Development, Characterization, and Subcellular Location of DNAse Activity in HL-60 Cells and Monocytes

By Pamela J. Roberts

The digestion of DNA from intact bacteria by human phagocytic cells was measured by the release of solubilized radiolabeled DNA. Two subclones from the human promyelocytic HL-60 cell line were unable to digest bacterial DNA unless they were previously induced to mature by incubation for several days with 1.25% dimethylsulfoxide (DMSO). The maximal capacity of DMSO-induced HL-60 cells to digest DNA was similar to that of monocytes purified from peripheral blood (PB) and much greater than that of neutrophils. The increasing capacity to digest DNA during maturation was associated with the development of acid DNAse activity, measured in a cell-free system, and slightly preceded development of 12-O-tetradecanoyl phorbol 13-acetate-stimulated respiratory burst activity. The acid DNAse had a pH optimum of 5.0 and did not require the presence of calcium or 2-mercaptopethanol (2-ME). A third subclone of HL-60 cells was able to digest DNA from intact bacteria without previous maturation, however, and this was associated with the presence of an alkaline DNAse which had a pH optimum between 7.0 and 8.0 and showed a dependence on calcium and 2-ME for maximal activity. The subcellular location of acid DNAse in DMSO-induced HL-60 cells was similar to that of monocytes in having a bimodal distribution on fractionated sucrose density gradients. The dense peak (mean density 1.195 g/mL) was located in the same region of the gradient as primary granule enzymes but the light peak (mean density 1.137 g/mL) did not codistribute with either plasma membrane, endoplasmic reticulum, or mitochondria, suggesting accumulation in a different organelle.

A MAJOR FUNCTION of phagocytic cells is the ingestion and killing of invading microorganisms. Effete autologous cells are also cleared from the body by phagocytes. Both effete cells and microorganisms are subsequently digested by phagocyte enzymes by processes that are not fully understood.1,2 Neutrophils and monocytes differ in their ability to digest some macromolecules. Neutrophils have the capacity to degrade bacterial protein, RNA, and peptidoglycans3-6 and to a much lesser extent DNA. Most ingested DNA remains as an undigested residue within the cell7,8 although some high molecular weight (mol wt) fragments are released.6 In monocytes, the deoxyribonuclease (DNAse) content is much higher6,8 and ingested DNA is rapidly degraded and released from the cell as small soluble fragments.

Many of the enzymes involved in killing and degradation are packaged into different types of granules within the phagocyte.9-11 In the neutrophil, neutral proteases,12,13 myeloperoxidase, lysozyme, and acid hydrolases are contained within the primary granules, whereas lactoferrin,8 vitamin B12 binding protein14 and further lysozyme are contained within the secondary granule compartment. Previous work in rabbit15 and guinea pig16 has demonstrated that ribonuclease and DNAse are also localized within the neutrophil granules and within the lysosomal fraction of human mononuclear phagocytes.7 Both peroxide-positive and peroxidase-negative granules have been described in the monocyte,17 the peroxide-positive granules also containing arylsulphatase and acid phosphatase.

In normal neutrophil18 and monocyte19 differentiation, the primary granule contents are produced before the secondary granules. Considerable insight into this differentiation has been obtained by study of the human promyelocytic cell line HL-60,19 recently reviewed by Collins.20 These cells have both neutrophilic and monocyctic potentiality. Dimethyl sulfoxide (DMSO) and retinoic acid induce differentiation predominantly down the neutrophil pathway; phorbol esters, dihydroxyvitamin D3, and interferon-γ (IFN-γ) induce differentiation predominantly down the monocyte pathway.20 Uninduced HL-60 cells express receptors for the Fc portion of immunoglobulin G11 and are able to phagocytose opsonized bacteria,22 but are unable to kill these effectively.22,23 DMSO induces bactericidal activity,23 which is associated with acquisition of components of the NADPH-dependent membrane oxidase system24 and the ability to mount a respiratory burst.23,24 Uninduced HL-60 cells contain substantial quantities of myeloperoxidase, but production of some other primary granule contents, such as lysozyme, β-galactosidase, and β-glucuronidase, increases with further differentiation whereas the production of others, such as collagenase and chymotrypsin-like cationic proteins, decreases.23,25,26 Neutrophil-like secondary granules and their contents are absent even in fully induced HL-60 cells.23 In this study, HL-60 cells have been used to study development of phagocyte DNase activity, its pH dependence, and subcellular localization and a comparison made with that of peripheral blood (PB) monocytes.

MATERIALS AND METHODS

Cell Preparation

Cell lines. Two DNase-negative subclones of HL-60 cells (clone 1, a gift from Dr R.C. Gallo, National Cancer Institute, Bethesda, MD; clone 2, a gift from Professor S. Shall, Department of Biochemistry, Sussex University, England) and a DNAse-positive subclone (clone 3, a gift from Dr Z.B. Gery, Institut de Recherche sur les Maladies du Sang, Inserm Unit 204, Paris, France) were...
grown in continuous culture at 37°C at cell densities of 1 to 5 x 10^5 cells/mL in RPMI 1640 medium containing 10% fetal calf serum (FCS), in a fully humidified atmosphere of 5% CO₂. The monocytic cell line U937 was grown under similar conditions. HL-60 cells were induced to differentiate by culture for up to 11 days in medium supplemented with dimethylsulfoxide (DMSO, 1.25% vol/vol) and this medium was renewed every 3 to 4 days. Under these conditions, cell viability as determined by trypan blue exclusion was greater than 86% on day 11. Morphological maturation was assessed on cytospin preparations stained with Leishman's stain. HL-60 cells were also incubated for 7 days in culture medium supplemented with 10 nmol/L 1,25-dihydroxycholecalciferol (1,25-dihydroxy-vitamin D₃) (Roche Products, Welwyn Garden City, Hertfordshire, England) in plastic tissue-culture dishes (14-cm diameter, Sterilin, Feltham, England).

PB Neutrophils and Monocytes

Monocytes and neutrophils were separated from heparinized venous blood (10 U preservative-free heparin/mL blood) by sedimentation of erythrocytes in the presence of 1% dextran sulfate, followed by centrifugation through a single cushion of Ficoll sodium metrizoate (density 1.077 g/mL). Contaminating erythrocytes were removed from neutrophil suspensions by lysis in hypotonic saline. Monocytes were further purified from lymphocytes by adherence to plastic tissue-culture dishes (plating density 2 x 10⁶/mL RPMI). Nonadherent cells and platelets were removed by gentle washing with RPMI. Adherent cells were gently removed by mechanical means and washed once before analysis. Purified cells were suspended in RPMI medium without phenol-red or sodium bicarbonate, with RPMI. Adherent cells were gently removed by mechanical means and washed once before analysis. Purified cells were suspended in RPMI medium without phenol-red or sodium bicarbonate, pH 7.4 (RPMI). Cytocentrifuge preparations of purified cells were stained with a combined stain for chloroacetate and α-naphthyl acetate esterase.

Phagocytosis and Digestion of DNA From Intact Bacteria

Phagocytosis and digestion of radiolabeled Escherichia coli was measured using a previously described technique outlined below; E. coli containing high specific activity (3H-thymine) DNA were heat-killed, opsonized with human immunoglobulin G (IgG), and suspended in RPMI.

Step 1: Phagocytosis. Radiolabeled bacteria were incubated with cells (10 bacteria: one phagocyte) at 37°C for 10 minutes with mixing by end-over-end rotation. Phagocytes were separated from uningested bacteria by centrifugation (170 g for 5 minutes) and resuspended in medium, and samples were taken to measure radioactivity.

Step 2: Solubilization of ingested bacterial DNA. Phagocytes from step 1 (1 x 10⁵/mL) were incubated for 2 hours more at 37°C and pelleted by centrifugation (170 g for 5 minutes). The supernatants, which contained bacterial products released from the cells, were saved, and the pellets were lysed with distilled water. Both supernatants and cell lysates were centrifuged (8,000 g for 4 minutes) and the resultant pellets were dissolved by boiling in 5% trichloroacetic acid (TCA) for 30 minutes. The radioactive content of supernatants and solubilized pellets was analyzed in a β-scintillation counter. Control samples without phagocytes showed that less than 2% of radioactivity was spontaneously released from bacteria.

Step 3: Analysis of digested DNA by solubility in 5% TCA. The supernatants and cell lysates were incubated in TCA (5% at 4°C for 30 minutes) and centrifuged (8,000 g for 4 minutes). The resultant pellets were solubilized in hot TCA, and radioactivity was measured as described in step 2. Both the amount of 5% TCA-soluble DNA and the total amount of DNA solubilized were expressed as a percentage of total ingested DNA to control for variable ingestion between samples.

Superoxide Production

Superoxide production in cells stimulated with 12-O-tetradecanoylphorbol 13-acetate (TPA) (100 ng/mL, Sigma, Poole, Dorset, England) was measured by the superoxide dismutase-inhibitable reduction of ferricytochrome c as previously described.

Cell-Free Assay of DNAse

Cultured cells and purified monocytes and neutrophils were washed twice in Dulbecco's phosphate-buffered saline (50 mmol/L, pH 7.4) and resuspended at 4 x 10⁷ cells/mL ice-cold hypotonic sucrose solution (6% wt/wt) containing phenylmethylsulfonyl fluoride (PMSF; 1 mmol/L) and aprotinin (100 U/mL, Sigma). Cells were incubated on ice for 15 minutes, followed by sonication for 15 seconds at an amplitude of 4 to 6 μm (Soniprep 150, MSE). The toxicity of the sucrose solution was adjusted to 12% (wt/wt), and the sonicates were centrifuged (600 g for 10 minutes at 4°C) to sediment unbroken nuclei. The DNA content of the sonicate was assayed by the method of Karsten and Wollenberger. DNAse was measured by the adapted method of Bornstein et al on 25 μL sonicate (equivalent to 1 x 10⁶ cells) in reaction mixtures containing: sodium acetate buffer (10 mmol/L, pH 5.1), magnesium acetate (653 μmol/L), human serum albumin (HSA, 16 μg/mL), 2-ME (1 mmol/L), sodium cholate (0.1% wt/vol), 1.5 μg DNA (type VIII from E. coli, Sigma) in sodium borate buffer (55 mmol/L, pH 8), and 100 ng purified (thymine-2-,13C)DNA from E. coli (specific activity 40.5 μCi/mg, Amersham International) in a total volume of 300 μL. Mixtures were incubated for 30 minutes at 37°C, and the reaction was stopped by addition of TCA (final concentration 5% vol/vol). Samples were incubated on ice for 60 minutes and centrifuged (8,000 g for 4 minutes) to remove the TCA precipitate. The radioactivity of the supernatant was measured in a β-scintillation counter and was expressed as a percentage of the total DNA in the assay mixture.

Subcellular Fractionation Studies

HL-60 cells (1.2 x 10⁵, clone 1, induced for 10 days with DMSO) and purified PB monocytes (2 x 10⁵ x 10²) were pelleted by centrifugation and washed twice in cold sucrose solution (11.2% wt/wt in 1 mmol/L EDTA, pH 7.4, and preservative-free heparin 5 IU/mL). The cells were suspended in cold sucrose (4 mL 11.2% supplemented with 1 mmol/L PMSF and 100 U/mL aprotinin) and disrupted in a Dounce homogenizer with 50 to 100 strokes of a tight-fitting (B) pestle (Kontes glass, Vineland, NJ). The homogenates were centrifuged at 600 g at 4°C for 10 minutes to remove the nuclei and unbroken cells. Under these conditions, the postnuclear supernatant (PNS) contained approximately 8% of total cell DNA. Three milliliters PNS was layered over 27 mL linear sucrose gradients (from 1% to 55% sucrose wt/wt, density 1.045 to 1.266 g/mL) over a 38 mL cushion of 60% sucrose. The gradients were centrifuged (25,000 rpm at 4°C) in an AH 627 swinging-bucket rotor in an OTD 55B Sorvall centrifuge (Du Pont, Stevenage, Herts, England) integrated angular velocity 4 x 10² sec⁻¹, bmax 125,000, bmax 54,000, average 89,900; 50 x 0.5 mL fractions were collected by upward displacement with 60% sucrose.

Analyses Conducted on Fractionated Cells

DNAse activity was measured as described, except that sodium cholate was omitted from the reaction mixture, on aliquots (2 to 10 μL) of the fractions and compared with that of the following components measured as follows: myeloperoxidase (EC 1.11.17.7) by
the peroxidation of O-dianisidine\textsuperscript{25}; cytochrome oxidase (EC 1.9.3.1) by the spectrophotometric method of Cooperstein and Lazzaro\textsuperscript{30}; adenylate cyclase (EC 4.6.1.1) by generation of cyclic AMP from ATP, using the method of Wisher and Evans\textsuperscript{40} minimally modified by addition of guanosine triphosphate (10 mmol/L) and sodium fluoride (10 mmol/L) to the incubation medium; cyclic AMP by a competitive binding assay (cyclic AMP assay kit, Amersham International); sulfatase (EC 3.1.6.1) by fluorometric analysis of liberation of 4-methyl-umbelliferone (4-MU) from 4-MU sulfate by the method of Canonico et al.\textsuperscript{55}

**RESULTS**

**Phagocytosis and Digestion of Radiolabeled Bacteria**

The proportion of morphologically mature HL-60 cells increased markedly in cultures incubated with DMSO, and after 11 days metamyelocytes comprised 42% of cells (Table 1). The capacity of HL-60 cells to phagocytose radiolabeled \textit{E. coli} was measured at intervals during DMSO-induced maturation and was compared with the ability of the cells to digest bacterial DNA. Uninduced HL-60 cells ingested IgG-opsonized \textit{E. coli} (23.257 ± 3.592 cpm/10\textsuperscript{6} cells, mean ± 1 SE, n = 4) and the capacity to phagocytose increased little during maturation (33.412 ± 6.084 cpm/10\textsuperscript{6} cells on day 11) (inset, Fig 1). The capacity to digest DNA from ingested bacteria was measured by the release of radioactive soluble DNA, and showed a differential development from that of phagocytosis. Uninduced HL-60 cells digested very little DNA, only 4% ± 2% (mean ± 1 SD, n = 6) of ingested DNA was released from the cells as 5% trichloroacetic acid (TCA)-soluble fragments. During maturation, DMSO-treated HL-60 cells developed an enhanced capacity to digest DNA that was maximal in cells cultured for 9 to 11 days. Figure 1 shows that most DNA released from mature HL-60 cells was soluble in 5% TCA and was therefore of very low mol wt.\textsuperscript{36}

**Comparison of Digestion of DNA From Intact \textit{E. coli} by DMSO-Induced HL-60 Cells With Phagocytes Derived From PB**

The ability of maximally matured HL-60 cells (9 to 11 days in DMSO) to release 5% TCA-soluble fragments of DNA from \textit{E. coli} was similar to that of freshly isolated PB monocytes and much greater than that of PB neutrophils (Table 2). In six experiments, DMSO-induced HL-60 cells released 34% ± 13% (mean ± 1 SD) of ingested DNA as 5% TCA-soluble fragments as compared with

![Fig 1. Phagocytosis (A) and digestion (B, C) of radiolabeled \textit{E. coli} by HL-60 cells, measured at intervals during DMSO-induced maturation. Shown are the total amount of soluble radiolabeled DNA (B) and low-mol-wt DNA (5% TCA-soluble) (C) released from the cells, expressed as a percentage of the absolute amount of ingested radioactivity (A) shown in inset. Data are the mean ± 1 SD of four experiments. Each sample contained 1 × 10\textsuperscript{6} viable cells.](image)

**Table 1. Viability, Morphology, and Cytochemical Characteristics of HL-60 Cells From Subclone 1 Induced to Differentiate With Dimethyl-Sulfoxide (1.25% vol/vol)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Exposure to DMSO (days)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Viability*</td>
<td>91 ± 3</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
</tr>
<tr>
<td>Myeloblast</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Promyelocyte</td>
<td>88 ± 2</td>
</tr>
<tr>
<td>Myelocyte</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Metamyelocyte</td>
<td>0</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0</td>
</tr>
<tr>
<td>Choroacetate esterase</td>
<td>&gt;95</td>
</tr>
<tr>
<td>Naphthyl-acetate esterase</td>
<td>0</td>
</tr>
</tbody>
</table>

Data shown are the mean ± 1 SE of seven experiments.

*Viability was measured by trypan blue exclusion. Morphology was assessed on cytocentrifuge preparations stained with Leishman's stain. Esterase staining was by the method of Yam and Li.\textsuperscript{39}

**Table 2. DNase Activity of HL-60 Cells Before and After Induction of Maturation, Compared With That of Monocytes and Neutrophils**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Solubilization of DNA by Intact Cells* (%) of ingested radioactivity</th>
<th>Cell-Free System Activity of Total Radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-60 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninduced</td>
<td>3 ± 2 (4)‡</td>
<td>3 ± 1 (6)</td>
</tr>
<tr>
<td>DMSO-induced</td>
<td>34 ± 13 (6)</td>
<td>24 ± 11 (10)</td>
</tr>
<tr>
<td>Vitamin D\textsubscript{3}-induced</td>
<td>ND</td>
<td>26, 48 (2)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>28 ± 6 (6)</td>
<td>35 ± 13 (9)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>9 ± 4 (6)</td>
<td>9 ± 8 (6)</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

*Release of 5% TCA-soluble radioactivity from ingested \textit{3H}-thymine labeled \textit{E. coli}. Absolute amount of ingested radioactivity was HL-60 cells, 19,637 ± 8,428; DMSO-induced HL-60 cells 30,064 ± 10,441; monocytes, 23,157 ± 6,768; neutrophils 20,624 ± 7,277 cpm/10\textsuperscript{6} cells.

†Solubilization of purified \textit{14C}-DNA to 5% TCA-soluble fragments at pH 5 by sonicators prepared from 10\textsuperscript{6} cells. Total amount of radioactivity added was 3,985 ± 1,073 cpm.

‡Data are the mean ± 1 SD of (n) experiments. Individual values are given when fewer than three experiments were performed.

$\text{HL-60 cells were induced for 7 to 11 days with 1.25% DMSO or for 7 days with 10 mmol/L 1 \alpha, 25-dihydroxycholecalciferol.}$

From PB

Monocytes and Neutrophils

HL-60 cells

Mono-
28% ± 6% released by monocytes and 9% ± 4% released by neutrophils. Despite acquisition of this monocyte characteristic by DMSO-induced HL-60 cells, they remained α-naphthyl acetate esterase (αNAE)-negative and most were positive for the granulocyte enzyme, chloroacetate esterase (Table 1).

**Characterization of Phagocyte DNAse**

The increase in solubilization of DNA from ingested bacteria during maturation of HL-60 cells is suggestive of an increase in activity of cellular DNAse. A cell-free system was devised to measure the DNAse activity in cell sonicates. The substrate in this assay was purified double-stranded DNA from *E. coli*. First, the pH optimum of DNAse in monocyte sonicates was determined. DNAse activity was highly pH dependent; the optimum was 5, with very little detectable activity between pH 6 and 8. The pH optimum of DNAse in sonicates prepared from DMSO-induced HL-60 cells (day 7 through 10) was then determined and compared with that of monocytes, neutrophils, and cells from the monocytoid cell line, U937 and was identical to that of the other cell types (Fig 2). The DNAse from monocytes, and DMSO-induced HL-60 cells did not show enhanced activity with 1 mmol/L calcium added (monocyte activity, 3,638 cpm 14C-DNA solubilized/10^6 cells without and 3,252 cpm/10^6 cells with 1 mmol/L calcium; activity of DMSO-induced HL-60 cells, 3,819 cpm/10^6 cells without and 3,605 cpm/10^6 cells with 1 mmol/L calcium), and activity was reduced only by 15% to 25% with 1 mmol/L EDTA added. Neither was the DNAse activity affected by 1 mmol/L 2-ME.

**Development of Acid DNAse Activity of HL-60 Cells During Maturation**

Uninduced HL-60 cells contained very little acid DNAse activity as measured in a cell-free system. In six experiments, uninduced cells solubilized only 3% of radioactive DNA (Table 2), levels that were comparable to those of PB neutrophils assayed in parallel. The acid DNAse activity of HL-60 cells increased, however, during DMSO-induced maturation to a maximum in cells induced for 8 to 12 days to reach a value that was about 70% of the activity of PB monocytes. The kinetics of development of DNase were studied in three experiments (Fig 3). Increased DNAse activity was apparent by day 2 and increased further as the cells matured. The DNAse activity in these experiments was quite variable in cells incubated with DMSO for longer than 8 days. HL-60 cells were also induced with dihydroxyvitamin D3 to examine whether cells that showed morphological characteristics of monocytes might have enhanced levels of DNAse activity as compared with those that resembled granulocytes. HL-60 cells induced with vitamin D3 became adherent, and about 70% were αNAE-positive after 7 days, with DNAse levels comparable to those of DMSO-induced HL-60 cells (Table 2). Because cell sonicates were used for these studies, endogenous DNA may have been interfering with the DNAse assay. There was no significant difference between the DNA content of samples from uninduced (4.2 ± 2.4 μg/10^6 cells; mean ± 1 SD, n = 3) vs compared with DMSO-induced cells (3.8 ± 1.5 μg/10^6 cells). Furthermore, control experiments showed that inclusion of this amount of endogenous DNA in the reaction mixture did not interfere with the DNAse activity of PNS containing 1.6 μg DNA/10^6 cells. Therefore, the DNA present in the samples probably did not contribute to the observed difference in DNAse activity between mature and immature cells.

**Kinetics of Development of DNAse and Oxidase Activity**

The ability of HL-60 cells to digest DNA either from intact bacteria or in a cell-free system developed with very
similar kinetics during DMSO-induced maturation and slightly precede development of TPA-stimulated superoxide production, a measure of phagocyte oxidase activity (Fig 4).

Comparison of DNAse Activity of Three Subclones of HL-60 Cells

The data so far presented refer to measurements made on cells derived from a frozen stock of cells from passage 30 of the original clone of HL-60 cells (clone 1, described in the Materials and Methods section). Cells from a second HL-60 subclone from another source (clone 2) gave similar results. A third clone of HL-60 cells was selected for further study because it tended to grow in aggregates rather than in single-cell suspension and had a greater degree of spontaneous maturation. The initial results (Table 3) suggested that DNAse activity was already developed in this unusual subclone because about 40% of DNA was released from ingested E. coli as 5% TCA-soluble fragments by uninduced cells whereas the other two clones had little capacity to digest DNA from intact bacteria in the uninduced state. Subsequent studies showed that the uninduced cells of clone 3 contained very little acid DNAse activity measured in a cell-free system (Table 3) and that acid DNAse activity developed in the cells in typical fashion on maturation with DMSO. Analysis of the pH optimum of DNAse of clone 3 cells showed considerable activity at pH 7 to 8 however (Fig 5), which might account for the digestion of DNA in the uninduced cells. Cells from clone 3 developed acid DNAse activity on induction with DMSO but retained their alkaline DNAse activity (Fig 5). The presence of both acid and alkaline DNAse in mature cells did not confer an enhanced ability to degrade DNA from ingested bacteria, and in three of four experiments shown in Table 3 mature cell function was less than that of uninduced cells.

To confirm that the unusual digestion of DNA by immature HL-60 cells was the result of alkaline DNAse, cells were preincubated for 30 minutes with mepacrine hydrochloride (10 μmol/L). This drug inhibits the digestion of DNA by monocytes probably by alkalinization of lysosomes, which inhibits acid hydrolases. Table 4 shows that although mepacrine enhanced the digestion of bacterial DNA by the alkaline DNAse-positive clone of HL-60 cells (clone 3), that of DMSO-induced cells from clone 1, monocytes, and IFN-γ-induced U937 cells was inhibited, confirming that the DNAse activity of immature HL-60 cells from subclone 3 was attributable to alkaline DNAse activity. In contrast to the acid DNAse present in DMSO-induced HL-60 cells from clones 1 and 2, the activity of the alkaline DNAse in clone 3 was increased threefold when 1 mmol/L calcium was added to the reaction mixture, was inhibited by 90% by 1 mmol/L EDTA and showed a 40% reduction in absence of 1 mmol/L 2-ME.

Subcellular Location of Acid DNAse

The subcellular distribution of acid DNAse in homogenates of purified PB monocytes was analyzed by fractionation on sucrose density gradients. The majority of the DNAse activity (70%) was distributed in the dense region of the gradient (Fig 6). In all of the four subcellular fractionation studies performed, distribution of DNAse in the dense...
### Table 3. Comparison of DNAse Activity of Three Clones of HL-60 Cells

<table>
<thead>
<tr>
<th>HL-60 Clone</th>
<th>Solubilization of DNA by Intact Cells*</th>
<th>Cell-Free System DNAse Activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninduced (% ingested radioactivity)</td>
<td>Uninduced (% total radioactivity)</td>
</tr>
<tr>
<td></td>
<td>Induced§</td>
<td>Induced</td>
</tr>
<tr>
<td>1</td>
<td>2.8 ± 0.8 (4)</td>
<td>38.0 ± 7.8 (4)</td>
</tr>
<tr>
<td>2</td>
<td>4.6 ± 0.6 (4)</td>
<td>34.8, 27.3 (2)</td>
</tr>
<tr>
<td>3</td>
<td>38.8 ± 6.1 (4)</td>
<td>28.0 ± 12.8 (4)</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

*Release of 5% TCA-soluble radioactivity from ingested 3H-thymine–labeled E. coli.
†Solubilization of purified bacterial DNA to 5% TCA-soluble fragments at pH 5 by sonicates prepared from 10⁶ cells. The absolute amount of 14C-DNA added was 4,100 ± 405 cpm.
§Cells were induced to mature by incubation for 7 to 11 days with 1.25% dimethylsulfoxide.

Analysis of a single subcellular fractionation of HL-60 cells induced for 10 days with DMSO (data not shown) indicated distribution of DNase identical to that observed in monocytes. The peak of DNase in the dense region of the gradient was at 1.198 g/mL as compared with 1.202 g/mL for myeloperoxidase and was completely distinct from the distribution of cytochrome c oxidase (peak density 1.170 g/mL). There was a clearly defined peak of activity at 1.142 g/mL similar to that observed in three of four monocyte gradients and a broad shoulder of activity in the light region of the gradient between 1.083 and 1.101 g/mL.

### DISCUSSION

In two subclones of HL-60 cells, we showed that the ability to digest DNA is maturation dependent. Immature HL-60 cells were able to phagocytose heat-killed IgG-opsonized E. coli but could not efficiently solubilize DNA from them. The capacity to digest DNA from intact bacteria developed after

### Table 4. Effect of Mepacrine Hydrochloride on Solubilization of DNA From Ingested Bacteria

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Release of 5% TCA-Soluble 3H-DNA (cpm/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Mepacrine*</td>
</tr>
<tr>
<td>HL-60 subclone 3 (uninduced)</td>
<td>1,733†</td>
</tr>
<tr>
<td></td>
<td>1,870</td>
</tr>
<tr>
<td>HL-60 subclone 1 (DMSO-induced)‡</td>
<td>367</td>
</tr>
<tr>
<td></td>
<td>311</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1,208</td>
</tr>
<tr>
<td></td>
<td>919</td>
</tr>
<tr>
<td>U937 cells (IFN-γ induced)</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>487</td>
</tr>
</tbody>
</table>

*Cells were preincubated either with 10 μmol/L mepacrine hydrochloride (a gift from Boots Chemical, Nottingham, England) or buffer for 30 minutes before incubation with radiolabeled bacteria.
†Data are from duplicate samples from a single experiment.
‡HL-60 cells from subclone 1 were induced to mature for 9 days with 1.25% DMSO.
induction of maturation with DMSO, with kinetics similar to development of acid DNAse activity, measured in a cell-free system. The increase in DNAse activity slightly preceded, and was therefore not dependent on, development of a functional superoxide-generating system. The increase in acid DNAse activity during DMSO-induced maturation is concomitant with the previously described increase in other degradative enzymes such as lysozyme, β-glucuronidase, and β-galactosidase.25

The capacity of DMSO-induced HL-60 cells to digest DNA was similar to that of PB monocytes and much greater than that of neutrophils, which have previously been reported to contain very little DNAse.6,4 This observation emphasizes the potential of the HL-60 cell line to develop characteristics of mononuclear cells while retaining characteristics of granulocytes, such as chloroacetate esterase activity, and DMSO should not be assumed to induce only features characteristic of the neutrophil.

The activity of DNAse in DMSO-induced HL-60 cells was highly pH dependent with an optimum around 5, similar to that of PB monocytes and neutrophils and to that of cells from the monocytoid cell line U937. An unusual clone of HL-60 cells containing considerable alkaline DNAse activity with a pH optimum between 7 and 8 was identified, however. The alkaline DNAse differed from the acid DNAse in that it was dependent on calcium and magnesium and required addition of 1 mmol/L 2-ME for maximal activity. Such an alkaline DNAse was not detectable in two other subclones of HL-60 cells, U937 cells, neutrophils, monocytes (described in the Results section), or cells from several T- and B-lymphocyte cell lines (data not shown), although all these cells contained acid DNAse activity. Although acid DNAse has widespread activity in human tissue and serum, alkaline DNAse is predominantly found only in serum,38 lymphocytes,39 and pancreatic tissue,40 other organs having low or no detectable activity.41 Alkaline DNAse appears not to have been previously described in phagocytes, and its role in these cells is unclear. Its presence in the clone of immature HL-60 cells was associated with the ability to solubilize DNA from ingested bacteria, a function usually associated with mature cells. These cells also acquired acid DNAse activity during DMSO-induced maturation however, so that the mature cells contained both acid and alkaline DNAse. This did not increase the ability of mature cells to solubilize DNA from intact bacteria, perhaps because a mechanism to acidify the phagosome also develops with maturation and reduces the pH of the phagocytic vacuole to below the optimum for sustained alkaline DNAse activity.

The subcellular location of acid DNAse activity in DMSO-induced HL-60 cells was similar to that of monocytes. Acid DNAse showed a bimodal distribution in both cell types. Most of the enzyme had a slightly lighter distribution in the gradient than the dense granules that contained myeloperoxidase. Because studies were not performed on purified granules, however, the exact location of the dense peak of DNAse was not determined. The minor peak of DNAse in the light region of the gradient (density 1.124 to 1.150 g/mL) was distinct from the mitochondrial marker cytochrome oxidase and did not codistribute with adenylate cyclase, a plasma membrane marker. A marker for endoplasmic reticulum, sulfatase c, had a bimodal distribution in the light region of the gradient. The light peak of DNAse coincided with a deep trough between the two peaks of sulfatase c, suggesting accumulation in a different organelle.

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