Inosine Monophosphate Dehydrogenase and Myeloid Cell Maturation

By Robert D. Knight, John Mangum, Diane L. Lucas, David A. Cooney, Ernest C. Khan, and Daniel G. Wright

In previous studies of purine ribonucleotide metabolism in the human myeloid leukemia cell line HL-60, we observed that there is a down-regulation of guanine ribonucleotide biosynthesis from the central intermediate, inosine monophosphate (IMP) and a depletion of intracellular guanosine triphosphate (GTP) and guanosine diphosphate (GDP) pools that occur during the induced maturation of these cells. We also found that inhibitors of IMP dehydrogenase, the enzyme that catalyzes the first step of guanylate synthesis from IMP, are potent inducers of HL-60 maturation.

Because of these observations we specifically investigated the activity of IMP dehydrogenase in HL-60 cells and in a new inducible human myeloid leukemia cell line, RDGD2-25, both during maintenance culture and during induced maturation of the cells. Enzyme activity was examined directly in cell extracts with a radiometric assay that measures free \( ^3\text{H}_{2}\text{O} \) formed from \([2-\text{H}]\text{IMP} \) during the conversion of IMP to XMP. Uninduced HL-60 and RDGD2 cells in maintenance culture were found to have high levels of IMPD activity (5.2 to 5.7 pmol IMP metabolized/10^7 cells/min) compared with normal neutrophils and monocytes that had been purified from blood (<1.5 pmol IMP metabolized/10^7 cells/min). However, when HL-60 and RDGD2-25 cells were induced to mature with retinoic acid (10^{-6} mol/L), dimethylformamide (6 x 10^{-2} mol/L), or a known IMPD inhibitor, tiazofurin (10^{-4} mol/L), IMPD activity in the cells fell by 51% to 80% within three to six hours. These changes in IMPD activity preceded detectable functional and antigenic maturation of the cells by at least 12 hours and were not temporally related to changes in cellular proliferation. These findings are consistent with the concept that the regulation of myeloid cell maturation may be influenced by intracellular concentrations of guanine ribonucleotides because IMP dehydrogenase activity is known to be rate limiting for the production of these nucleotides.

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The neoplastic cells of acute myelogenous leukemia are characterized by an inability to undergo normal terminal differentiation. However, defects of differentiation in leukemic cells are not necessarily complete. Leukemic cells may display variable degrees of maturity in individual patients, and they may retain a capacity for spontaneous maturation when placed in primary culture. Furthermore, a number of continuously maintained, myeloid leukemia cell lines have been established that undergo maturation when exposed to a variety of chemical agents in vitro.

Understandably these cell lines have attracted considerable investigative interest since they appear to provide an opportunity to define basic mechanisms underlying the blockade of terminal differentiation in leukemia.

The HL-60 cell line, originally isolated from the leukemic blasts of a patient with acute promyelocytic leukemia, has been the object of particularly extensive investigation. This cell line can be induced experimentally to undergo a form of terminal differentiation with which the cells acquire functional and morphological characteristics of mature neutrophils. In previous studies of HL-60 cells we showed that discrete changes in the intracellular production and supplies of guanine ribonucleotides guanosine triphosphate [GTP] and guanosine diphosphate [GDP] are associated with induced maturation of these cells. Intracellular concentrations of guanine ribonucleotides were found to fall during HL-60 cell maturation, and this change could be attributed to diminished nucleotide biosynthesis. Furthermore, changes in guanylate production during induced maturation were not accompanied by comparable changes in adenylate production or in intracellular adenosine nucleotide concentrations.

Studies of purine nucleotide biosynthesis using radiolabeled precursors to examine both the de novo and purine salvage pathways during HL-60 cell maturation focused our attention upon the first step of guanylate biosynthesis from the central intermediate inosine monophosphate (IMP), catalyzed by the enzyme IMP dehydrogenase (IMPD). This step appeared to be the point at which guanylate biosynthesis is selectively down-regulated during induced maturation of these cells. We therefore examined the effects of known inhibitors of IMPD upon HL-60 cells and found that these agents (eg, mycophenolic acid, 3-deazaguanosine, and 2′-β-D-ribofuranosylthiazole-4-carboxamide [tiazofurin]) not only selectively inhibit guanylate biosynthesis in the cells, as expected, but also induce the cells to mature.

These findings were of particular interest because of previous reports by others suggesting that IMP dehydrogenase activity in cells may be relevant to neoplastic transformation and/or to cellular maturity. Rat hepatoma cells and human leukemic blasts have been found to express high levels of IMPD activity when compared to normal, differentiated cells of the same tissues. Moreover, such differences in IMPD activity, which distinguish normal from neoplastic cells, could not be explained simply by differences in cellular proliferation.

In the studies reported here, we used a radiometric assay of IMPD to study the activity of this enzyme directly in HL-60 cells and in a new human myeloid leukemia cell line, RDGD2-25.

Cells were studied both in maintenance culture and during induced maturation. Results confirmed our impression that a down-regulation of IMPD occurs early during the induced

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maturation of myeloid leukemia cells, and they lend support to the concept that the intracellular supplies of guanine ribonucleotides, which are determined by the activity of this rate-limiting enzyme for guanylate synthesis, may have a role in the regulation of myeloid cell maturation.

MATERIALS AND METHODS

Human myeloid leukemia cell lines. Continuous suspension cultures of the HL–60 cell line were established initially from seed cultures obtained from the laboratory of Dr Robert C. Gallo (National Cancer Institute, Bethesda, MD). Additional stocks cryopreserved at passage 11 following the original establishment of this line, were obtained from the American Type Culture Collection (Rockville, MD). Cells were maintained in RPMI 1640 medium supplemented either with 10% heat-inactivated (56 °C for 30 minutes) fetal bovine serum (Reheis Chemical, Phoenix, AZ) or with insulin (6 μg/mL), transferrin (5 μg/mL), and selenium (3 μmol/L) (Sigma, St. Louis). In our studies serum-free medium supplemented with insulin, transferrin, and selenium is referred to as ITS medium. Cells were cultured in 75-cm² tissue culture flasks (Falcon Plastics, Becton Dickinson, Oxnard, CA) and incubated at 37 °C in a continuously maintained environment with 7% CO2 and 100% humidity. Cell counts in culture specimens were determined by hemocytometer chamber counting, and the viability of cells was assessed by dye exclusion using 0.1% trypan blue (MA Bioproducts, Walkersville, MD). Morphology of cells was examined with cytospin preparations stained by Wright-Giemsa. For maintenance cultures, cells were passaged by resuspending them in fresh nutrient media at a concentration of 2 x 10⁶ cells/mL every three to five days. Cells were studied at maintenance passages 20 to 30. The RDFD2 cell line was isolated from the peripheral blood blasts of a 56-year-old man with acute myelogenous leukemia (FAB class M1). Shortly after diagnosis the patient died from a cerebral hemorrhage and was found to have diffuse leukemic infiltration of the brain, lymph nodes, and central organs. Leukocytes were obtained for culture at the time of diagnosis when the WBC count was 188,000/µL (71% blasts, 5% promyelocytes or myelocytes, and 2% lymphocytes). Leukemic blasts were peroxidase positive and α-naphthyl butyrate esterase negative. These cells were separated from blood by Hypaque-Ficoll gradient centrifugation and then cultured in RPMI 1640 supplemented with 20% fetal bovine serum (Reheis), ITS, and 10% PHA-stimulated, human lymphocyte-conditioned media (LCM) at a cell concentration of 1 x 10⁶/mL. After three weeks initial culture at 37 °C with 7% CO₂, additional medium was added and then renewed weekly by demidepletion with gradual withdrawal of the fetal bovine serum (FBS) and LCM. By eight weeks cells were continuously maintained (averaging doubling time ~ 56 hours) in RPMI, ITS, and 10% FBS. These cells showed a limited but stable capacity to undergo induced myeloid maturation. This initial RDFD2 line was then cloned by limiting dilution, and derivative lines were isolated, which demonstrated either limited degrees of induced maturation or prominent myeloid maturation when exposed to inducing agents. Two such derivative lines, RDFD2–25 (inducible) and RDFD2–4 (noninducible), were examined in the studies reported here. Like the HL–60 cells, RDFD2 cells were resuspended in fresh nutrient media every three to five days. These cells were studied at maintenance passages 20 to 40.

Induced maturation of HL–60 and RDFD2 cells. Before IMDP levels were studied in leukemic cell lines during induced maturation, the cells were adapted to and maintained in serum-free ITS media for at least three passages. Cells in log-phase growth (two to three days following media change) were induced to mature by inoculation into fresh ITS media supplemented with a variety of inducing compounds. Final concentrations of inducing agents were retinoic acid (Sigma), 1.0 μmol/L; dimethylformamide (Eastman Kodak, Rochester, NY), 60 mmol/L; and 2β-D-ribofuranosylthiazole-4-carboxamide (or tiazofurin, obtained from the Drug Synthesis and Chemistry Branch, DCT, NCI, NIH, Bethesda, MD), 1.0 μmol/L. Induced maturation of the cells was assessed by previously described methods for determining NBT reduction, phagocytosis of opsonized yeast, and reactivity to the monoclonal antibody (MoAb) OKM1 (Ortho Diagnostics, Raritan, NJ) detected by FACS analysis.

For certain studies HL–60 cells were incubated for four days with and without 5 mmol/L α-difluoromethyl ornithine (DFMO) (Merrell Dow, Cincinnati), which arrests cellular proliferation but does not induce maturation. For these studies cells were placed into fresh ITS media with and without DFMO every 24 hours as described previously.

Measurement of IMP dehydrogenase in cell extracts. Cell pellets (containing 5 x 10⁶ to 5 x 10⁷ cells) were disrupted by sonication in 1.0 mL Tris buffer (0.1 mol/L Tris-HCl, 0.02 mol/L KC1, 1.0 mmol/L dithiothreitol, pH 8.1) over ice. Sonicates were then centrifuged at 12,000 x g, and IMPD was extracted from the supernatant by acid precipitation with 0.5 mL of 1.0 mol/L sodium acetate, pH 5.0, added to 1.0 mL of supernatant. After a two-minute repeat centrifugation at 12,000 x g, the pelleted precipitate was redissolved in 500 μL Tris buffer, pH 8.1. Insoluble materials were eliminated by recentrifugation and the supernatant extract used as an enzyme source. Total protein concentrations in cell sonicates and in cell extracts were determined by the Bio-Rad colorimetric method (Bio-Rad Laboratories, Richmond, CA).

IMPD activity in cellular extracts was then measured by a radiometric assay, modified from methods previously reported by Cooney et al. This assay is based upon the recovery of tritiated water produced during the IMPD-mediated generation of XMP from [2-3H]IMP. For the assay 100-μL aliquots of cell extracts were incubated at 37 °C with 50 μL of allipurinol (20 μmol/L); 50 μL of 0.1 mol/L uridine (Sigma), and 5 μL of [2-3H]IMP [200 cpm/mL; 60 mmol/L; and 2-13-D-ribofuranosylthiazole-4-carboxamide (or tiazofurin, obtained from the Drug Synthesis and Chemistry Branch, DCT, NCI, NIH, Bethesda, MD), 1.0 μmol/L. In a total reaction volume of 205 μL the IMPD reaction was terminated after incubation at 23 °C for zero to 120 minutes by applying 100-μL aliquots of the reaction mixtures to replicate 6 x 30 mm Dowex-1-C1⁻ columns (Bio-Rad), followed by elution of the columns with 1.9 mL distilled H₂O and collection of eluants in scintillation vials with 20 mL Hydrofluor (National Diagnostics, Summerville, NJ). In this assay, anion exchange resin columns, which absorb unreacted [2-3H]IMP while H₂O is eluted, were used instead of saturated KOH solution as originally described to separate the reaction products. This modification improved the reproducibility and efficiency of the assay in our hands; nonetheless, results were similar with both versions of the radiometric assay. The radioactivity of released tritium was measured in a beta-scintillation counter and converted to pmol of IMP metabolized per minute. Enzyme activity was expressed both in relationship to the number of cells extracted and to total extracted protein added to the reaction mixture.

The specificity of the dehydrogenation reaction of IMP to XMP was confirmed by reverse phase and ion exchange chromatography, and by comparing reaction conditions with and without added NAD. Breakdown of the radiolabeled IMP to inosine by nonspecific phosphatase activity was minimal (5% of total radioactivity) to undetectable.

Measurement of intracellular purine ribonucleotide concentrations. Purine ribonucleotides in perchlorate extracts of 2 x 10⁶ cell pellets were analyzed by reverse phase HPLC using an anion exchange, microparticulate column (Partisil-10 SAX; Whatman, Clifton, NJ) as described previously. Ribonucleotide (GTP and From www.bloodjournal.org by guest on September 14, 2017. For personal use only.
ATP) concentrations (nmol/10^7 cells) were derived from chromatographic peaks detected by UV absorbance at 254 nm, which were related to standards chromatographed at known concentrations.

**Purification of normal peripheral blood neutrophils and monocytes.** Neutrophils and monocytes were separated from venous blood samples (50 to 100 mL) obtained from normal volunteers. Neutrophils (>95% purity) were isolated by dextran sedimentation of whole blood followed by centrifugation on Hypaque-Ficoll density gradients (Ficoll-Paque; Pharmacia, Piscataway, NJ) and hypotonic lysis of residual erythrocytes. Blood monocytes (>90% purity) were isolated from the buoyant mononuclear cells, recovered from Hypaque-Ficoll gradient-separated blood, by counter-flow elutriation as described previously.

Presentation and statistical analysis of results. Unless otherwise noted, results are expressed as mean values ± the standard error of the mean for replicate measurements. P values for the statistical significance of differences were determined by the Student's t test.

**RESULTS**

**IMP dehydrogenase activity in human myeloid leukemia cells lines and in normal blood leukocytes.** IMPD activity was readily detected in extracts of HL-60 cells harvested from maintenance culture. Dehydrogenation of IMP by extracts of 2 x 10^7 HL-60 cells was linear during the first 20 minutes of incubation with radiolabeled substrate but slowed thereafter. Therefore attention was given to using incubation times and sample dilutions that would conform to linear reaction conditions. Under such conditions IMPD activity was directly related both to the numbers of cells from which extracts were prepared (within a range of 1.0 to 3.0 x 10^7 cells) and to the protein content of cell extracts (within a range of 0.2 to 3.0 mg cell protein).

Consistent with previous reports that leukemic blast cells have higher levels of IMPD than do mature leukocytes, both HL-60 and RDFD2-25 cells were found to have substantially higher levels of IMPD activity than did purified, normal blood neutrophils or monocytes. Levels of IMPD activity expressed as pmol IMP metabolized/10^7 cells/minute (± SEM, n = 6) were 5.7 ± 0.5 and 5.2 ± 0.2 for HL-60 and RDFD2-25 cells respectively, compared with 1.1 ± 0.4 and 1.4 ± 0.4 for neutrophils and monocytes; IMPD levels expressed as pmol IMP metabolized/mg cell protein/min (± SEM, n = 6) were 27.6 ± 2.2 and 20.6 ± 0.8 for HL-60 and RDFD2-25 cells, compared with 4.5 ± 1.7 and 7.5 ± 2.3 for neutrophils and monocytes.

**IMPD-dehydrogenase activity in myeloid leukemia cells during induced maturation.** Substantial decreases in IMPD activity were observed in HL-60 cells by three hours of incubation with agents that induced maturation of the cells (Figs 1A and 1B); these decreases in IMPD activity were apparent both when enzyme activity was expressed in relationship to cell numbers and when expressed in relationship to milligrams of extracted protein assayed. The most rapid decline in IMPD activity was observed in cells treated with tiazofurin; in these cells IMPD levels were decreased by more than 75% within six hours and remained low thereafter (The slight rise in IMPD levels between six and 24 hours apparent in Figs 1A and 1B is not statistically significant). The decline in IMPD activity in cells treated with retinoic acid and dimethylformamide was somewhat more gradual than in tiazofurin-treated cells but was fully established by 12 hours of incubation with the inducing agents. In all cases substantially decreased IMPD levels were detectable at least 12 to 18 hours before cellular maturation was apparent either by measurement of phorbol diester-stimulated NBT reduction, by measurement of the myeloid surface antigen OKM1 (Figs 2B and 2C), or by measurement of phagocytic activity (data not shown).

Changes in IMPD levels during induced maturation of HL-60 cells were not related temporally to changes in cellular proliferation. IMPD levels fell markedly during the first 12 hours of induction (Figs 1A and 1B) before significant changes in the cell growth rate (P < 0.05) were observed (Fig 2A). Furthermore, DFMO, which markedly inhibits HL-60 cell growth but does not induce maturation, was found to cause either decreased IMPD levels in the cells or changes in intracellular guanine ribonucleotide concentrations, unlike the maturation inducers, retinoic acid and dimethylformamide (Table 1).

Decreased IMPD levels in association with induced myeloid maturation were also observed in another human leukemia cell line, RDFD2-25. This line was isolated from a patient with acute myelogenous leukemia, FAB class M1 (see “Materials and Methods” section) and is unrelated to the HL-60 line. As in HL-60 cells, IMPD activity in RDFD2-25 cells was decreased by more than 50% within six hours of incubation with retinoic acid, dimethylformamide, or tiazofurin and remained at these diminished levels during
IMP DEHYDROGENASE AND MYELOID CELL MATURATION 637

was found to be resistant to maturation cloning technique, which was segregated subsequent culture. A separate strain of RDFD2, RDFD2-4, which were response to retinoic acid or dimethylformamide (Table 2), following the eighth passage of this line by a limiting dilution induction. These open squares: cells incubated with tiazofurin (10' mmol/L). Results are mean values from triplicate measurements.

<table>
<thead>
<tr>
<th>Incubation Conditions</th>
<th>IMPD Activity*</th>
<th>Intracellular [GTP] (nmol/10^7 cells)†</th>
<th>Cellular Proliferation (Percent increase in cell concentration by four days)‡</th>
<th>Myeloid Maturation (NBT reduction by cells after four days cultured)§</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>6.3 ± 0.5</td>
<td>12.3 ± 1.1</td>
<td>326 ± 40</td>
<td>12 ± 2</td>
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<tr>
<td>Retinoic acid (10^-4 mol/L)</td>
<td>1.8 ± 0.3</td>
<td>6.8 ± 0.7</td>
<td>74 ± 23</td>
<td>173 ± 12</td>
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<tr>
<td>Dimethylformamide (60 mmol/L)</td>
<td>1.7 ± 0.4</td>
<td>5.4 ± 0.5</td>
<td>95 ± 30</td>
<td>115 ± 11</td>
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<tr>
<td>DFMO (5 mmol/L)</td>
<td>7.7 ± 0.3</td>
<td>12.7 ± 1.5</td>
<td>53 ± 27</td>
<td>7 ± 1</td>
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</table>

*IMPD activity in cells after 24-hour incubation with or without inducing agents, expressed as pmol IMP metabolized/10^7 cells/min (mean ± SEM; n = 6).
†GTP concentrations measured in perchlorate extracts of cell pellets prepared after 24-hour incubation of cells with or without inducing agents; results represent means ± SEM from four replicate studies.
‡Measurements of cellular proliferation represent the percent increases in cell concentrations during four days' culture from an initial seeding concentration of 3 x 10^5 cells/mL; [percent increase = absolute increase in cell concentration / 10^6 (cells/mL)] / 3.0 x 100); results represent means ± SEM; n = 4.
§NBT reduced by 10^6 cells stimulated with phorbol diester (Δ OD units at 595/30 min); mean ± SEM, n = 4.
|Control cells not exposed to inducing agents.|

DISCUSSION

Inosine monophosphate dehydrogenase (IMPD) catalyzes the first step unique to guanylate biosynthesis from the central purine intermediate, IMP, whereby IMP is converted to XMP. This irreversible reaction is known to be subject to both negative and positive feedback controls and to be rate limiting for the formation of guanine ribonucleotides in cells under physiologic conditions. We investigated the activity of this enzyme in two human myeloid leukemia cell lines, HL-60 and RDFD2-25, and in normal peripheral blood neutrophils and monocytes using a radiometric assay. We found both myeloid cell lines to have substantially higher levels of IMPD than purified normal blood neutrophils or monocytes. In addition, we observed that induced maturation of the leukemic cell lines is associated with a reduction in the activity of IMPD toward those levels observed in normal, terminally differentiated neutrophils and monocytes. Moreover, decreased IMPD activity was found to be a very early feature of induced maturation. A significant decline in

Fig 2. (A) Increase in cell numbers during 72-hour culture for HL-60 cells incubated without inducing agents (closed circles), with retinoic acid, 10^-4 mol/L (open circles), with dimethylformamide, 60 mmol/L (open triangles), or with tiazofurin, 10^-4 mol/L (open squares). Results represent mean values ± SEM from four replicate studies; data points marked with an asterisk (*) are significantly different from control data for cells incubated without inducing agents (P < 0.05). (B and C) Acquisition of functional maturity by HL-60 cells incubated with inducing agents: (B) NBT reduction by 10^6 cells stimulated with phorbol myristate acetate (100 ng/mL) for 30 minutes (NBT reduction is expressed as Δ OD units at 595/30 minutes); (C) Percent of cells expressing the OKM1 antigen. Cross-hatched horizontal bars represent limits of mean values ± 2 SD from six separate studies for control, uninduced cells examined at 0, 12. 24, and 48 hours of incubation. Open circles: cells incubated with retinoic acid (10^-4 mol/L); open triangles: cells incubated with dimethylformamide (60 mmol/L); open squares: cells incubated with tiazofurin (10^-4 mol/L). Results are mean values from triplicate measurements.
IMPD activity was evident by three to six hours of incubation of HL-60 and RDFD2-25 cells with inducing agents and preceded the functional and antigenic maturation of these cells by at least 12 to 18 hours. Diminished IMPD activity in HL-60 and RDFD2 cells treated with tiazofurin was not unanticipated, since this agent is known to inhibit IMPD directly and selectively. However, the mechanism by which IMPD is down-regulated in these cells when exposed to retinoic acid or dimethylfor- tiamide is unclear. Nonetheless, these findings are consistent with our previously reported observation that guanine ribonucleotide biosynthesis from IMP is reduced in HL-60 cells during induced maturation in part because of an apparent block at the IMPD-mediated step.

Several additional observations in our present studies lend further weight to the conclusion that reduced levels of IMPD activity and myeloid cell maturation are related. Decreased IMPD levels were not observed in a strain of RDFD2 cells that is resistant to maturation-induction when these cells (RDFD2-4) were incubated with the inducing agents retinoic acid and dimethylformamide. Moreover, changes in IMPD activity observed with cellular maturation could be dissociated from altered rates of cell division. Not only did IMPD levels fall in the myeloid cell lines before the decline in cell growth rate that accompanies induced maturation, but IMPD levels did not fall in cells exposed to DFMO, which blocks cellular proliferation but does not induce matura- tion.

Becker and Lohr have reported previously that the circulating blasts of patients with acute myelogenous leukemia have high levels of IMPD activity compared with those of normal blood leukocytes recovered from healthy subjects. Although our methods for measuring IMPD differ from those used in these earlier studies, our results with HL-60 and RDFD2 cells are quantitatively similar to those reported for freshly isolated leukemic blast cells.

A relationship between the activity of IMPD and neoplastic transformation has been suggested by Jackson et al. These investigators found that rat hepatoma cells had substantially higher levels of IMPD activity than did normal liver cells. IMPD activity in hepatoma cells was also greater than that in normal regenerating liver tissue with similar cell growth rates. Although we were unable to compare the IMPD levels of leukemic cells with those of a comparably homogenous preparation of normal myeloid progenitor cells, our studies support the idea that a high level of IMPD activity in cells is a biochemical marker of cellular immaturity. Not only do HL-60 and RDFD2 cells have high levels of IMPD compared with their normal, terminally differentiated blood cell counterparts, but they also express diminished IMPD activity in association with induced maturation, whereby the block in terminal differentiation characteristic of these neoplastic cells in maintenance culture is circumvented or partially removed. At the same time it is important to point out that the induction conditions examined in our studies are among those that promote a form of cellular maturation reminiscent of neutrophil differentiation. We did not examine induction conditions associated with cellular maturation to macrophage-like or eosinophil-like cells, and our results may not necessarily apply to such conditions.

While diminished IMPD activity may be simply a biochemical consequence of cellular maturation, there is reason to believe that the activity of this enzyme may have a role in regulating the maturation process by controlling intracellular supplies of guanine ribonucleotides. Several lines of evidence support this latter possibility. First, we have shown previously that IMPD inhibitors are potent inducers of maturation in human myeloid cell lines. Second, we have shown in more recent studies that the maturation-inducing effects of IMPD inhibitors on HL-60 cells can be blocked by preventing the depletion of guanine ribonucleotide pools that ordinarily results from IMPD inhibition; this is accomplished by incubating cells with guanosine or guanine to provide the cells with a substrate for guanylate synthesis by direct salvage, bypassing the synthetic pathway from IMP.

In cell-free extracts, IMPD is known to be subject to negative feedback inhibition by XMP and GMP and to require NAD as a cofactor. These characteristics of the enzyme have been used as the basis for designing nucleoside analogues that function as selective IMPD inhibitors, such as tiazofurin. In intact cells, however, this enzyme may be subject to a variety of additional controls at the level of enzyme synthesis, degradation, or posttranslational modification, and it is possible that the changes observed in IMPD

<table>
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<tr>
<th>Table 2. IMP Dehydrogenase Activity, Intracellular GTP Concentrations, and Induced Myeloid Maturation in RDFD2-25 and RDFD2-4 Cells</th>
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<tbody>
<tr>
<td>RDFD2-25</td>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>RA (10⁻⁶ mol/L)</td>
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<tr>
<td>DMF (60 mmol/L)</td>
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<tr>
<td>tiazofurin (10⁻⁴ mol/L)</td>
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<tr>
<td>IMPD activity*</td>
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<tr>
<td>5.2 ± 0.2 (nmol/10⁷ cells)</td>
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<tr>
<td>Intracellular GTP</td>
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<tr>
<td>11.8 ± 1.2 (nmol/10⁷ cells)</td>
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<tr>
<td>NBT reduction‡</td>
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<td>11 ± 6</td>
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<tr>
<td>Phagocytosis§</td>
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<tr>
<td>14 ± 4</td>
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<tr>
<td>RDFD2-4</td>
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<td>RA (10⁻⁶ mol/L)</td>
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<td>tiazofurin (10⁻⁴ mol/L)</td>
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<td>IMPD activity*</td>
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<td>5.3 ± 0.7 (nmol/10⁷ cells)</td>
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<td>Intracellular GTP</td>
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<tr>
<td>11.2 ± 1.5 (nmol/10⁷ cells)</td>
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<td>NBT reduction‡</td>
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<td>12 ± 4</td>
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<tr>
<td>Phagocytosis§</td>
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<td>11 ± 3</td>
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</table>

*Percent of cells capable of ingesting opsonized yeast after four days' incubation with and without inducing agents (mean values ± SEM; n = 4).
‡Percent of cells reducing NBT when stimulated by phorbol diester after four days' incubation with and without inducing agents (mean values ± SEM; n = 4).
§Percent of cells capable of ingesting opsonized yeast after four days' incubation with and without inducing agents (mean values ± SEM; n = 4).
activity in cells treated with retinoic acid or dimethylformamide reflect such control mechanisms. In any case, our studies suggest that such controls of IMPD activity deserve further investigation, since they may be relevant both to normal and to disordered regulation of myeloid cell maturation. Our studies also support the suggestion of others\textsuperscript{11,23} that IMPD is an appropriate target for the design of new chemotherapies that may have selective antineoplastic effects.

REFERENCES

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