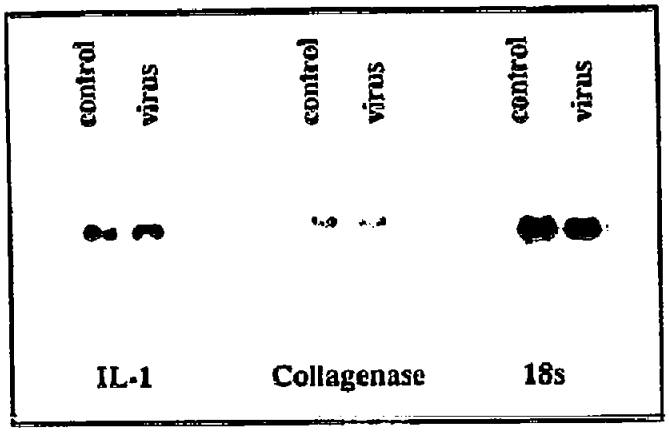

Fig. 3. Northern blot analysis of interleukin-1α, collagenase, and 18 S mRNA expression by virus-infected human airway epithelial cells. The data shown are representative of three separate experiments.

epithelial cell is the primary site of influenza infection, and the epithelial layer is the site of the most intense inflammation in the influenza-infected airway. Thus the production of the strongly chemotactic cytokine interleukin-8 is likely to be important in the pathogenesis of virus-induced airway inflammation.

Other airway epithelial products are also chemotactic. Metabolism of arachidonic acid by 5-lipoxygenase in dog tracheal epithelial cells produces leukotriene B_4 (LTB_4) [14], which is strongly chemotactic. However, the predominant lipoxygenase in human airway epithelium is 15-lipoxygenase [15]. This enzyme allows human airway epithelium to produce 8,15-dihydroxyeicosapentaenoic acid, which is also chemotactic but is much weaker in this respect than either LTB_4 or interleukin-8. It is also unknown whether production of lipoxygenase metabolites of arachidonic acid is stimulated by viral infection, as we have shown for interleukin-8.

The mechanism by which viral infection induces interleukin-8 gene expression has not yet been determined. Although interleukin-1 is known to stimulate interleukin-8 gene expression [16,17] in airway epithelial cells [6], we do not believe this was the mechanism in our study as interleukin-1 gene expression was not induced by viral infection.

Airway inflammation is strongly associated with the functional abnormalities of asthma [18], and may contribute to the similar functional abnormalities of airway secretion and bronchoconstriction seen with viral infections. The production of interleukin-8 by the epithelium may be an early step in the initiation of virus-induced airway pathology.

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