Functional Heterogeneity of Isopycnic Fractions of Rat Alveolar Macrophages

SHANE J. O'NEILL,* SUSAN K. HOEHN, ESTHER LESPERRANCE, AND DANIEL J. KLASS
Section of Respiratory Diseases, Department of Medicine, University of Manitoba, Winnipeg, Manitoba R3E 0Z3, Canada

Received 4 January 1984/Accepted 29 June 1984

Rat alveolar macrophages separated into four isopycnic fractions on Percoll gradients were functionally heterogeneous in bacterial phagocytosis, intracellular killing, superoxide anion release, and lysosomal enzyme activity but not in hydrogen peroxide release. With increasing alveolar macrophage density, phagocytosis, intracellular killing, superoxide anion release, and lysozyme activity increase, and acid hydrolase activity decreases.

Macrophages harvested from the same anatomical site can differ strikingly with regard to function, stage of differentiation, biochemical characteristics, and many other features (5). We have separated suspensions of rat alveolar macrophages (AMs) into four distinct fractions on the basis of buoyant densities of the AMs on discontinuous gradients of Percoll. AMs from each isopycnic fraction were tested for ability to phagocytize and kill Staphylococcus aureus 502A and for lysosomal enzyme activity and zymosan-stimulated superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) release to determine whether these properties are affected by cell density.

Bronchoalveolar cells were harvested by saline lavage of male Sprague-Dawley rats (200 to 250 g) and layered at a concentration of 1.4 × 10^7 viable cells onto discontinuous gradients of Percoll (Pharmacia Fine Chemicals, Dorval, Quebec) formed from solutions of 1, 3, 4, 6, and 8% Percoll (density, 1.010 to 1.075). The gradients were centrifuged at 400 × g for 30 min at 4°C. The cells were harvested at the different interfaces of the Percoll solutions, washed three times in Hank's balanced salt solution, and counted with a hemocytometer. Cell viability was determined by exclusion of 1% trypan blue, and differential counts were performed on Wright-stained cytocentrifuge preparations. The cells were suspended in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) at a concentration of 10^6 viable cells per ml in petri dishes (Falcon [diameter, 35 mm]; Becton, Dickinson & Co., Baltimore, Md.), and cultured for 3 h in a 5% CO_2 incubator at 37°C.

To study bacterial phagocytosis and intracellular killing by AMs, we used a modified version (S. J. O'Neill, E. Lesperance, and D. J. Klass, Am. Rev. Respir. Dis., in press) of a recently described dual-isotope radiometric assay (8). Briefly, S. aureus 502A was labeled with [14C]phenylalanine by incubation in 20 ml of tryptic soy broth containing 6 μCi of [14C]phenylalanine (specific activity, 496 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) at 37°C for 16 h. The number of adherent AMs in a given monolayer was quantitated by inverted-phase microscopy (1). AM monolayers were then challenged with 10^8 14C-labeled S. aureus organisms. After a 1-h incubation at 37°C in a 5% CO_2 incubator, extracellular staphylococci in one-half of the dishes were lysed with the murolytic enzyme lysostation (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 20 μg/ml. Lysostation was removed with three washes in Hank's balanced salt solution, and AMs in all dishes were then hypotonically lysed. Viable bacteria in the lysates were labeled by incubation with 0.22 μg of [3H]thymidine (specific activity, 2.7 mCi/mg; Amersham) for 30 min and harvested on filters (pore size, 0.22 μm; Millipore Corp., Mississauga, Ontario). Radioactivity on the filter was counted as disintegration per minute by dual-isotope analysis in a liquid scintillation spectrometer (Searle Analytic Inc., Des Plaines, Ill.). For each experiment, a range of 14C-labeled bacterial suspensions containing 10^5 to 10^7 organisms per ml was labeled with [3H]thymidine, harvested and counted as above, and CFUs were measured. 3H and 14C disintegrations per minute were plotted against numbers of CFU, and the resultant standard curve was used to determine the numbers of bacteria in the samples. Extracellular and intracellular live and dead bacteria were enumerated in the samples by differential labeling and lysostation treatment. All bacteria were labeled with [14C]phenylalanine, whereas only viable bacteria were labeled with [3H]thymidine. Therefore, the 14C disintegrations per minute represent the total number of bacteria and 3H disintegrations per minute represent the number of viable bacteria in a sample. By algebraic notation, where a = 3H-labeled bacteria in samples not treated with lysostation = total viable bacteria in samples, b = 14C-labeled bacteria in samples not treated with lysostation = total bacteria in samples, and c = 3H-labeled bacteria in samples treated with lysostation = viable intracellular bacteria: phagocytosis = b - (a - c)/number of AMs in monolayer (i.e., total intracellular bacteria/number of AMs in monolayer); intracellular killing efficiency = [b - a]/([b - a] - c) × 100 (i.e., [(number of bacteria killed/total intracellular bacteria) × 100].

To determine the lysosomal enzyme activity of each isopycnic fraction, AMs (10^6) from each fraction were washed and suspended in 0.2% (wt/vol) Triton X-100. The activities of β-glucuronidase, acid phosphatase, α-galactosidase, and N-acetyl glucosaminidase were determined by using 4-methylumbelliferyl substrates, as previously described (3). Lysozyme activity was determined by the rate of hydrolysis of Micrococcus lysodeikticus (Sigma Chemical Co.) measured by decrease in absorbance at 450 nm (12). Crystalline egg white (grade 1) lysozyme (Sigma Chemical Co.) was used as a standard. Opsonized zymosan-stimulated H_2O_2 and O_2^- release were measured by the fluorimetric method of Ruch et al. (9) and by ferricytochrome c reduction (6), respectively. Results are expressed as mean ± standard deviation. Statistical significance was evaluated by using Student's t test for unpaired data.

* Corresponding author.
TABLE 1. Bacterial phagocytosis, intracellular killing, lysozyme activity, and hydrogen peroxide release by isopycnic fractions of AMa

<table>
<thead>
<tr>
<th>Percoll solution fraction (% of total cells recovered)</th>
<th>Bacterial phagocytosis (mean no. of bacteria per AM)</th>
<th>Intracellular killing efficiency (%)</th>
<th>Lysozyme activity</th>
<th>H2O2 release</th>
<th>O2− release</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole population</td>
<td>19.2 ± 4.3</td>
<td>39.7 ± 7.1</td>
<td>1.19 ± 0.23</td>
<td>5.3 ± 1.1</td>
<td>7.2 ± 1.3</td>
</tr>
<tr>
<td>1%-3% Interface (17 ± 2%)</td>
<td>3.2 ± 1.9</td>
<td>7.5 ± 2.6</td>
<td>0.97 ± 0.18</td>
<td>5.1 ± 0.9</td>
<td>3.9 ± 1.2</td>
</tr>
<tr>
<td>3%-4% Interface (54 ± 7%)</td>
<td>17.4 ± 3.3</td>
<td>35.8 ± 5.6</td>
<td>1.05 ± 0.27</td>
<td>4.8 ± 1.3</td>
<td>6.3 ± 0.8</td>
</tr>
<tr>
<td>6%-8% Interface (14 ± 3%)</td>
<td>23.7 ± 5.2</td>
<td>44.8 ± 6.8</td>
<td>1.21 ± 0.32</td>
<td>5.6 ± 1.9</td>
<td>9.6 ± 1.5</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation of 12 experiments. Bold-faced values indicate P < 0.001 versus whole population values by the t test for unpaired data.

Of the cells initially applied to the Percoll gradient, 79 ± 8% were recovered. The cell distribution pattern (Table 1) was characterized by a large proportion of cells at the 3%-4% interface. The least-dense fraction (1%-3%) was enriched in nonviable (47%) and ciliated columnar cells. The two intermediate-dense fraction interfaces were enriched for AMs, and the highest density fraction interface (6%-8%) was enriched for lymphocytes (12%). The size, degree of cytoplasmic vacuolation, and number of intracytoplasmic inclusions of the AMs tended to decrease as their density increased. Table 1 demonstrates that bacterial phagocytosis and intracellular killing increased with increasing AM density. We hypothesized that enhanced intracellular killing by higher-density AMs might be related to increased lysosomal enzyme activity or oxygen-dependent microbicidal mechanisms in these fractions. Lysozyme activity and O2− release (Table 1) increased, acid hydrolase activity (Table 2) decreased, and zymosan-stimulated H2O2 release (Table 1) remained constant with increasing AM density. Thus, enhanced lysozyme activity and O2− release with increasing AM density may partly explain the enhanced microbicidal activity of the denser AM fractions. The relationship between AM density and lysosomal enzyme activity seen in our study is in accordance with a previous report (2) demonstrating heterogeneity in acid hydrolase and lysozyme activity in isopycnic fractions of rabbit peritoneal exudate macrophages. The least-dense fractions were enriched for acid hydrolase activity, and the highest-density fractions were enriched for lysozyme activity. The enhanced O2− release with increasing AM density is in accordance with the findings of Holian et al. (4).

Marked functional heterogeneity exists within whole populations of alveolar and peritoneal macrophages in the expression of Fc receptor activity (7). Moreover, mouse peritoneal macrophages of the 1C-21 line which were separated into isopycnic fractions on discontinuous gradients of

Percoll exhibited enhanced antibody-dependent phagocytosis of chromium-labeled sheep erythrocytes with increasing cell density (10). The density-dependent enhancement of bacterial phagocytosis seen in our study seems likely to be mediated at least in part by enhanced expression of Fc receptor activity by the higher-density fractions.

AM subfractions, separated on the basis of cell density, are known to be heterogeneous in stimulated migration, O2− release, pinocytosis (4), antitumor function (13), suppression of lymphocyte mitogenesis, and the production of interleukin I (11). Our findings provide evidence for functional heterogeneity in AM subfractions in staphylococcal phagocytosis, intracellular killing, and lysosomal enzyme release.

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LITERATURE CITED


6. Johnston, R. B., C. A. Godzik, and Z. A. Cohn. 1978. Increased superoxide anion production by immunologically activated and

TABLE 2. Acid hydrolase activity of isopycnic fractions of AMA

<table>
<thead>
<tr>
<th>Percoll solution fraction</th>
<th>β-Glucuronidase</th>
<th>Acid phosphatase</th>
<th>α-Galactosidase</th>
<th>N-Acetyl glucosaminidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole population</td>
<td>51 ± 5</td>
<td>199 ± 23</td>
<td>12 ± 3</td>
<td>262 ± 35</td>
</tr>
<tr>
<td>1%-3% Interface</td>
<td>67 ± 6</td>
<td>264 ± 26</td>
<td>20 ± 24</td>
<td>361 ± 48</td>
</tr>
<tr>
<td>3%-4% Interface</td>
<td>54 ± 6</td>
<td>209 ± 23</td>
<td>14 ± 3</td>
<td>274 ± 39</td>
</tr>
<tr>
<td>4%-6% Interface</td>
<td>36 ± 6</td>
<td>126 ± 17</td>
<td>7 ± 2</td>
<td>152 ± 18</td>
</tr>
<tr>
<td>6%-8% Interface</td>
<td>22 ± 4</td>
<td>62 ± 9</td>
<td>3 ± 2</td>
<td>65 ± 9</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation of duplicate values, expressed as nanomoles of 4-methylumbelliferyl released by 0.2% Triton X-100 by 106 cells per hour. Bold-faced values indicate P < 0.001 versus whole population values by the t test for unpaired data. The unfractonated cell population and the four isopycnic fractions had comparable amounts of protein (191 ± 223 µg) per 106 cells.


