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Autocrine regulation of interleukin-8 by interleukin-1α in respiratory syncytial virus-infected pulmonary epithelial cells *in vitro*

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SUMMARY

Respiratory epithelial cells infected with respiratory syncytial virus (RSV) produce interleukin-8 (IL-8); however, the mechanisms of RSV-induced regulation of IL-8 are poorly understood. In the present study, the regulation of IL-8 by RSV was evaluated using pulmonary type II-like epithelials (A549). Live purified RSV (pRSV) induced a significant increase in IL-8 after 8 hr of exposure, while conditioned supernatants from pRSV-infected A549 cells (cRSV) induced IL-8 production in fresh A549 cultures within 4 hr of infection. Furthermore, cRSV that had been rendered non-infectious by ultraviolet-irradiation (UV-cRSV) or ribavirin treatment also induced an increased production of IL-8 in fresh A549 cells, suggesting that RSV induced the synthesis of a soluble mediator(s) which in turn enhanced the synthesis of IL-8. We have previously shown that RSV-infected A549 cells produce IL-1α, IL-1-β and tumour necrosis factor-α (TNF-α), which by themselves are known to induce the synthesis of IL-8. Preincubation of UV-cRSV or simultaneous incubation of pRSV with recombinant IL-1 receptor antagonist almost completely blocked (95–98%) the production of IL-8 by A549 cells. Furthermore, incubation with neutralizing antibodies against IL-1α, IL-1β and TNF-α showed that IL-1α was the predominant soluble mediator that enhanced the mRNA expression and synthesis of IL-8. IL-1β and TNF-α induced the synthesis of IL-8 at 24 hr, but partially inhibited the synthesis at 48 hr. In summary, these experiments provide direct evidence for an autocrine mechanism of enhanced IL-8 production in RSV-infected epithelial cells that is primarily mediated by IL-1α. In clinical settings, inhibitors of IL-1α may be useful in suppressing inflammation due to IL-1α as well as IL-8.

INTRODUCTION

Respiratory syncytial virus (RSV) is the most common cause of lower airway disease of infancy requiring hospitalization.[1] It is also the most common virus that is associated with the development of otitis media.[2] In the early phase of inflammation associated with RSV infection, neutrophils probably play an important role in the airway injury.[3] As evidence, neutrophil has been shown to be the predominant leucocyte in the lower airway and middle ear during the early phase of RSV infection.[4,5] The mechanism of migration of neutrophils into RSV-infected airway is not well understood, but it is most likely mediated by interleukin-8 (IL-8) as it is a chemokine with a potent chemoattractant property, and is detected in significant quantities in nasal secretions and middle ear fluids of children with RSV infection.[6,7] Furthermore, plasma samples of children with RSV bronchiolitis have been shown to contain increased quantities of IL-8.[8]

In the RSV-infected lower airway, a variety of cells, such as alveolar macrophages, fibroblasts, and epithelial and endothelial cells, are a potential source of IL-8. Among these cells, the respiratory epithelial cell is likely to be the most important source of IL-8 since it is the primary target for RSV infection, and is present in far larger numbers as it forms the lining of the entire respiratory tract. Hence, increasing attention has been focused on the mechanisms of regulation of IL-8 in RSV-infected airway epithelial cells.[9–12] Recent studies have shown that the induction of IL-8 by RSV in A549, a human type II-like epithelial carcinoma cell line, is due to activation of the IL-8 gene transcription factors, namely, nuclear factors (NF)-κB and NF-IL-6;[13–15] and is dependent on oxidant tone regulated by molecules other than nitric oxide.[16] Using the same cell line, Fiedler *et al.* also have shown that RSV-infected epithelial cells produce IL-8-inducing soluble mediator(s), however, the identity of the mediator(s) was unknown.[12]

IL-8 synthesis can be induced by soluble cytokines such as IL-1α, IL-1β, tumour necrosis factor-α (TNF-α) and

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interferon-γ (IFN-γ).[17,18] Since our recent studies have shown that RSV-infected A549 epithelial cells synthesize IL-1α, IL-1β and TNF-α,[19] we postulated that any of these cytokines could regulate the synthesis of IL-8. The present study provides direct evidence for the enhanced synthesis of IL-8 in RSV-infected respiratory epithelial cells that is mediated primarily by IL-1α in an autocrine fashion.

MATERIALS AND METHODS

Cell cultures

HEp2 and A549 cells were purchased from the American Type Culture Collection (Rockville, MD). These cells were grown as monolayers in minimal essential medium (MEM) supplemented with 6% heat-inactivated fetal calf serum (FCS), 100 U/ml of penicillin, 100 μg/ml of streptomycin, and 2 mM of glutamine (Gibco-BRL, Grand Island, NY), and incubated at 37° in 5% CO_2.

Virus pools

Purified RSV pool (pRSV). Human Long strain (A1) RSV was grown in HEp-2 cells, and purified by precipitation in polyethylene glycol and separation on sucrose density gradients as previously reported.[19] This preparation of virus is referred to as purified RSV (pRSV) pool. The titre of the pool, as determined by a plaque assay on Hep-2 monolayers, was 10^8 plaque-forming units (PFU)/ml. The pool contained ≈50% of sucrose. One millilitre of 1:100 working dilution of the pool (sucrose = 0·5%) to infect 10^6 A549 cells resulted in the multiplicity of infection (MOI) of 1.

Conditioned RSV pool (cRSV). Monolayers of A549 cells grown in 24-well tissue culture plates were infected with pRSV at a MOI of 1 in media containing MEM and 2% FCS, and then incubated at 37° in 5% CO_2. When the cells exhibited cytopathic effect at 48 hr, the supernatant was aspirated and centrifuged at 2000 *g* to remove large cellular debris. This preparation is referred to as conditioned RSV (cRSV) pool. The titre of infectious virus in cRSV was $\approx 10^{5.5}$ PFU/ml. A 60% vol.:vol. working dilution of the pool to infect A549 cells resulted in the MOI of ≈1.

Control media. These were prepared from uninfected cell cultures, which were processed in the same manner. All samples of virus and control media were aliquoted, quick-frozen in liquid nitrogen, and stored at −70° until needed.

Virus inactivation

The virus samples were rendered non-infectious by two different methods: cRSV was exposed to a 254-nm ultraviolet (UV) source (Mineralight lamp, UVP, San Gabriel, CA) at 10-cm distance for 3 min on ice; and intracellular virus growth was abolished by premixing the virus samples with 11·2 μg/ml of ribavirin (ICN, Irvine, CA) prior to infection of cells. The lack of live virus infectivity of these preparations was confirmed by culturing them in HEp-2 monolayers and observing for viral cytopathic effect for 5 days.

Cytokine assays

Supernatants collected from A549 cell cultures following incubation with RSV pools or other stimuli were analysed by quantitative enzyme-linked immunosorbent assay (ELISA) for IL-1α, IL-1β, IL-8 and TNF-α (Quantikine Kits, R & D Systems, Minneapolis, MN). The double-sandwich ELISA were performed following the instructions and reagents supplied by the manufacturer. The ranges of detection were 7–2000 pg/ml for IL-1α, 3·9–250 pg/ml for IL-1β, 4·7–2000 pg/ml for IL-8 and 10–1000 pg/ml for TNF-α.

Cytokine neutralization assays

Based on the quantities of IL-1α, IL-1β, and TNF-α detected in cRSV, neutralizing antibodies against the respective cytokines or recombinant human IL-1 receptor antagonist (rIL-1ra) were added to cRSV in more than 10-fold excess of the recommended concentrations. The final concentrations of antibodies against IL-1α, IL-1β and TNF-α were 150 μg/ml, 150 μg/ml and 75 μg/ml, respectively, while the concentration of rIL-1ra was 2 μg/ml. As control, murine IgG1 control antibody was used. The neutralizing antibodies were mixed with the respective recombinant cytokines to confirm their biological activities. All of these reagents were purchased from R & D Systems. The final volume dilution of cRSV was 60%. The mixtures were preincubated for 3 hr at 37°. One hundred and fifty microlitres of the suspension was then inoculated into wells containing A549 monolayers in a 96-well plate, and incubated for 24 hr at 37° in 5% CO_2. The supernatants were then aspirated and assayed for IL-8. A549 cultures similarly stimulated in 24-well plates were collected and analysed for mRNA of IL-1α, IL-1β, IL-8 and TNF-α by RNase protection assay or quantitative polymerase chain reaction (PCR).

RNase protection assay

Human IL-8 cDNA template (a kind gift from Dr J. Navaro, Galveston, TX) was radioactively transcribed with T7 polymerase by using [α-^{32}P] CTP (T3/T7 Transcription Kit, Clontech, Palo Alto, CA) to generate an IL-8 RNA probe (244 base pairs). A human G3PDH DNA template, as supplied in the kit as an internal control, was used to generate a G3PDH RNA probe (404 base pairs). The transcribed RNA probe was purified on 6% urea polyacrylamide gel, and then using a Ribonuclease Protection Assay Kit (Ambion, Austin, TX), the probe was used for solution hybridization with RNA extracted from A549 cells. Unhybridized RNA was removed by treatment with RNase A/T1. The protected hybridized RNA was precipitated with ethanol, resuspended in loading buffer, and analysed by electrophoresis on a 6% polyacrylamide gel. The gel was then dried overnight and exposed to X-ray film (X-OMAT, Kodak, Rochester, NY). The density of the target bands on the film was then analysed by Ultroscan laser densitometer (LKB-Producter AB, Bromma, Sweden).

Detection of mRNA for IL-8 and G3PDH by PCR

RNA was extracted from A549 cells by treatment with Ultraspec RNA (Biotecx, Houston, TX). Following the manufacturer's instructions, a GeneAmp RNA PCR Kit (Perkin Elmer Cetus, Norwalk, CT) was used to perform the reverse transcription (RT) and PCR on RNA from 10^6 cells. Briefly, the RT reaction mixture consisted of 5 mM $MgCl_2$, PCR buffer (50 mM of KCl, 10 mM Tris–HCl, 1 mM deoxyribonucleoside triphosphates (dNTPs), 1 U/ml RNAase inhibitor, 2·5 U/μl Moloney murine leukaemia virus reverse transcriptase and 2·5 mM oligo d(T) in a total volume of 20 μl. The cDNA was synthesized and dissociated from the RNA by heating at 42° for 60 min, 90° for 5 min, and cooling at 4° for 5 min. PCR

was performed on 2 μl of this sample in a 50-μl reaction mixture consisting of 1·5 mM $MgCl_2$, PCR buffer, 0·16 mM dNTPs, 1·25 U/50 μl of Taq polymerase and 0·15 mM each of sense and antisense primers for IL-8 and G3PDH (Clontech). The reaction was carried out with a hot start at 95° for 2 min, followed by 33 cycles at 95° for 45 seconds (denaturation), 60° for 45 seconds (annealing), and 72° for 60 seconds (extension), and a final amplification at 72° for 10 min in a DNA thermal cycler (Perkin Elmer, Norwalk, CT). To assure the conditions for the PCR assay, cDNA fragments for IL-8 and G3PDH (Clontech) were used under the same conditions. The resultant PCR products, together with ϕX174/*Hae*III DNA markers (Sigma, St. Louis, MO), were analysed by electrophoresis on a 6% polyacrylamide gel (Novex, San Diego, CA) which was then stained with ethidium bromide. The DNA was visualized by examining the gel under UV light, and photographed with Polaroid 55 film. The density of the target bands on the film was then analysed by Ultroscan laser densitometer. The base-pair sizes of the PCR products for IL-8 and G3PDH were 303 and 983, respectively.

Statistical analysis

All ELISA optical density values were compared by non-parametric Mann–Whitney rank sum test, and the difference between the groups was considered significant if the *P*-value was less than 0·05. All statistical and data manipulations were performed on a personal microcomputer using the Sigmastat (Jandel Scientific, San Rafael, CA) statistical software package.

RESULTS

Enhancement of IL-8 production by RSV

Compared with cells exposed to the control media, pRSV-exposed cells produced significantly increased quantities of IL-8 within 24 hr (mean control 0·32 versus pRSV 0·94 ng/ml), and peaked at 48 hr of incubation (mean control 0·44 versus 20·40 ng/ml; Fig. 1a). The quantity of IL-8 at 48 hr of pRSV exposure was comparable with that induced by exogenous TNF-α (Fig. 1a). After 48 hr, due to further cell detachment and destruction caused by virus infection, the assay could not be continued. RSV infection and replication were essential for the production of IL-8 or IL-8-enhancing mediator(s) since extracellular inactivation of infectivity of purified virus by UV irradiation, or intracellular inhibition of virus replication by ribavirin treatment did not enhance the production of IL-8 (Fig. 1b,c).

Enhancement of IL-8 production by supernatants from pRSV-infected cells

Further experiments were performed to determine whether the increased production of IL-8 was due to a soluble mediator(s) secreted by pRSV-infected cells. Fresh A549 cells were incubated with 60% vol./vol. dilution of conditioned supernatant collected from live pRSV-exposed A549 cell cultures (cRSV) or control media. The cRSV enhanced the production of IL-8 as early as 4 hr, which was quicker than that following exposure with pRSV (Fig. 1a). Moreover, UV-cRSV was also able to induce the production of IL-8 when incubated with fresh A549 cells (Fig. 1b). These observations suggested that

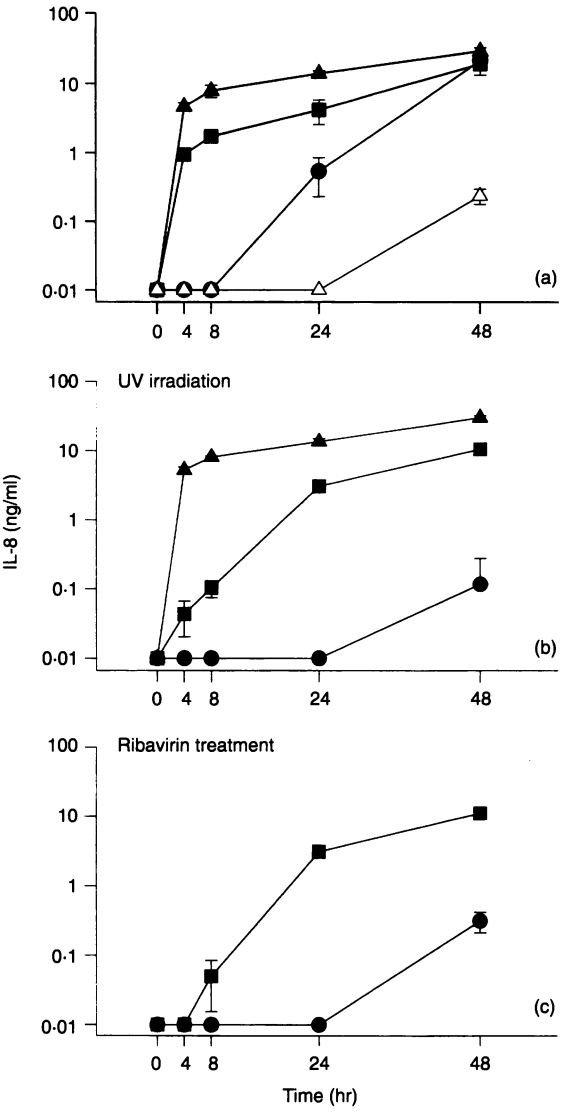

Figure 1. Time–course of IL-8 accumulation (mean ± SD of triplicate observations), as determined by ELISA, in the supernatants of A549 cell cultures grown in 24-well plates (100% confluent monolayers). The cells were exposed to a variety of stimuli: 50 pg/ml of TNF-α (▲), pRSV at MOI = 1 (●), cRSV (■), and control media (△). (b) depicts responses to UV-irradiated stimuli, while (c) depicts responses to ribavirin-treated stimuli.

the conditioned supernatant contained a preformed soluble mediator(s) with IL-8-inducing ability, which is distinct from the infectious virus.

Cytokine detection in RSV-infected A549 cell cultures

Exogenous IL-1α, IL-1β, TNF-α and IFN-γ are known to induce the production of IL-8 in A549 cells. We have previously reported the kinetics of synthesis of IL-1α, IL-1β and TNF-α in pRSV-infected A549 cultures.[19] In the present study, the supernatants at 48 hr of pRSV exposure contained mean concentrations of 120 pg/ml of IL-1α, 20 pg/ml of IL-1β and 75 pg/ml of TNF-α. These quantities of cytokines are probably sufficient to induce the production of IL-8 since dose dilution studies showed that as little as 10 pg/ml of the recombinant

forms of these cytokines were capable of inducing IL-8 production in A549 cells (data not shown). IL-1α, IL-1β and TNF-α were not detected in significant quantities in the pRSV pools, or in the supernatant from uninfected cells. Neither IFN-γ protein nor its mRNA were detectable in RSV-infected cells and supernatants (data not shown).

Blocking of UV-cRSV-induced production of IL-8 by neutralization of IL-1α, IL-1β and TNF-α

Because exogenous IL-1α, IL-1β and TNF-α can enhance production of IL-8, and because RSV-infected A549 cells produced these cytokines, it was possible that any or all of these cytokines were responsible for the enhanced IL-8 production following RSV infection. Therefore, the IL-8-enhancing property of these cytokines was evaluated by blocking their bioactivities with the respective neutralizing antibodies or rIL-1ra, a protein that inhibits the activities of IL-1α and IL-1β by competitively binding with the cellular IL-1 receptors. Incubation of recombinant cytokines with the respective blockers resulted in a complete blockage of IL-8 production (data not shown). UV-cRSV was preincubated with saturating concentrations of rIL-1ra or neutralizing antibodies against IL-1α, IL-1β and TNF-α. Twenty-four hours later, the IL-8 mRNA and protein were analysed.

Preincubation of UV-cRSV with rIL-1ra resulted in an almost complete blockage (98%) in IL-8 production (Fig. 2a). Assays of cytokine neutralization with antibodies showed that this blockage was primarily due to IL-1α. On the other hand, neutralization of IL-1β resulted in a trend towards increased production of IL-8 by 145% that was statistically insignificant, while neutralization of TNF-α significantly increased the production of IL-8 by 845% (Fig. 2a). However, when all three antibodies were added together, the production of IL-8 was almost completed blocked.

The pattern of detection of the mRNA for IL-8 paralleled the detection of its protein. The relative quantity of IL-8 mRNA was elevated within 4 hr of incubation of cells with UV-cRSV (Fig. 3). With the addition of neutralizing antibodies for IL-1α, the mRNA expression for IL-8 in cells was completely blocked, while antibodies for IL-1β and TNF-α increased the levels of IL-8 transcripts by 145% and 185%, respectively (Fig. 3). None the less, with the addition of all three antibodies together, the mRNA expression was completely blocked. These results suggested that in the first 24 hr of infection, IL-1α is the primary mediator in UV-cRSV that induces the synthesis of IL-8, while IL-1β and TNF-α individually have an inhibitory role.

Blocking of live pRSV-induced production of IL-8 by neutralization of IL-1α, IL-1β and TNF-α

The activities of blockers of IL-1α, IL-1β and TNF-α were next evaluated in live pRSV-exposed cell cultures. IL-1α antibody and rIL-1ra almost completely blocked the production of IL-8 at 24 hr and 48 hr (Fig. 2b,c), suggesting that the IL-8 production was almost exclusively mediated by RSV-induced IL-1α in an autocrine manner. Analysis of mRNA expression also showed that the inhibitory effect of IL-1α antibody was at the level of IL-8 gene transcription (Fig. 4).

Similar to the results of UV-cRSV, addition of TNF-α antibody during live pRSV infection produced a significant enhancement of IL-8 (859%) at 24 hr (Fig. 2b). However, at 48 hr, there was a partial inhibition (42%; Fig. 2c). IL-1β antibody caused a less noticeable enhancement at 24 hr (149%), as well as a less noticeable inhibition at 48 hr (86%). However, with the reduction in the virus infection dose by half (MOI = 0·5), the blocking effects of TNF-α and IL-1β antibodies at 48 hr were comparable (Fig. 2c). These results suggested that following live RSV infection, IL-1α is actively synthesized which in turn regulates the synthesis of IL-8. Furthermore, in the first 24 hr of infection, IL-1β and TNF-1α individually have an inhibitory effect on IL-8 production, while by 48 hr of infection, these cytokines have a partially enhancing effect similar to IL-1α.

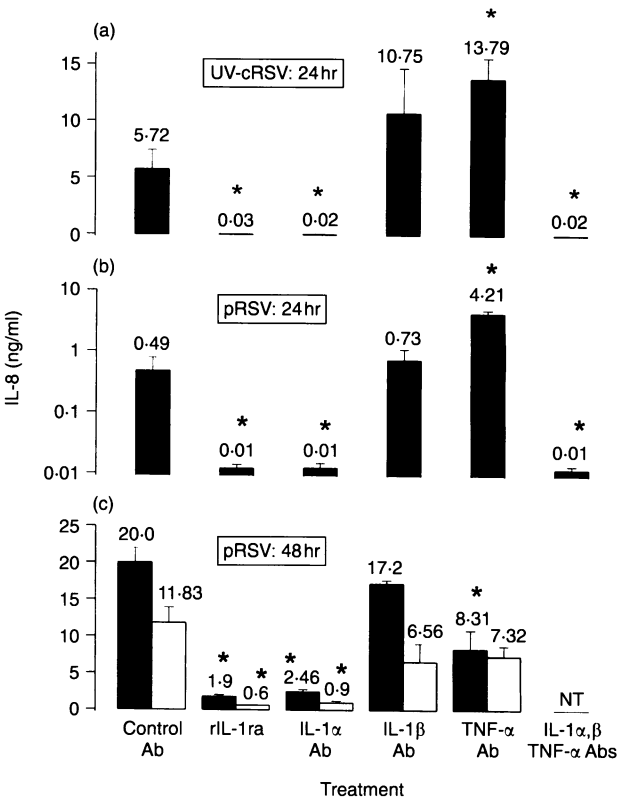

Figure 2. Comparison of IL-8 accumulation (mean ± SD of triplicate observations), as determined by ELISA, in supernatants of A549 cell cultures grown in 96-well plates (100% confluency) that were stimulated with UV-cRSV or pRSV and simultaneously treated with a variety of cytokine modulators. (a) shows stimulation with UV-cRSV for 24 hr, while (b) and (c) show stimulation with pRSV at 24 and 48 hr, respectively (closed bars = MOI of 1, open bars = MOI of 0·5). The amount of IL-8 produced by control cells was subtracted from each treatment group. *Compared with control antibody, $P < 0{\cdot}05$. NT = not tested, Ab = antibody.

DISCUSSION

The present study confirms the previous observation that RSV up-regulates the production of IL-8 in respiratory epithelial cells of lower airway origin. Moreover, the present study for the first time provides evidence that specific soluble mediators are involved in the regulation of RSV-induced production of

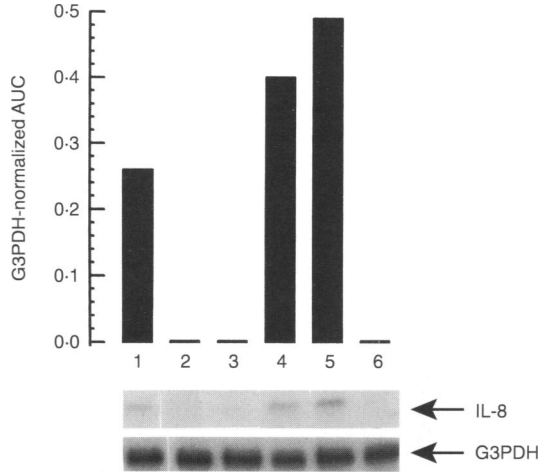

Figure 3. Quantification of mRNA for IL-8 and G3PDH (internal control) as determined by RNase protection assay at 4 hr of exposure to various stimuli. Lane 1, UV-cRSV + IgG control antibody; lane 2, sham control media; lane 3, UVcRSV + IL-1α antibody; lane 4, UVcRSV + IL-1β antibody; lane 5, UVcRSV + TNF-α antibody; and lane 6, UVcRSV + IL-1α, IL-1β and TNF-α antibodies. The results shown are representative of experiments performed twice.

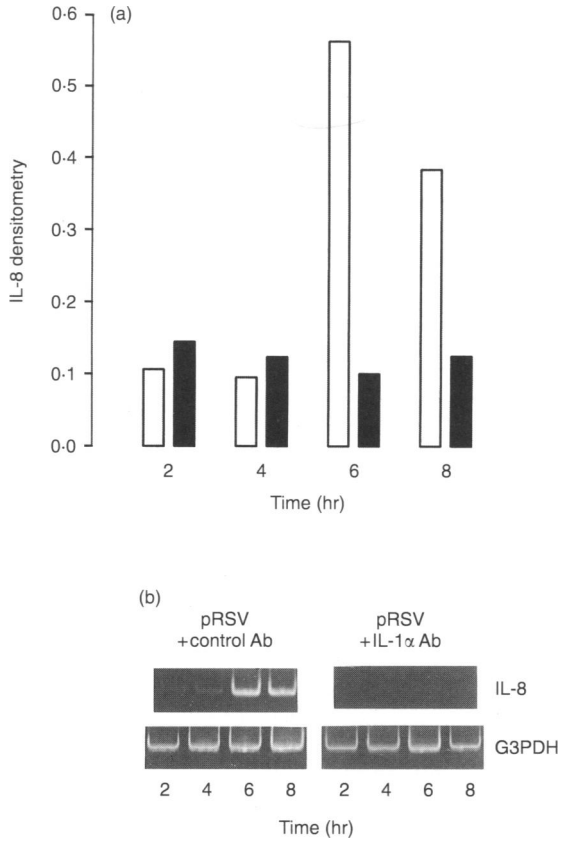

Figure 4. Time-course of mRNA expression of IL-8 as determined by densitometric analysis of (G3PDH-normalized AUC) of RT-PCR products of RNAs extracted from A549 cells exposed to pRSV (MOI = 1) + control antibody (open bars) or IL-1α antibody (closed bars). The results shown are representative of experiments performed twice.

IL-8 through an autocrine mechanism. There were several lines of evidence. First, conditioned supernatants from RSV-infected A549 cells which had been rendered non-infectious by UV-irradiation or ribavirin treatment were capable of inducing IL-8 production. Second, purified RSV-infected cells released IL-1α, IL-1β and TNF-α into the supernatant, which by themselves are known to induce IL-8 production. Live virus infection was required for the initial production of IL-8-inducing soluble mediators that were released in the supernatant since incubation of cell cultures with UV-irradiated purified virus or pretreatment of cell cultures with ribavirin inhibited the enhancement of IL-8 production. Third, it was shown that IL-1α and/or IL-1β may be the predominant IL-8-inducing mediator(s) since rIL-1ra almost completely inhibited the enhancement of IL-8 production by supernatants from RSV-infected cells. Fourth, using cytokine-neutralizing antibodies, it was shown that IL-1α was the predominant soluble mediator that enhanced the gene expression and the synthesis of IL-8.

Recent studies have explored the mechanisms of regulation of IL-8 during RSV infection. Mastronarde *et al.* observed that antioxidants dimethyl sulphoxide and 5,5-dimethyl-1-pyrroline-*N*-oxide inhibited IL-8 production but not viral replication.[16] The effect of these antioxidants on the production of IL-8-inducing cytokines such as IL-1α needs to be evaluated. Fiedler *et al.* made observations similar to ours – production of an IL-8-enhancing soluble mediator(s) occurred during RSV infection.[12] However, they could not identify the specific mediators(s). Neither IL-1β nor TNF-α was detected in the supernatant collected at 24 hr of RSV infection at a MOI of 1, while IL-1α was not investigated. The apparent lack of detection of IL-1β or TNF-α in the supernatant may not be surprising since our previous studies have shown that very little of these cytokines, including IL-1α, can be detected by ELISA in the supernatant within the first 24 hr of infection, but they can be detected at later time-points.[19] Furthermore, the current study shows that even at the period of low levels of detection of IL-1α, the gene transcription of IL-8 can be blocked by IL-1α antibody. The reasons for this observation may be that the commercial ELISA kits may not detect some of the early biologically active isoforms or that minute quantities of bioactive cytokines produced may be below the detection limits of the ELISA kits.

Indeed, in human umbilical vein endothelial cells infected with *Ricketssia conorii*, Kaplanski *et al.* could not detect IL-1α, IL-1β, or TNF-α in the culture supernatants, but IL-1 receptor antagonist or IL-1α antibody significantly blocked the production of IL-6 and IL-8.[20] Moreover, Shreeniwas *et al.* have shown that the umbilical vein endothelial cells subjected to hypoxic injury produced IL-1α that was detected using the D10 bioassay, which in turn regulated the expression of endothelial–leucocyte adhesion molecule-1 and intercellular adhesion molecule-1.[21]

Unlike IL-1α, the role of IL-1β and TNF-α in the regulation of IL-8 by RSV infection was found to be more complex in our experiments. As suggested by the cytokine neutralization assays, in the first 24 hr of infection, IL-1β and TNF-α had an inhibitory effect on IL-8 synthesis at the gene transcription level. However, additional post-transcriptional, translational, or post-translational mechanisms cannot be excluded. The inhibitory effect was short lasting since at 48 hr of infection an IL-8-enhancing activity was seen. The inhibitory effect of

IL-1β and TNF-α on IL-8 synthesis was specific to RSV stimulation, as recombinant forms of these cytokines were otherwise enhancers of IL-8. Perhaps RSV influences certain interactions among IL-1α and IL-1β and TNF-α by either positively or negatively influencing the expression of cytokine receptors, and/or a variety of inhibitors and promoters of cytokine synthesis; the influence varying according to the duration of RSV infection.

The contradictory behaviour of cytokines has been previously reported. Using human myelomonocytic cells, Orlando *et al.* have shown that TNF-α markedly reduces the binding of IL-1 to its cellular receptors by causing the release of type II IL-1 decoy receptor, which in turn neutralizes the binding activity of exogenous IL-1 in the supernatant.[22] IFN-β, a cytokine that is also produced by RSV-infected epithelial cells,[23] can enhance IL-8 mRNA expression in response to bacteria lipopolysaccharide priming in fibroblasts.[24] However, it suppresses IL-8 production in fibroblasts that had been simultaneously stimulated with IL-1α.[25] Additional studies are required to investigate the interactions between RSV and the epithelial cells that influence the biological activities of IL-1α and IL-1β and TNF-α, and the ultimate effect of these interactions on inflammation and host defence.

In conclusion, respiratory epithelial cell, a primary target cell for RSV infection, may be the most important cell that initiates and further regulates the inflammatory responses in RSV infection. Our studies show that RSV-infected respiratory epithelial cells release IL-1α, which in turn regulates the synthesis of IL-8. Previous studies have also shown that IL-1α regulates the epithelial cell expression of intercellular adhesion molecule-1 and class I major histocompatibility molecules.[19,23] In this respect, IL-1α may be the most important cytokine that modulates the early inflammatory processes. Hence, clinical use of inhibitors of IL-1 may lead to reduction of inflammation during RSV infection. On the other hand, production of IL-1 may be advantageous to the host by promoting an immunological response that results in the clearance of virus-infected cells.

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