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Chemotactic Cytokines in the Established Adult Respiratory Distress Syndrome and At-risk Patients*

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Neutrophils have been implicated in the pathogenesis of the adult respiratory distress syndrome (ARDS) but an identical syndrome has been described in neutropenic patients, suggesting an alternative pathogenic pathway, possibly involving monocytes (which possess much of the histotoxic potential of neutrophils). Since there is no clear role for the chemotaxes C5a or LTB4 in ARDS, we were interested in the role of the new supergene family of chemotactic cytokines, some members of which (interleukin-8 [IL-8], ENA-78) are specifically chemotactic for neutrophils and others (monocyte chemotactic protein-1 [MCP-1], macrophage inflammatory protein-1 [MIP-1α]) chemotactic for monocytes. Using specific sandwich ELISAs, the levels of these peptides were measured in bronchoalveolar lavage (BAL) samples of 15 patients with established ARDS and as control subjects, 10 patients ventilated for reasons other than ARDS. Since the generation of these cytokines might be expected to precede the development of a significant inflammatory response, in a fashion that might predict the development of ARDS, levels of these cytokines were also measured in blood and BAL of 29 patients "at-risk" of ARDS. This group included 16 patients with major multiple trauma (ISS 16-54), 6 patients with perforated bowel, and 7 with acute pancreatitis. Seven patients subsequently developed ARDS. Results are summarized in Table 1.

In the at-risk group, blood levels of these peptides were variably elevated, and there was no relationship with subsequent ARDS development. However, there was a clear relationship between high levels of IL-8 (but not ENA-78) in BAL samples (most of which were performed within 2 h of the trauma incident) and ARDS. Immunocytochemistry findings showed that the alveolar

Table 1—Results*

<table>
<thead>
<tr>
<th>At-Risk Patient Groups</th>
<th>BAL</th>
<th>Blood</th>
<th>BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ARDS Progression</td>
<td>No ARDS Progression</td>
<td>ARDS Progression</td>
</tr>
<tr>
<td>IL-8</td>
<td>3.06 (±2.65)</td>
<td>0.05 (±0.01)</td>
<td>5.13 (±)</td>
</tr>
<tr>
<td>ENA-78</td>
<td>0.09 (±0.05)</td>
<td>0.06 (±0.02)</td>
<td>NS</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0.09 (±0.04)</td>
<td>0.16 (±0.09)</td>
<td>NS</td>
</tr>
<tr>
<td>MIP-1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*NS = not statistically significant
† p < 0.01
‡p < 0.001
§ p < 0.0001
macrophage represented at least one important source of BAL IL-8 in the patients who subsequently developed ARDS. In established ARDS, both neutrophil chemotaxins 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**

Richard B. Goodman, M.D.; and Thomas R. Martin, M.D., F.C.C.P.

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 histone homologies with the 78-amino acid epithelial cell-derived neutrophil activator, ENA-78, and the GRO subfamily of chemottractants. 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 by 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 AMs 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 A<sub>2</sub> of Human Neutrophils by Tumor Necrosis Factor**

David A. Bass, M.D., F.C.C.P.; Michael C. Seeds, Ph.D.; David F. Jones, M.D.; Floyd H. Chilton, Ph.D.; and Sue A. Bauldry, Ph.D.

Tumor necrosis factor primes polymorphonucleotides (PMN) for receptor-mediated stimulation (eg, by C5α or IMLP), 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, LT<sub>B</sub>) using PMN-labelled for 30 min with 3H-AA (which labels primarily PC and PI phospholipids). Thus, primed PMN could provide a source of the AA and LT<sub>B</sub>, which occurs during ARDS. Many aspects of such events remain uncertain, including the mass of AA released by such cells and the specific phospholipase A<sub>2</sub> 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 gas chromatography/mass spectrometry. Resting PMN contained 6 to 18 pmols free AA per 2.5 x 10<sup>6</sup> PMN, which was doubled after fMLP stimulation. Primed PMN, stimulated by fMLP, released 360 to 620 pmols 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 PLA<sub>2</sub>(s) responsible for AA release in such cells are unknown. The AA release from PMN is known to be Ca<sup>2+</sup>-dependent. Two Ca<sup>2+</sup>-dependent PLA<sub>2</sub>(s), defined in other cells, are candidate enzymes for the activity in primed PMN: (1) an 85 kDa cytosolic PLA<sub>2</sub> (cPLA<sub>2</sub>) and (2) a 14 kDa group 2 PLA<sub>2</sub> which is secreted by some cells (sPLA<sub>2</sub>). Enzymatic assays could distinguish sPLA<sub>2</sub> and cPLA<sub>2</sub> by sensitivity to acid or diithiothreitol, and by sensitivity to anti-sPLA<sub>2</sub> and anti-cPLA<sub>2</sub> blocking antibodies. Both sPLA<sub>2</sub> and cPLA<sub>2</sub> activities were present in PMN disrupted by N<sub>c</sub> 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 PLA<sub>2</sub> proteins of both the sPLA<sub>2</sub> and cPLA<sub>2</sub> types. The presence of mRNA for the cytosolic PLA<sub>2</sub> in PMN was identified by reverse transcriptase PCR, indicating the PMN enzyme is homologous to the cPLA<sub>2</sub> cloned from U937 macrophages. Although enzymatic assays and Western blots indicated

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