Phagocytosis and Intracellular Killing by Peripheral Blood Monocytes of Patients With Monocytic Leukemia

By R. van Furth, P. C. J. Leijh, T. L. van Zwet, and M. I. van den Barselaar

This article concerns a study on the endocytic functions of circulating monocytes from 12 patients with acute or chronic monocytic leukemia. The results show that phagocytosis and intracellular killing of *Staphylococcus aureus* are impaired in only two patients and that the opsonic activity of the serum of all patients is normal. With respect to the intracellular killing of ingested *Staphylococcus aureus*, an interesting phenomenon was found in that the cells of patients with monocytic leukemia proved to be in a state of activation, as shown by the finding that patients' monocytes with normal phagocytosis killed about 64% of the ingested bacteria in the absence of extracellular stimulation by serum factors. When extracellular serum was present, the mean killing index rose to only 69%. This is unlike the situation seen in monocytes from healthy donors, where no killing occurs in the absence of extracellular serum and extracellular stimulation by serum factors is mandatory for optimal intracellular killing.

**Materials and Methods**

**Patients**

Four patients with acute and eight patients with chronic monocytic leukemia were studied. The clinical diagnosis was made by the department of Clinical Hematology, and morphological investigation of the bone marrow was performed by the Laboratory of Haematomorphology (Dr. P. Lopes Cardozo). Some characteristics of the patients, including those of the circulating monocytes investigated with methods described elsewhere, are summarized in Table 1.

**Monocytes**

Monocytes were collected by differential centrifugation of blood on a Ficoll-Hypaque gradient (Ficoll Pharmacia, Inc., Uppsala, Sweden; Hypaque, Winthrop Laboratories, Sterling Drug, Inc., New York), and the interface layer was prepared as described elsewhere.

**Granulocytes**

Granulocytes of healthy donors were collected and prepared for the opsonin assay as already described.

**Opsonins Assay**

The opsonic activity of patient serum was determined with granulocytes of healthy donors as described elsewhere.

**Bacteria**

*Staphylococcus aureus* (type 42D) that were not killed by 2 hr of incubation in a medium with 100% fresh serum were used in all experiments. The bacteria were cultured overnight, harvested by centrifugation at 1500 g for 10 min, washed with phosphate-buffered saline, and resuspended to a concentration of 10^7 bacteria/ml in Hank's balanced salt solution (HBSS) containing 0.1% gelatin.

**Serum**

Serum was prepared by clotting blood from healthy donors (blood group AB) for 1 hr at room temperature and centrifugation at 1100 g for 20 min; 2-ml aliquots were stored at –70°C. Patients’ sera were stored at –20°C.

**Phagocytosis Assay**

Phagocytosis of *S. aureus* by monocytes was determined as the decrease in the number of viable extracellular bacteria during incubation under rotation (4 rpm) of 5 x 10^6 monocytes/ml and 5 x 10^6 bacteria/ml in the presence of 10% fresh serum, as described elsewhere.

**Intracellular Killing Assay**

Intracellular killing of *S. aureus* by monocytes was determined as the decrease in the number of viable intracellular bacteria. After 3
Table 1. Characteristics of Monocytes From Patients With Monocytic Leukemia

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Disease</th>
<th>Total Number of Leukocytes (x 10^9/liter)</th>
<th>Total Number of Monocytes (x 10^9/liter)</th>
<th>Proportion of Monocytes With Peroxidase Activity (%)</th>
<th>Esterase Activity (%)</th>
<th>Fcy Receptors (%)</th>
<th>C3b Receptors (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>AMoL</td>
<td>6</td>
<td>1</td>
<td>19.0</td>
<td>74.0</td>
<td>100.0</td>
<td>98.8</td>
</tr>
<tr>
<td>2</td>
<td>AMoL</td>
<td>20</td>
<td>5</td>
<td>0</td>
<td>7.0</td>
<td>97.3</td>
<td>94.6</td>
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<td>3</td>
<td>AMoL</td>
<td>7</td>
<td>6</td>
<td>26.5</td>
<td>92.5</td>
<td>99.5</td>
<td>91.0</td>
</tr>
<tr>
<td>4</td>
<td>AMoL</td>
<td>15</td>
<td>9</td>
<td>15.8</td>
<td>0</td>
<td>55.0</td>
<td>85.3</td>
</tr>
<tr>
<td>5</td>
<td>AMoL</td>
<td>16</td>
<td>10</td>
<td>27.2</td>
<td>7.3</td>
<td>92.7</td>
<td>97.7</td>
</tr>
<tr>
<td>6</td>
<td>AMoL</td>
<td>85</td>
<td>68</td>
<td>5.3</td>
<td>0.5</td>
<td>93.5</td>
<td>84.5</td>
</tr>
<tr>
<td>7</td>
<td>AMoL</td>
<td>88</td>
<td>79</td>
<td>49.0</td>
<td>99.7</td>
<td>99.5</td>
<td>100.0</td>
</tr>
<tr>
<td>8</td>
<td>AMoL</td>
<td>120</td>
<td>110</td>
<td>0</td>
<td>96.0</td>
<td>99.0</td>
<td>100.0</td>
</tr>
<tr>
<td>9</td>
<td>CMoL</td>
<td>3</td>
<td>1</td>
<td>98.0</td>
<td>4.7</td>
<td>94.7</td>
<td>92.0</td>
</tr>
<tr>
<td>10</td>
<td>CMoL</td>
<td>6</td>
<td>1</td>
<td>99.5</td>
<td>71.0</td>
<td>95.0</td>
<td>73.0</td>
</tr>
<tr>
<td>11</td>
<td>CMoL</td>
<td>12</td>
<td>9</td>
<td>1.0</td>
<td>4.5</td>
<td>73.0</td>
<td>94.0</td>
</tr>
<tr>
<td>12</td>
<td>CMoL</td>
<td>70</td>
<td>20</td>
<td>84.0</td>
<td>21.0</td>
<td>98.0</td>
<td>92.0</td>
</tr>
</tbody>
</table>

*Patients are listed according to increasing numbers of monocytes per liter blood.
AMoL, acute monocytic leukemia; CMoL, chronic monocytic leukemia.

min of phagocytosis at a bacteria-to-monocyte ratio of 1:1, the extracellular bacteria were removed by differential centrifugation (110 g) and two washes with phosphate-buffered saline. Next, the monocytes with intracellular bacteria were reincubated at 37°C, and after 0, 60, and 120 min, the number of viable intracellular bacteria was determined microbiologically as described elsewhere.43

RESULTS
Phagocytosis of S. aureus by Monocytes

The phagocytosis of S. aureus in the presence of 10% fresh serum by monocytes from healthy donors is a very rapid process with an almost exponential decrease in the number of viable extracellular bacteria during the first 60 min of incubation, which results in a phagocytic index of 94.8% (± 6.7% SD) at 60 min; at 120 min, this index amounted to 98.2% (± 1.2% SD) (Fig. 1). In the absence of serum, no ingestion of S. aureus by monocytes was detected.

Phagocytosis studies performed under similar conditions, but with a suspension of 80% monocytes and 20% lymphocytes from healthy donors prepared by elutria tion centrifugation showed the same rate of ingestion (phagocytic index of 96.7% at 60 min and 98.3% at 120 min) as that observed for a monocyte-lymphocyte suspension obtained from the interphase layer of a Ficoll-Hypaque gradient containing about 25% monocytes and about 75% lymphocytes.5 Similar studies in a population with more than 90% peripheral blood lymphocytes obtained by elutria tion centrifugations and at a bacteria-to-cell ratio of 1:1 showed no decrease in the number of extracellular bacteria. These results indicate that adherence of bacteria to lymphocytes, if any, does not affect the rate of phagocytosis.

The ingestion of S. aureus by monocytes from 7 of the patients in the presence of 10% serum showed a pattern comparable to the ingestion by normal monocytes (Fig. 1); in 3 cases (patients 3, 5, 6), ingestion was slightly impaired, and in 2 (patients 4, 11), almost no ingestion was observed (Fig. 1). Incubation of the patients' monocytes with S. aureus in the absence of serum did not lead to a reduction in the number of viable extracellular bacteria, indicating that no bactericidal substances are released from the monocytes during 2 hr incubation. Incubation of patients' monocytes and S. aureus in the presence of serum, but without rotation, gave no decrease in the number of bacteria in the supernatant (i.e., standing control).

The opsonic activity of serum from patients with...
AMoL or CMoL, studied at concentrations of 1% and 10%, was normal in all cases (Table 2).

**Intracellular Killing of S. aureus by Monocytes**

The average number of viable cell-associated bacteria after 3 min of phagocytosis of opsonized S. aureus by patients' monocytes (with exclusion of patients 4 and 11, with impaired ingestion; Fig. 1) amounted to $1.9 \times 10^6$ bacteria/$5 \times 10^6$ monocytes. For the monocytes with impaired ingestion (patients 4 and 11), the average number of viable cell-associated bacteria after 3 min of phagocytosis amounted to $3.3 \times 10^5$ bacteria/$5 \times 10^6$ monocytes. If it is assumed that in these two cases the bacteria were extracellularly attached to the monocytes, calculation shows that 17.4% of the viable cell-associated bacteria are extracellular. For monocytes of healthy donors, this percentage is about 10.4.

Incubation of monocytes containing ingested S. aureus in the presence of 10% fresh serum led to a decrease in the number of viable intracellular bacteria comparable to that found for monocytes of healthy donors (Fig. 2), except that monocytes showing impaired phagocytosis (patients 4, 11) did not kill ingested bacteria.

If the incubation was performed in the absence of extracellular serum, i.e., a condition under which monocytes of healthy donors are unable to kill ingested S. aureus, the decrease in the number of viable intracellular bacteria was almost equal to that obtained in the presence of serum (Fig. 3). The mean killing index was 63.7% without serum and 69.0% with serum present. This indicates that monocytes from patients with AMoL or CMoL are activated in such a way that they do not need an extracellular stimulus for maximal intracellular killing.

![Fig. 2. Intracellular killing of S. aureus in the presence of extracellular serum by monocytes from patients with AMoL or CMoL. Numbers refer to patients in Table 1; N, mean value for 15 normal donors. Hatching indicates the range of the standard deviations of the values of the normal donors.](image)

![Fig. 3. Intracellular killing indices for monocytes from patients with AMoL or CMoL after 60 min of incubation in the presence of serum or Hanks' balanced salt solution (HBSS). Numbers refer to patients in Table 1.](image)
Correlation Between Monocyte Functions

To assess correlation between the parameters under study—the percentage of cells with peroxidase activity, esterase activity, Fcγ receptors, and C3b receptors (data from Table 1), the phagocytosis index, and the intracellular killing index (data from Figs. 1 and 2)—correlation coefficients were calculated after arcsine transformation of the data. The results, given in Table 3, show that phagocytosis and intracellular killing of S. aureus are correlated with each other and with the percentage of cells with Fcγ receptors (Table 3).

DISCUSSION

The present results show that the phagocytosis and killing of ingested bacteria by monocytes from patients with AMoL and CMoL do not differ greatly from those of monocytes from healthy donors. The opsonic activity of the serum of these patients was also normal. Two of the 12 patients formed exceptions with respect to phagocytosis and bactericidal activity. Analysis of all data showed correlation only between the percentage of cells with Fcγ receptors, phagocytosis, and intracellular killing of S. aureus.

Comparison of the intracellular killing of monocytes from patients with AMoL and CMoL in the absence and presence of extracellular serum showed that under the latter condition the killing index only increases slightly (Table 4). Monocytes from normal donors do not kill ingested S. aureus in the absence of serum, and optimal intracellular killing is only achieved when at least 5% fresh serum is present extracellularly (Table 4). The serum factors responsible for this stimulation are IgG and C3b. The same phenomenon was found for granulocytes, but for these cells the basal intracellular killing in the absence of serum was much higher than in normal monocytes (Table 4).

The pattern of intracellular killing shown by monocytes from patients with AMoL or CMoL might mean that these monocytes are activated. In a small number of patients with monocytic leukemia whose monocytes showed intracellular killing activity, the O2 uptake was studied during stimulation with opsonized heat-killed S. aureus or with phorbol myristate acetate and appeared to be not higher than in monocytes from healthy donors (unpublished observations). The mechanism underlying increased intracellular killing by monocytes from AMoL and CMoL patients is not known.

Since leukemic cells are often assumed to be immature and functionally impaired, it is remarkable that the monocytes of most of the AMoL and CMoL patients showed such good endocytic function. Furthermore, in vitro incubation of monocytes in the presence of 3H-thymidine during 6 hr, a marker indicating DNA synthesis, gave a mean labeling index of 0.9% (range 0%–5.5%) for the monocytes from all 12 patients. If we reserve the term blast cell for cells that divide, the circulating monocytes in AMoL and CMoL are not monoblasts (or promonocytes) but mature nondividing monocytes.

The clinical relevance of our findings might be that in AMoL and CMoL, the monocytes are highly active and might replace the granulocytes functionally in some respects when relative or absolute granulopenia develops. The findings could also indicate that macrophages deriving from these functionally activated monocytes are more active than those found under normal conditions, which might explain why patients with untreated AMoL or CMoL have (severe) infectious complications less frequently than patients with other kinds of leukemia. Further studies are required to identify the factors that in these patients activate circulating monocytes to kill ingested bacteria.

Table 4. Comparison of the Intracellular Killing Indices of Monocytes and Granulocytes for Staphylococcus aureus

<table>
<thead>
<tr>
<th>Mean Killing Index at 60 min (%)</th>
<th>In the Absence of Serum</th>
<th>In the Presence of Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal monocytes*</td>
<td>6</td>
<td>75</td>
</tr>
<tr>
<td>AMoL/CMoL monocytes</td>
<td>63</td>
<td>69</td>
</tr>
<tr>
<td>Normal granulocytes†</td>
<td>36</td>
<td>84</td>
</tr>
</tbody>
</table>

*Data from reference 4.
†Data from reference 9.
REFERENCES

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7. Leijh PCJ, van den Barselaar MT, van Zet TL, Dubbelde-man-Rempt Y, van Furth R: Kinetics of phagocytosis of *Staphylo-
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