macrophage represented at least one important source of BAL IL-8 in the patients who subsequently developed ARDS. In established ARDS, both neutrophil chemotaxis were elevated together with MCP-1, but not MIP-1α. These data suggest that different chemotactic cytokines are employed at different stages of the evolution of ARDS. BAL IL-8 may represent a very early marker of the inflammatory response leading to ARDS which is of potential predictive value in at-risk groups.

Lipopolysaccharide-stimulated Human Alveolar Macrophages Produce mRNA for a Spectrum of α-Chemokines*

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Interleukin-8 (IL-8) is a potent inflammatory mediator in the lung. Alveolar macrophages (AM) are the primary lung cells producing IL-8 in response to endotoxin. However, IL-8 only accounts for a portion of the polymorphonuclear (PMN) chemotactic activity produced by LPS-stimulated AM. Recently, we purified to homogeneity the PMN chemotactic activity produced by LPS-stimulated porcine AM and identified two proteins. The full-length cDNAs identify the first protein, AM-derived chemotactic factor-I (AMCF-I), as the porcine homologue of IL-8. The identity of the second protein, AMCF-II, remains unknown, but shares its highest homologies with the 78-amino acid epithelial cell-derived neutrophil activator, ENA-78, and the GRO subfamily of chemotactants. Thus, we sought to determine whether LPS-stimulated human AM transcribe the mRNAs for ENA-78 and GRO, as well as IL-8.

Human AM were incubated in the presence or absence of 1 mg/ml LPS for 2, 4, and 8 h. Total RNA was isolated at each time point. Detection of the mRNAs for IL-8, ENA-78, and GRO was performed by two methods: polymerase chain reaction (PCR) and Northern analysis. For PCR detection, aliquots of total RNA at each time point were treated in the presence or absence of reverse transcriptase. PCR was performed using primers for IL-8, ENA-78, and GRO. The signals for each chemokine are LPS-inducible and depend on reverse transcriptase, indicating that we are detecting their mRNAs rather than their genomic DNA. We confirmed the identities of ENA-78 and GRO by eluting their PCR bands from agarose, ligating them into a plasmid vector, expanding them in Escherichia coli, and sequencing them. This further identified the GRO PCR product as GRO-beta. Using the PCR-cloned cDNAs for IL-8, ENA-78, and GRO as templates, we labelled antisense polynucleotide probes for PCR for use in Northern analysis. We observed a time-dependent and LPS-inducible accumulation of each of the mRNAs for IL-8, ENA-78, and GRO. Thus, human AM transcribe the mRNAs for ENA-78 and GRO-beta as well as IL-8. GRO and ENA-78 may account for the remaining chemotactic activity seen in human AM supernatants after antibody neutralization of IL-8. These observations have implications for the design of α-chemokine inhibitors. Strategies designed to block LPS-induced inflammation in the lung should consider ENA-78 and GRO in addition to IL-8.

Priming of Phospholipases A₂ of Human Neutrophils by Tumor Necrosis Factor*

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Tumor necrosis factor primes polymorphonucleotides (PMN) for receptor mediated stimulation (eg, by C5a or fMLP), which might contribute to the pathogenesis of ARDS. We previously reported that primed PMN, stimulated with fMLP, have increased release of free arachidonic acid (AA) and eicosanoids (5-HETE, LTB₄) using PMN-labelled for 30 min with ³⁵H-AA (which labels primarily PC and PI phospholipids). Thus, primed PMN could provide a source of the AA and LTB₄, which occurs during ARDS. Many aspects of such events remain uncertain, including the mass of AA released by such cells and the specific phospholipase A₂ involved.

To determine the effect of priming on receptor-stimulated AA release, we quantitated the mass of AA released by stimulation of resting or TNF-primed PMN using negative ion chemical ionization mass spectrometry. Resting PMN contained 6 to 18 pmol free AA per 2.5 × 10⁶ PMN, which was doubled after fMLP stimulation. Primed PMN, stimulated by fMLP, released 360 to 620 pmol AA, with peak AA occurring 1 to 2 min poststimulation, followed by a rapid decline. Thus, primed PMN release about 20-fold more AA than unprimed PMN after receptor stimulation.

The PLA₂ responsible for AA release in such cells are unknown. The AA release from PMN is known to be Ca²⁺-dependent. Two Ca²⁺-dependent PLA₂(s), defined in other cells, are candidate enzymes for the activity in primed PMN: (1) an 85 kD cytosolic PLA₂, (cPLA₂), and (2) a 14 kD group 2 PLA₂ which is secreted by some cells (sPLA₂). Enzymatic assays could distinguish sPLA₂ and cPLA₂ activities by sensitivity to acid or diethiothreitol, and by sensitivity to anti-sPLA₂ and anti-cPLA₂ blocking antibodies. Both sPLA₂ and cPLA₂ activities were present in PMN disrupted by N₂ cavitation or by sonication. Both activities were approximately doubled by priming the PMN prior to disruption. Using several antibodies to probe Western blots, we identified PLA₂ proteins of both the sPLA₂ and cPLA₂ types. The presence of mRNA for the cytosolic PLA₂ in PMN was identified by reverse transcriptase PCR, indicating the PMN enzyme is homologous to the cPLA₂ cloned from U937 macrophages. Although enzymatic assays and Western blots indicated

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