Release of Interleukin-1 by Peripheral Blood Mononuclear Cells in Patients with Tuberculosis and Active Inflammation

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Peripheral blood monocytes from patients with active tuberculosis and acute inflammatory disease showed spontaneous interleukin-1 production when compared with those from control patients or healthy controls. Moreover, interleukin-1 production appeared to be a more specific indicator of active disease than were other commonly used indices, such as the erythrocyte sedimentation rate and serum C-reactive protein levels.

Spontaneous interleukin-1 (IL-1) production by monocytes and macrophages has been described in patients with both infectious and inflammatory diseases such as leprosy (10), scleroderma (1), and sarcoidosis (5). In the present study, we examined IL-1 production by peripheral blood monocytes in patients with active tuberculosis and a variety of acute infectious and inflammatory diseases. The study focused on three questions. (i) Was there indeed spontaneous IL-1 release by circulating monocytes? (ii) Did this relate to disease state? (iii) Did this correlate with other known indices of underlying disease, specifically erythrocyte sedimentation rate and the acute-phase response? Our results show that spontaneous IL-1 production by monocytes can be detected in patients with both acute and chronic infectious and inflammatory diseases. Furthermore, this appeared to correlate with the activity of the disease. Spontaneous IL-1 production was associated with elevation of C-reactive protein (CRP) levels and with erythrocyte sedimentation rate (ESR). However, these parameters were occasionally increased in some patients with apparently inactive or resolved conditions. Thus, the spontaneous production of IL-1 by monocytes may be a more specific indicator of active inflammation.

A total of 34 subjects were studied. These were divided into four groups as follows: group I, active tuberculosis (age range, 36 to 65 years); group II, acute bacterial or inflammatory conditions (age range, 16 to 73 years); group III, noninflammatory, inactive, or resolved conditions (age range, 26 to 70 years); group IV, healthy controls (age range, 22 to 67 years). Characteristics of the defined groups are summarized in Table 1.

Anticoagulated venous blood was diluted 1:2 with normal saline, and mononuclear cells were isolated with standard Hypaque-Ficoll separating medium. The interface cells were washed three times in RPMI 1640 culture medium. The cells were next suspended to 3 × 10⁶/ml in RPMI 1640 containing 10% fetal bovine serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. A 2-ml portion of the suspension was added to sterile plastic culture dishes (60 mm; Costar, Cambridge, Mass.), which were then incubated for 12 h at 37°C under an atmosphere of 5% CO₂ and 100% humidity. After incubation, the nonadherent cells were removed by vigorous washing with warm RPMI 1640, and then 2 ml of serum-free RPMI 1640 was added to each dish. Some cultures were also stimulated with 500 μg of zymosan particles. After an additional 24 h of incubation, supernatants were collected, centrifuged (500 × g, 10 min), and then stored at −20°C. Adherent cells were quantitated by scraping the plates in 1 ml of cold RPMI 1640 and then counting the suspension with a hemacytometer.

IL-1 was assayed by the standard mouse thymocyte proliferation assay (7). Briefly, a suspension of 5 × 10⁵ CBA/J mice (Jackson Laboratory, Bar Harbor, Maine) thymocytes in 0.1 ml of RPMI 1640-fetal bovine serum containing 5 μg of phytohemagglutinin and antibiotics was placed into each well of a 96-well sterile culture dish. Equal volumes of log serially diluted test supernatant were added to triplicate wells. Positive control wells contained dilutions of IL-1 active supernatant from lipopolysaccharide-stimulated CBA/J resident peritoneal macrophages or commercially obtained human IL-1 (Cistron, Pinebrook, N.J.). After 66 h of incubation, each well was pulsed with 0.5 μCi of tritiated thymidine, and cells were then harvested at 72 h with a MASH II multiwell harvester. Total incorporation was determined by liquid scintillation spectrophotometry.

An IL-1 unit was arbitrarily defined as the amount of supernatant causing an increase in thymidine incorporation of 3,000 cpm. This was calculated by linear regression analysis of the values obtained from serial dilutions.

Interleukin-2 (IL-2) activity was also assayed in monocytes culture supernatants by using the IL-2-dependent cytotoxic T-lymphocyte line (CTLL-2) described by Gillis et al. (3). Briefly, 5 × 10⁵ CTLL-2 cells in 0.1 ml of RPMI 1640-fetal bovine serum mixed with an equal volume of serially diluted test supernatant were distributed into each well of 96-well culture dishes. After 24 h of incubation, the wells were pulsed with 0.5 μCi of [³H]thymidine for 6 h and then harvested as described above. Positive controls consisted of dilutions of standard IL-2 containing supernatants prepared from concanavalin A-stimulated mouse spleen cells.

The standard Westergen ESR was measured by using anticoagulated blood. In addition, sera was obtained from cleft specimens and was subjected to automated rate nephelometry (Beckman Instruments, Inc., Brea, Calif.) for determination of CRP C-reactive protein (8).

The Student t test was used to compare control and experimental groups. Values of P > 0.05 were considered not significant.

The ESRs were not significantly different among hospitalized individuals (groups I, II, and III) (Fig. 1). However, the ESRs were clearly elevated compared with those of healthy

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controls (group IV). Similar results were obtained upon comparison of the serum levels of the acute-phase protein CRP (Fig. 2). Although there was a tendency for CRP levels to be higher in patients with acute inflammatory diseases, no statistical difference could be detected between patient groups. However, the mean CRP level was significantly elevated compared with that of healthy controls.

Interesting results were obtained when we examined monocyte production of IL-1. Five of six (67%) patients with tuberculosis (group I) showed spontaneous IL-1 production ($P < 0.005$), one patient showed borderline but detectable IL-1 activity, and no activity was detectable in one of the six patients (Fig. 3). All patients with acute infectious or inflammatory diseases (group II) showed spontaneous IL-1 synthesis ($P < 0.001$). In contrast, patients with inactive or resolved conditions (group III) produced very low levels of, or no, IL-1, as did healthy controls (group IV). When we specifically examined healthy controls over the age of 50 (50 to 67 years), we likewise saw no spontaneous IL-1 production (Fig. 3, legend).

When monocyte cultures were stimulated with zymosan, no significant differences could be observed among groups (data not shown). Similar results were reported by Watson et al. (10), who demonstrated a loss of significant differences in IL-1 production by lipopolysaccharide-stimulated monocytes from leprosy patients and controls; yet spontaneous production of IL-1 was clearly different among groups. It is noteworthy that this group also reported that monocytes from leprosy patients were more adherent, because we also observed greater adherence by monocytes of patients with tuberculosis ($8.5 \pm 4.0 \times 10^5$ per dish) than by those of controls ($2.5 \pm 1.9 \times 10^5$ per dish).

We did not detect significant IL-2 activity in any of the monocyte culture supernatants, indicating that all interleukin activity could be attributed to IL-1. IL-1-inhibitory activity was also measured in urine samples from all patients and controls, and no significant differences were observed.

Our results are in accord with previous reports demonstrating spontaneous production by IL-1 monocytes in patients with leprosy and scleroderma (1, 10). We extend these findings by showing that spontaneous IL-1 production by circulating monocytes can occur in patients with both acute and chronic diseases. Moreover, IL-1 production appears to better correlate with disease activity than do other indices such as ESR and CRP. The latter has been suggested as

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**TABLE 1. Summary of patient groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
<th>Disease or complaint</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Active tuberculosis</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>Pneumonia</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Septic arthritis</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Decubitus ulcer</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>Nonspecific back pain</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Prostatic carcinoma</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Tuberculosis, treated</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Resolved deep venous thrombosis</td>
</tr>
<tr>
<td>IV</td>
<td>17</td>
<td>Healthy controls</td>
</tr>
</tbody>
</table>

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**FIG. 1.** Westergren ESR of patient and control groups. For an explanation of groups I through IV, see Table 1.

**FIG. 2.** Serum CRP levels of patient and control groups. Values were determined by automated rate nephelometry. For an explanation of groups I through IV, see Table 1.

**FIG. 3.** Spontaneous production of IL-1 by circulating monocytes. Adherent mononuclear cells were incubated for 12 h in serum-free medium, and then supernatants were collected and assayed for IL-1 activity. Supernatants were also tested for IL-2, and no significant activity could be detected. The counts per minute plus or minus the standard error of the mean obtained for IL-1 activity among groups was as follows: I, 1,430 ± 389; II, 1,872 ± 381; III, 21 ± 21; IV, 124 ± 59 (controls over age 50, 72 ± 42). Values were obtained with a 1:8 dilution of supernatants. For an explanation of groups I through IV, see Table 1.
useful for monitoring disease activity in certain patient groups (4, 6). This is probably because CRP production is stimulated by IL-1 (9). However, CRP most likely remains elevated longer than the initial IL-1 stimulus. Since IL-1 is a primary mediator of physiologic responses such as fever and the acute-phase response, (2, 9), it would be reasonable to assume that it would be a sensitive indicator of disease activity. In preliminary studies, we have found that patients with sarcoidosis likewise show spontaneous IL-1 production at an incidence similar to that shown by patients with tuberculosis. As more efficient and sensitive assays for IL-1 are developed, it may be possible to measure this parameter for the detection of occult diseases or for monitoring the effectiveness of pharmacologic therapy.

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LITERATURE CITED