Phagocytosis by Human Monocytes

By Martin J. Cline and Robert I. Lehrer

The blood monocytes of mammals are phagocytic cells which are almost certainly related to the phagocytic tissue macrophages of the reticuloendothelial system. Morphologic and cytochemical studies indicate that mononuclear cells isolated from the peripheral blood or peritoneal cavity of mammals can differentiate, under appropriate circumstances, into large cells resembling tissue macrophages. Such phagocytic mononuclear cells, including the blood monocyte, may have a number of roles in normal physiologic processes, such as defense functions against microorganisms, the processing of antigenic information, the removal of damaged or effete red cells, and perhaps tissue repair and modeling of bone. At least some of these functions require particle ingestion as an initial event.

Detailed studies of the metabolism and phagocytic activity of human monocytes have not been reported, presumably because of the difficulties of isolating pure populations of these cells. In 1966, Bennett and Cohn described a method of isolating monocytes in high purity and good yield from the peripheral blood of horses. These investigators accurately predicted that their albumin flotation technic could be applied to monocytes of other species, including man. This report describes our studies of the phagocytic capacity of human monocytes isolated by their technic. The morphologic and metabolic changes of such monocytes cultured in vitro and in response to specific stimuli will be reported subsequently.

Methods

Leukocyte and Serum Preparations

Monocytes were isolated from the heparinized blood of 18 hematologically normal subjects by a minor modification of the method of Bennett and Cohn. All procedures were carried out using sterile technic. After sedimentation of the red cells with 3 per cent dextran in saline, the leukocyte-rich supernatant was centrifuged at 150 g for 10 minutes.
Fig. 1.—Isolated monocytes maintained in vitro for 4 hours. × 1200.

The white cell pellet was resuspended in 35 per cent albumin,* and phosphate-buffered saline was added to adjust the albumin concentration to 27 per cent. The suspension was centrifuged at 2400 g. for 36 minutes at 12 C. (temperature control was critical). The pellicle containing monocytes and lymphocytes was removed, suspended in Hanks' balanced salt solution containing 20 per cent fetal calf serum and centrifuged at 150 g. The cell button was washed in the same medium and suspended at a cell concentration of 2 × 10⁶/ml. in Tissue Culture Medium-199 (TC-199) containing 30 per cent fetal calf serum, penicillin, 100 units/ml., and streptomycin, 50 μg./ml. The suspension was then distributed in appropriate containers. For morphologic studies, Leighton tubes containing coverslips were used; for studies of RNA, protein and DNA synthesis, T-flasks containing 10 ml. of cell suspension were employed; and for measurement of C¹⁴O₂ generation from glucose-1-C¹⁴, cells were added to the external chamber of Erlenmeyer flasks containing a center well. After being gassed with 5 per cent CO₂, the cells (monocytes and lymphocytes) were allowed 90 minutes at 37 C. to adhere to coverslips or the walls of their containers. They were then washed free of nonadherent lymphocytes with warm Hanks' salt solution at

37 C, and resuspended in TC-199. Serum, metabolic inhibitors or test particles were then added to the supernatant medium. In occasional experiments the mixture of monocytes and lymphocytes was added to capped plastic tubes and rotated at 30 rpm in an incubator at 37 C.

In 33 separate experiments the purity of the monocyte preparations varied between 55 and 99.5 per cent. Contaminating cells were usually neutrophils and eosinophils; lymphocytes were almost never seen. In experiments involving measurements of metabolic changes following phagocytosis, monocyte populations of at least 95 per cent purity were used. An example of an isolated monocyte population is shown in Figure 1.

Human serum was obtained from a blood group AB normal subject and stored at -60 C. Human IgG (7S gamma globulin) was prepared from the sera of normal subjects or patients with multiple myeloma by methods previously described. Human IgM was prepared similarly from the sera of patients with macroglobulinemia.

**Preparation of Particles**

Polystyrene particles, 1.3 μ in diameter, were suspended in Hanks' solution at a final concentration of 0.5-1.0 mg/ml.

An 18-hour culture of *Candida albicans* in Sabouraud's medium was washed with saline by centrifugation and either used directly or after being killed by heating at 100 C. for 10 minutes. *Cryptococcus neoformans* were isolated from a patient with cryptococcal meningitis and cultured on agar slants. Before use, the microorganisms were washed from the slant with Hanks' solution, then washed three times by centrifugation with the same solution. The fungi were added to the leukocyte preparations in a ratio of 2:1 or 4:1.

*Escherichia coli* or *Staphylococcus aureus* 502A, grown for 18 hours in Trypticase soy agar, were washed in Hanks' solution and added to leukocyte suspensions in a ratio of 15:1.

Blood group O Rh-positive red cells were washed with buffered saline and incubated for 2 hours with serum containing anti-D antibody in high titer (positive Coombs' test at a dilution of 1:1000). The red cells were then washed thrice and added to leukocyte preparations in a final concentration of 0.5 per cent (v/v).

Injured neutrophils were prepared as follows. Leukocyte preparations consisting primarily of neutrophils and metamyelocytes were isolated from patients with inflammatory diseases as previously described. The granulocytes were suspended in Eagle's minimal essential medium containing 20 per cent autologous serum and were incubated for 60 minutes after the addition of saline, *E. coli* endotoxin, 50 μg/ml, or serum from a multiply transfused patient containing a high titer of leukoagglutinins which reacted with 90 per cent of the leukocyte populations tested. Incubation was continued for 45 minutes, and the granulocytes were collected by centrifugation and extensively washed with Hanks' solution containing fetal calf serum. The granulocytes, suspended in TC-199 containing 20 per cent serum, were added to the monocyte preparations in a final ratio of 1:1.

**Phagocytosis of Particles**

Monocyte preparations were incubated at 37 C for 60 minutes in the presence or absence of metabolic inhibitors before the addition of test particles. In most experiments the cells were fixed to a glass surface at the time of addition; in a few experiments they were in suspension in plastic tubes. In studies involving the use of radioisotopes, the radioactive compound was added along with test particles. After the addition of particles, incubation was continued for 30 to 120 minutes; the coverslips were then removed and studied by phase contrast microscopy or fixed with methanol and stained with Giemsa. For measurements of RNA or protein synthesis, cells were harvested from T-flasks essentially as described by Bennet and Cohn.
**Metabolic Studies**

Incorporation of H3-uridine into acid-precipitable RNA and of C14-amino acids into protein was measured as previously described.13,14 The production of C1402 from glucose-1-C14 was determined by a previously described method,14 modified by the direct addition of Hyamine® as a CO2-absorber to the center well of a modified Erlenmeyer flask.

**RESULTS**

**Particle Ingestion**

Preparations of human monocytes ingested a variety of test particles, including polystyrene spherules, _C. albicans_, _C. neoformans_, _E. coli_, _Staph. aureus 502A_ and red blood cells sensitized with incomplete 7S (anti-D) antibody. However, they failed to phagocytize either red cells aged in vitro under sterile conditions in Alsever’s solution for 48 hours at 37 C. or neutrophils injured with endotoxin or leukoagglutinins. The efficiency of phagocytosis varied with the test particle and the experimental conditions employed.

_Polystyrene Particles_. Monocytes were more efficient than neutrophils in ingesting polystyrene particles. At 30 minutes after the addition of these particles to the incubation medium, an average of 93 per cent of monocytes had ingested five or more beads each, compared with an average of 73 per cent of neutrophils and only 54 per cent of eosinophils. Monocytes in suspension and adhering to a glass surface were equally effective in ingesting polystyrene particles. Phagocytosis proceeded in the absence of serum and over a broad pH range (Fig. 2).

_Bacteria_. Monocytes phagocytized both _E. coli_ and _Staph. aureus 502A_ in vitro (Table 1). In the absence of serum, ingestion of these microorganisms was considerably reduced. Monocytes were effective phagocytes both when adherent to a glass surface and in free suspension. The percentage of monocytes containing more than 10 bacteria each was greater in the sus-

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*Packard Instrument Co., La Grange, Illinois.*
Table 1.—Phagocytosis of Bacteria by Monocytes and Neutrophils

<table>
<thead>
<tr>
<th>Cell Preparation</th>
<th>Incubation Medium</th>
<th>Bacteria</th>
<th>Phagocytic Leukocytes</th>
<th>Monocytes</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Phagocytic</td>
<td>% &gt; 10 Organisms</td>
<td>% Phagocytic</td>
</tr>
<tr>
<td>Suspension</td>
<td>TC-199 + serum</td>
<td><em>Escherichia coli</em></td>
<td>68 ± 4</td>
<td>12 ± 3</td>
<td>79 ± 2</td>
</tr>
<tr>
<td>Adherent to glass</td>
<td>TC-199 + serum</td>
<td><em>Escherichia coli</em></td>
<td>72 ± 2</td>
<td>1.5 ± 1</td>
<td>71 ± 13</td>
</tr>
<tr>
<td>Adherent to glass</td>
<td>TC-199 + serum</td>
<td><em>Escherichia coli</em></td>
<td>7 ± 3</td>
<td>0</td>
<td>52 ± 10</td>
</tr>
<tr>
<td>Suspension</td>
<td>TC-199 + serum</td>
<td><em>Staphylococcus aureus</em></td>
<td>91 ± 3</td>
<td>78 ± 7</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>Adherent to glass</td>
<td>TC-199 + serum</td>
<td><em>Staphylococcus aureus</em></td>
<td>91 ± 4</td>
<td>55 ± 5</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>Adherent to glass</td>
<td>TC-199 + serum</td>
<td><em>Staphylococcus aureus</em></td>
<td>52 ± 3</td>
<td>6 ± 3</td>
<td>88 ± 10</td>
</tr>
</tbody>
</table>

Pension cultures, probably reflecting a more efficient contact between particles and leukocytes in suspension. The percentage of phagocytic monocytes was, in general, slightly less than the percentage of phagocytic neutrophils, and the percentage of monocytes containing a large number of ingested bacteria was considerably less than the corresponding percentage of neutrophils. Monocytes thus appeared to be somewhat less efficient phagocytes of bacteria under the experimental conditions employed.

Yeast. Monocytes efficiently ingested both *C. albicans* and *C. neoformans*, provided serum or serum factors were either present in the suspending medium or preincubated with the yeast (Table 2). In the latter studies,
Monocytes incubated with Cryptococcus neoformans in the presence of serum; rosettes of yeast around central monocytes are prominent. × 1600.

$1 \times 10^8$ washed *C. albicans* were incubated for 60 minutes with 0.5 ml. of serum or with IgG (10 mg./ml.) or human albumin (10 mg./ml.). The yeast was washed extensively and resuspended in Hanks' solution before being added to the monocyte cultures. Our studies indicated that IgG, but not albumin, effectively opsonized Candida for phagocytosis. They also indicated that phagocytosis probably does not require complement components, since purified protein fractions were used for incubation with the yeast and no complement could be detected in the supernatant medium of the washed monocyte preparations.

Monocytes adherent to glass were approximately as efficient as neutrophils in the ingestion of *C. albicans*, and an average of 99 per cent of monocytes were phagocytic in the presence of serum and at a ratio of yeast to monocytes of 4:1 or 5:1. Monocytes were significantly more effective than neutrophils in the ingestion of *C. neoformans*. After 90 minutes, an average of 65 per cent of monocytes were phagocytic, compared with only 6 per cent of neutrophils ($p < 0.01$). As shown in Figure 3, rosettes of yeast around the phagocytic monocytes were usual, but it was easy to identify phagocytized yeast within vacuoles. Detailed studies of the ingestion and killing of yeast by monocytes and other human leukocytes will be reported subsequently.
PHAGOCYTOSIS

Table 3.—Phagocytosis of Red Cells by Human Monocytes

<table>
<thead>
<tr>
<th>Incubation Medium</th>
<th>Red Cells Type</th>
<th>Pretreated with</th>
<th>% Monocytes with Ingested Red Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC-199</td>
<td>0 Rh+</td>
<td>Saline</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>TC-199</td>
<td>0 Rh+</td>
<td>Anti-D serum</td>
<td>65.0 ± 4.0</td>
</tr>
<tr>
<td>TC-199 + serum</td>
<td>0 Rh+</td>
<td>Anti-D serum</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>TC-199 + albumin</td>
<td>0 Rh+</td>
<td>Anti-D serum</td>
<td>63.0 ± 6.0</td>
</tr>
<tr>
<td>TC-199 + IgG</td>
<td>0 Rh+</td>
<td>Anti-D serum</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>TC-199 + IgM</td>
<td>0 Rh+</td>
<td>Anti-D serum</td>
<td>58.0 ± 7.0</td>
</tr>
<tr>
<td>TC-199</td>
<td>0 Rh−</td>
<td>Anti-D serum</td>
<td>1.5 ± 0.5</td>
</tr>
</tbody>
</table>

Red Cells. After in vitro exposure for periods up to 36 hours, less than 0.5 per cent of monocytes phagocytized unsensitized fresh red cells or red cells aged in vitro for 48 hours. Under similar conditions, monocytes effectively phagocytized Rh-positive erythrocytes that had been sensitized with the appropriate antibody (Table 3, Fig. 4). They also phagocytized Coombs'-positive red cells (coated with IgG) from a patient with hemolytic anemia associated with ingestion of α-methyl DOPA. Such phagocytosis was always accompanied by the formation of rosettes of red cells around a central monocyte (Fig. 4).

The requirements for ingestion of sensitized red cells differed considerably from those for yeast, since serum, even in concentrations of 5 per cent, had a markedly inhibitory effect on erythrophagocytosis. Some of the details of this inhibition were delineated for the Rh system. As shown in Table 3, IgG, but not IgM or albumin, inhibited phagocytosis of Rh-positive sensitized red cells in concentrations as low as 10 μg./ml. Preparations of IgG from normal serum and from the sera of patients with multiple myeloma appeared to be equally effective as inhibitory agents.

When monocytes were incubated with fresh unsensitized Rh-positive red cells in the presence of a serum with a high titer (1:2000) of anti-D for periods up to 4 hours, only a small fraction (mean of 11 per cent) of the monocytes were phagocytic, presumably because of the relatively large amounts of non-antibody gamma globulin in the serum.

In the absence of serum there was a broad pH optimum for erythrophagocytosis (Fig. 2). In these experiments, monocytes appeared to require surface contact for erythrophagocytosis. When monocytes in suspension were rotated in plastic tubes with sensitized red cells, less than 1 per cent were phagocytic over a 4 hour period. Prominent rosettes of red cells were observed around monocytes (but not neutrophils), but phagocytic white cells were rarely seen. In an attempt to demonstrate the requirement for surface contact, we repeated the studies with red cells and monocytes pel-
Fig. 4.—Phagocytosis of antibody (7S)-coated red cells by a monocyte; rosettes of red cells, as well as red cells within vacuoles, are seen. × 1200.

Injured Neutrophils. The mechanism of disposal of injured neutrophils is unknown. Two possible mechanisms are autolysis by released lysosomal enzymes and removal by other phagocytic cells. We therefore undertook to determine whether monocytes would phagocytize injured granulocytes in vitro. Two types of granulocyte injury were studied: endotoxin and antibody injury.

Granulocytes were incubated with endotoxin (50 μg./ml.) or with high-titer leukoagglutinin which did not directly kill the cell (as evidenced by trypan blue exclusion). The cells were washed free of nonadherent endo-
Table 4.—Effect of Metabolic Inhibitors on Phagocytosis by Human Monocytes

<table>
<thead>
<tr>
<th>Metabolic Inhibitor</th>
<th>Concentration</th>
<th>Ingestion of Sensitized Red Cells ( % of control)</th>
<th>Ingestion of Polystyrene Particles ( % of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen atmosphere</td>
<td>--</td>
<td>83 ± 3</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>Potassium cyanide</td>
<td>1 x 10⁻³ M</td>
<td>90 ± 7</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>2 x 10⁻² M</td>
<td>3 ± 1</td>
<td>56 ± 12</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>1 x 10⁻⁴ M</td>
<td>8 ± 3</td>
<td>39 ± 18</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>5 µg./ml.</td>
<td>95 ± 8</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>Puromycin</td>
<td>4 x 10⁻⁵ M</td>
<td>77 ± 9</td>
<td>91 ± 4</td>
</tr>
<tr>
<td>Cortisol</td>
<td>7 x 10⁻⁵ M</td>
<td>87 ± 3</td>
<td>--</td>
</tr>
<tr>
<td>Colchicine</td>
<td>1 x 10⁻⁵ M</td>
<td>78 ± 16</td>
<td>--</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>1 x 10⁻⁵ M</td>
<td>42 ± 17</td>
<td>99 ± 2</td>
</tr>
</tbody>
</table>

Toxin or antibody and reincubated with monocytes already adherent to glass coverslips. In these experiments we were unable to demonstrate phagocytosis of granulocytes during incubations lasting as long as 3 hours; however, we did find significantly more antibody-injured (12 ± 1 per cent) and endotoxin-injured (15 ± 4 per cent) granulocytes in contact with monocytes than uninjured granulocytes (6 ± 2 per cent). Despite increased contact between injured neutrophils and monocytes, rosettes of the type seen with antibody-coated red cells and yeast were not observed in these preparations.

In another type of experiment neutrophils were prelabeled with H³-uridine, washed and exposed to endotoxin or saline for 45 minutes, and re-washed. The labeled endotoxin-injured neutrophils and control cells were then added to monocyte preparations. Although contact of the endotoxin-treated neutrophils with monocytes was increased (10.5 per cent vs. 1.5 per cent), release of tritium-labeled nucleotides into the suspending medium was not augmented.

Metabolic Requirements for Phagocytosis

As an approach to the study of the metabolic requirements for particle ingestion by monocytes, the cells were preincubated with a variety of metabolic inhibitors before the addition of polystyrene beads or sensitized red cells. To distinguish between inhibition of phagocytosis and simple killing by the inhibitors, all cell preparations were stained with trypan blue at the conclusion of the experiment. Significant uptake of supravital stain (up to 7 per cent) was seen only with the inhibitors of glycolysis. These inhibitors also produced morphologic distortions of the monocytes in Giemsa-stained preparations and caused the cells to lose contact with the glass surface.

In general, the metabolic inhibitors tested had similar effects on the phagocytosis of polystyrene beads and sensitized red cells, although the ingestion of erythrocytes was more sensitive to inhibition of glycolysis. Detailed stud-
Table 5.—Effect of Phagocytosis on Metabolism of Human Leukocytes

<table>
<thead>
<tr>
<th>Cell Preparation</th>
<th>( \text{H}^3)-Uridine Incorporation into RNA* (c.p.m./10^6 cells/hr.)</th>
<th>Glucose-1-C(^{14}) Incorporation into RNA (c.p.m./10^6 cells/hr.)</th>
<th>C(^{14})-Amino Acid Incorporation into RNA (c.p.m./10^6 cells/hr.)</th>
<th>Glucose Utilization ((\mu)g./10^6 cells/hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monocyte (2 (\times) 10^6/ml.; adherent to glass)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9395 ± 1520</td>
<td>93 ± 7</td>
<td>271.0 ± 32.0</td>
<td>240 ± 80</td>
</tr>
<tr>
<td>Phagocytic</td>
<td>6983 ± 1280</td>
<td>222 ± 19</td>
<td>285.0 ± 64.0</td>
<td>210 ± 60</td>
</tr>
<tr>
<td>Neutrophil (2 (\times) 10^7/ml.; free suspension)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>727 ± 140</td>
<td>82 ± 8</td>
<td>40.6 ± 4.1</td>
<td>—</td>
</tr>
<tr>
<td>Phagocytic</td>
<td>1430 ± 284</td>
<td>1260 ± 65</td>
<td>33.7 ± 3.5</td>
<td>—</td>
</tr>
</tbody>
</table>

*Different methods of analysis of radioactivity were used in experiments with neutrophils and monocytes. Therefore, the absolute values for \(\text{H}^3\)-uridine incorporation and C\(^{14}\)O\(_2\) production by these types of leukocytes are not strictly comparable.

Metabolic Changes Accompanying Phagocytosis

The following metabolic events were measured in phagocytic monocytes: glucose utilization, production of C\(^{14}\)O\(_2\) from glucose-1-C\(^{14}\), and incorporation of \(\text{H}^3\)-uridine into RNA and of C\(^{14}\)-amino acids into protein. For comparison of monocytes with other types of phagocytic leukocytes, previously published data on neutrophils\(^{14}\) is also presented. As seen in Table 5, particle ingestion by monocytes was associated with enhanced production of C\(^{14}\)O\(_2\) from glucose-1-C\(^{14}\) (a semiquantitative measurement of the hexose-monophosphate shunt) but no detectable increase in incorporation of \(\text{H}^3\)-uridine or C\(^{14}\)-amino acids or glucose consumption. In neutrophils phagocytosis was accompanied by a more striking increment in C\(^{14}\)O\(_2\) production and increased incorporation of \(\text{H}^3\)-uridine and production of lactic acid.

Discussion

Several studies of mononuclear cells from mammalian species have shown that the metabolic behavior and phagocytic capacities of these cells are likely to depend on the conditions and time of cultivation in vitro and the extent of differentiation toward a cell type resembling the tissue macrophage.\(^1\) Our preliminary observations suggest that over the course of several
days human monocytes in culture will also differentiate into cells morphologically similar to large macrophages. Our initial studies, reported here, were restricted to the first few hours after monocyte isolation, at which time the cells were morphologically indistinguishable from the circulating monocytes of the blood. Such cells can be compared with circulating neutrophils isolated under similar conditions in their ability to phagocytize a variety of particles and in their environmental and metabolic requirements for phagocytosis. Certain similarities and differences in the behavior of these two cell types were interesting, particularly those concerning the varieties of particles ingested. Both monocytes and neutrophils effectively phagocytized E. coli, Staph. aureus, inert plastic beads, and C. albicans, and neither cell type ingested antibody- or endotoxin-injured neutrophils. Monocytes, however, efficiently ingested antibody-sensitized red cells and Cryptococci, whereas neutrophils either failed to do so or showed low levels of activity. The selective binding of IgG-coated red cells by monocytes (as opposed to neutrophils) has been noted by other investigators, as has the inhibition of such binding by free IgG. On the basis of such observations, LoBuglio and Jandl suggested that the monocyte possesses unique receptor sites for IgG. We found that binding of IgG-coated red cells by monocytes, with subsequent formation of rosettes around the central leukocyte, was almost always accompanied by erythrophagocytosis. LoBuglio and Jandl suggested that adherence of the erythrocyte to the surface of the monocyte results in spherocytosis and increased susceptibility to osmotic lysis. It is obvious that erythrophagocytosis depends on the experimental conditions employed and may be an important additional mechanism of removal of IgG-coated red cells.

The observed capacity of monocytes to ingest Cryptococci, together with the relative inability of neutrophils to do so, may be relevant to the normal defense reactions against this fungus. If monocytes are important or critical in the removal of Cryptococci, it would perhaps explain the known susceptibility of patients with certain disorders of the lymphoreticuloendothelial system (e.g., Hodgkin's disease) to cryptococcal infections and the relative rarity of such infections in patients with disorders of granulocyte function. The situation with C. albicans appears to be quite different. The neutrophil apparently is the principal leukocyte involved in the phagocytosis and killing of this fungus. 

Monocytes resemble neutrophils, eosinophils, and unicellular microorganisms in having a broad pH optimum for phagocytosis. They resemble neutrophils in their ability, both when free in suspension and adherent to glass, to ingest polystyrene particles. The observation that ingestion of antibody-coated red cells occurred only when the monocytes were adherent to glass is of interest and may be relevant to the fact that erythrophagocytosis is rarely observed in the peripheral blood of either normal subjects or patients with hemolytic anemia.

In their metabolic requirements for phagocytosis, monocytes appear to
resemble neutrophils; that is, they require an intact glycolytic metabolism
and are not affected by inhibition of oxidative metabolism and of RNA syn-
thesis. Monocytes are somewhat more sensitive to inhibitors of protein syn-
thesis than are neutrophils. Cohn and Benson\(^1\) have suggested that cultured
monocytes become increasingly susceptible to the action of 2,4-dinitrophenol
with time. This phenomenon may be related to the transformation of the
monocytes to macrophage-like cells that require aerobic conditions for phag-
ocytosis. Thus, the transformed monocyte and the tissue macrophage more
closely resemble primitive unicellular organisms such as the amoeba\(^18\) than
they resemble the neutrophil and circulating monocyte.

In a limited number of studies of mononuclear cells from patients with
hematologic diseases, we observed a wide range of phagocytic capacities
against a variety of particles. We could not predict on morphologic grounds
which cells would be effective phagocytes. Thus, bizarre atypical mono-
cytes from one patient with infectious mononucleosis more effectively phago-
cytized sensitized red cells than did the morphologically normal monocytes
from the peripheral blood of a patient with reticulum cell sarcoma. The same
monocytes from the patient with infectious mononucleosis phagocytized \textit{C. albicans} as efficiently as did normal monocytes. We plan to do additional
studies on the metabolic activity and phagocytic capacity of monocytes from
patients with a variety of hematologic disorders.

**Summary**

Human monocytes isolated from peripheral blood phagocytized polystyrene
ingestion of fungi and bacteria required serum or serum fractions. Ingestion
of antibody-coated erythrocytes was inhibited by whole serum or IgG, but
not by IgM or albumin. Adherence to a surface appeared to be critical for
erythrophagocytosis but not for ingestion of bacteria.

Phagocytosis occurred over a broad pH range and in the presence of in-
hibitors of oxidative metabolism and of RNA synthesis. Phagocytosis was
significantly depressed by inhibitors of glycolysis (iodoacetate and fluoride)
and by chloroquine at $1 \times 10^{-8}$M. Particle ingestion by monocytes was ac-
companied by enhanced production of C\textsubscript{14}O\textsubscript{2} from glucose-1-C\textsubscript{14}, but was
not associated with a detectable increase in total glucose utilization, incor-
poration of radioactive uridine into RNA, or incorporation of amino acids
into protein.

**SUMMARIO IN INTERLINGUA**

Monocytos human isolato ab sanguine peripheric se monstrava capace a phagocytisar
ingestion de fungos e bacterios requireva le presentia de sero o de fractiones de sero. Le
ingestion de erythrocytos a revestimento anticorporeo esseva inhibite per sero integre o
per IgG sed non per IgM o albumina. Adherentia al superficie pareva esser de importancia
critic pro le erythrophagocytose sed non pro le ingestion de bacterios.
Phagocytose occurreva in un large area de pH e in le presentia de inhibitores de metabolismo oxidative e del synthesse de acido ribonucleic. Le phagocytose esseva significativemente deprimite per inhibitores de glycolyse (iodoacetato e fluoruro) e per chloroquina a $1 \times 10^{-5}$ M. Le ingestion del particulas per monocytos esseva accompaniate de un augmentate production de C14O2 ab glucosa-1-C14, sed illo non esseva associate con un detegibile augmento in le utilisation total de glucosa, le incorporation de uridina radioactive ad in acido ribonucleic, o le incorporation de amino-acidos ad in proteina.

REFERENCES


Phagocytosis by Human Monocytes

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