Imbalance in the Nucleotide Pools of Myeloid Leukemia Cells and HL-60 Cells: Correlation with Cell-Cycle Phase, Proliferation, Differentiation, and Transformation

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ABSTRACT

Myeloblasts from the blood of patients with chronic myeloid leukemia (CML) in a blastoid crisis were shown to have an imbalance in the ribonucleotide pools compared with normal blood neutrophils. This imbalance includes decreased ratios of purine:pyrimidine, adenine:guanine, and uracil:cytosine nucleotides as well as an increased relative concentration and a changed composition of the uridine diphosphate (UDP) sugars, with relatively more UDP-N-acetylgalactosamines. Similar, more prominent deviations were found in HL-60 promyelocytic leukemia cell line cells. We have used HL-60 cells to investigate the relationships between these changes in the ribonucleotide pools and myelocyte proliferation, maturation, and/or transformation to the malignant state.

When HL-60 cells were separated by elutriation centrifugation into fractions enriched in G1, S phase, or G2 + M, we found only differences in the amount of nucleotides per cell (G1 + M > S phase > G2) corresponding with the increase in cell volume but not in the qualitative composition of the nucleotides. Therefore, throughout this study, the nucleotide content of all cells was calculated per unit of cell volume.

When HL-60 cells were induced to myeloid differentiation with dimethyl sulfoxide, proliferation stopped after 3 days. After 6 days, 70–90% of the cells had matured into cells capable of nitro blue tetrazolium reduction upon stimulation with phorbol myristate acetate. During the maturation process, the mean volume of the HL-60 cells decreased, and the nucleotide content and the purine:pyrimidine and adenine:guanine nucleotide ratios increased. The composition of the UDP sugars changed dramatically, with a decrease of UDP-N-acetylgalactosamines and an increase of UDP-hexoses. Similar changes were detected in HL-60 cells that stopped proliferating without dimethyl sulfoxide-induced maturation, except that the UDP sugar composition showed an increase of UDP-N-acetylglucosamines and a decrease of UDP-hexoses.

Careful examination of these results indicates that the decreased ratio of purine:pyrimidine nucleotides and the decreased ratio of uracil:cytosine nucleotides observed in CML myeloblasts may be regarded as specific changes caused by transformation of myelocytes to the malignant state. The increased amount of UDP-N-acetylgalactosamines and total UDP sugars in the CML cells may also be connected with the transformation process. All other deviations in the nucleotide pattern of transformed myelocytes in comparison to that of mature, normal neutrophils can be explained by the state of proliferation and/or immaturity of CML myeloblasts and HL-60 cells. The abnormal UDP sugar concentrations in CML cells form a rationale for the use of d-glucosamine as cytostatic drug, although not specifically for transformed cells.

INTRODUCTION

Recently, several reports have been published about abnormal purine and pyrimidine metabolism in lymphoid and myeloid leukemic cells compared with normal peripheral lymphocytes and neutrophils, respectively. The abnormalities include enzyme activities (for review, see Refs. 1 and 2) as well as metabolite concentrations (3, 4).

During our own investigations on the purine and pyrimidine nucleotide content of leukemic versus normal cells, we have found an imbalance in the nucleotide pool of myeloblasts from CML patients during a myeloblast crisis. This imbalance might be associated with differences in cell cycle phase, cell proliferation, maturation stage, and/or with the transformation process. To study this question, a model is available in the promyelocytic cell line HL-60 (5); the composition of the nucleotide pool in these cells was comparable to that detected in CML myeloblasts. Because HL-60 cells can be induced to maturation into myelocytes and granulocytes in the presence of polar compounds such as DMSO but also by compounds interfering with the purine and/or pyrimidine metabolism such as 6-thioguanine, hypoxanthine, and inhibitors of IMP dehydrogenase (6–9), we have used the HL-60 cell line to study the imbalance in the nucleotide pool of myeloblasts in more detail.

MATERIALS AND METHODS

Chemicals

The nucleotides used as chromatographic standards were obtained from Sigma Chemical Co. (St. Louis, MO). Human albumin (60 g/liter) and tridistilled water were obtained from CLB (Amsterdam, The Netherlands). Fetal calf serum was obtained from Flow Laboratories (Ayrshire, Scotland) and RPMI 1640 medium with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid from Gibco, Ltd. ( Paisley, Scotland). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden) and Percoll suspensions of different specific gravities were made as described (10). All other chemicals used were of analytical grade and obtained from Merck (Darmstadt, West Germany).

Isolation of Cells

Myeloblasts. Blast cells from patients with CML in a blastoid crisis were obtained from Sigma Chemical Co. (St. Louis, MO). Human albumin (60 g/liter) and tridistilled water were obtained from CLB (Amsterdam, The Netherlands). Fetal calf serum was obtained from Flow Laboratories (Ayrshire, Scotland) and RPMI 1640 medium with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid from Gibco, Ltd. (Paisley, Scotland). Percoll was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden) and Percoll suspensions of different specific gravities were made as described (10). All other chemicals used were of analytical grade and obtained from Merck (Darmstadt, West Germany).

CML Myeloblasts. Blast cells from patients with CML in a blastoid crisis were obtained from 10–20 ml of heparinized blood by centrifugation over Ficoll-Isopaque (specific gravity, 1.077 g/cm3 at 20°C; 290 mosm). The cells from the interface were collected and treated with RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum. This cell suspension was used for immunological typing or as a starting point for the purification of lymphocytes and myeloblasts.

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The abbreviations used are: CML, chronic myeloid leukemia; CLB, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service; DMSO, dimethyl sulfoxide; McAb, monoclonal antibody; NBT, nitro blue tetrazolium; PBS, phosphate-buffered saline (140 mM NaCl, 9.2 mM NaH2PO4, 1.3 mM Na2HPO4, pH 7.4); PMNA, phorbol myristate acetate; STZ, serum-treated zymosan.

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2 To whom requests for reprints should be addressed, at % Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, P.O. Box 9406, 1006 AK Amsterdam, The Netherlands.
sions contained >90% myeloid cells at different stages of maturation. HL-60 Cells. Cultures of HL-60 cells were maintained in humidified air/5% CO₂ at 37°C in RPMI 1640 medium containing glutamine (2 mm), penicillin (100 units/ml), streptomycin (100 μg/ml) and heat-inactivated fetal calf serum (10%, v/v). Cells were kept in exponential growth by dilution to 5 × 10⁶ cells/ml every 2–3 days. For induction of maturation, the cells were diluted 1:2 from a density of 2 × 10⁷ cells/ml 1 day before the addition of DMSO and brought to a density of 5 × 10⁶ cells/ml just before the addition of DMSO (1.25%, v/v). For nucleotide analysis, the medium was changed by centrifugation (500 × g; 7 min; room temperature) and the cells were resuspended in supplemented PBS at a concentration of about 20 × 10⁶ cells/ml (10).

Elutriation Centrifugation. HL-60 cells were separated into fractions with cells at different cell cycle phases by elutriation centrifugation (12). The elutriation medium was supplemented PBS, and the medium flow was kept constant at 17 ml/min during the entire run. The elutriation medium was kept at 10°C and the centrifuge at 15°C. When the cells had been introduced into the elutriation chamber (Beckman JE-6 rotor), the speed of the centrifuge was decreased from 4000–3000 rpm. Thereafter, 15 fractions of 100 ml were collected, with a decrease of the rotor speed of 100 rpm after each fraction.

**Immunological Typing**

CML Myeloblasts. Blast cells with a density <1.077 g/cm³ from patients with CML in a blastoid crisis were characterized for antigenic markers with antibodies (mono- or polyclonal) against hematopoietic differentiation antigens (13) at the Department of Immunohematology of the CLB. Patients with blast cells negative for lymphoid markers (especially the common acute lymphocytic leukemia antigen) but positive for myeloid markers were considered to be suffering from a myeloid blast crisis of their CML.

HL-60 Cells. HL-60 cells were cultured in the presence or absence of DMSO (1.25%, v/v) were fixed with 1% paraformaldehyde for 5 min. A panel of McAbs was used to characterize the differentiated and nondifferentiated state of the cells. This panel consisted of McAbs directed against the nonmorphologic part of the HL-DA complex (Ia), the Fc receptor (CLB FcRgran 1) (14), and the β chain of the LFA-1/Mo-1/p150,95 molecule (CLB-LFA1/1) (15), and the granulo- and monocyte McAbs B2.12, B4.3, B13.9, B2.13, CLBgran 10, and 63D3 (13, 16, 17). All McAbs were used in the indirect immunofluorescence test, with a fluorescein isothiocyanate-labeled goat anti-mouse Ig (G17-15-F, CLB). The percentages of fluorescent cells and the fluorescence intensity were measured on an Epics flow cytometer (Coulter Electronics, Dunstable, United Kingdom).

**DNA Flow Cytometry.** Nuclei for flow cytometric DNA analysis were prepared according to the method of Vindeløv et al. (18). The flow cytometer was an Ortho Spectrum III (Ortho Instruments, Westwood, MA).

Nitro Blue Tetrazolium Slide Test. To judge the capacity of individual cells to generate superoxide after activation by PMA, a slide test with NBT dye was performed as described previously (19). Cells (10⁶/ml) in PBS with 5 mg of albumin/ml, 0.5 mg of NBT/ml, 0.6 mM CaCl₂, and 1.0 mM MgCl₂ were stimulated with PMA (100 ng/ml). Cells that generate superoxide reduce NBT to the insoluble, dark-blue formazan and can therefore be recognized microscopically. At least 200 cells were scored as formazan-positive or -negative and judged morphologically by microscopic examination after May-Grünwald/Giemsa staining (in the same microscopic preparation; see Ref. 19).

**Oxygen Consumption.** The oxygen consumption of the cells was measured with a Clark-type electrode (20). In contrast to normal blood neutrophils, HL-60 cells were stimulated with a combination of PMA (100 ng/ml) and STHZ (1 mg/ml), because this combination was found to enhance the oxygen consumption of the DMSO-induced HL-60 cells to a higher degree than either stimulus alone.

**Morphology and Viability of the Cells.** Morphological examination of the cell suspensions was performed on cytopsin preparations (Shandon-Elliot centrifuge) stained with May-Grünwald/Giemsa (minimally 200 cells scored). The relative cell volume was defined as the channel fluorescence test, with a fluorescein isothiocyanate-labeled goat anti-mouse (G17-15-F, CLB). The percentages of fluorescent cells and the fluorescence intensity were measured on an Epics flow cytometer (Coulter Electronics, Dunstable, United Kingdom).

The CML myeloblasts and the HL-60 cells also showed deviations in the amount and the composition of the UDP sugars (Table 1 and Fig. 1). Again, the deviations were more pronounced in the HL-60 cells than in the CML myeloblasts.

In principle, the imbalance in the content and composition of the nucleotide pools in CML myeloblasts as compared to normal neutrophils may be caused by the fact that the CML cells constitute a population of dividing cells, by the more immature character of the CML cells, or by the transformed state of the CML cells. Based on the similarity of the imbalance found in CML myeloblasts and HL-60 cells, we have used the HL-60 cell line to investigate the possible reasons for this imbalance, because HL-60 cells can be forced into a condition of nondivision as well as into a more differentiated but still transformed state.

**Cell Cycle Studies**

First, a possible relationship between the imbalance in the nucleotide pool and a specific phase of the cell cycle was investigated. HL-60 cells were separated into 15 fractions by means of elutriation centrifugation (Fig. 2). The total recovery of about 70% is normal for elutriation experiments. The first six fractions contained only cell debris and dead cells. From fractions 7 through 15, the size of the cells increased, with the mean volume of the cells in fraction 15 being more than twice that of the cells in fraction 7. The viability of the HL-60 cells was >90% in fractions 7–14 and 85% in fraction 15. Mitoses were only observed in cells of fractions 13–15. DNA histograms of
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Table 1  Nucleotide pattern of myeloblasts from CML patients, neutrophils from healthy donors, and HL-60 cells under various growth conditions

Nucleotides (pmol/unit of cell volume) were measured in neutralized perchloric acid extracts of myeloid cells with a high-performance liquid chromatography system as described under "Materials and Methods." The results are given as mean ± SE of n experiments. Statistical evaluation was performed with Student's t test; values are given only if significant. Asterisk between columns indicate levels of significance between values in the respective columns; asterisks after the last column indicate levels of significance between normal neutrophils and DMSO-induced HL-60 cells.

<table>
<thead>
<tr>
<th></th>
<th>CML myeloblasts (n = 13)</th>
<th>Normal neutrophils (n = 10)</th>
<th>Exponentially growing (n = 8)</th>
<th>Five paired experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Noninduced</td>
<td>DMSO induced</td>
</tr>
<tr>
<td>AMP + ADP + ATP</td>
<td>57.7 ± 8.2 **</td>
<td>95.3 ± 2.7 **</td>
<td>57.0 ± 2.8 **</td>
<td>78.4 ± 3.9 *</td>
</tr>
<tr>
<td>GMP + GDP + GTP</td>
<td>20.3 ± 1.5 **</td>
<td>27.0 ± 1.1 **</td>
<td>18.3 ± 0.7 **</td>
<td>23.6 ± 0.8</td>
</tr>
<tr>
<td>UDP + UTP</td>
<td>10.4 ± 2.2 *</td>
<td>9.5 ± 0.4</td>
<td>15.9 ± 1.3</td>
<td>13.5 ± 0.9</td>
</tr>
<tr>
<td>CDP + CTP</td>
<td>2.8 ± 0.3 **</td>
<td>2.2 ± 0.1</td>
<td>6.9 ± 0.4 **</td>
<td>6.0 ± 0.5</td>
</tr>
<tr>
<td>UDPNAG°</td>
<td>5.8 ± 0.2 **</td>
<td>4.0 ± 0.2</td>
<td>8.7 ± 1.4 **</td>
<td>13.8 ± 1.9</td>
</tr>
<tr>
<td>UDPG</td>
<td>2.4 ± 0.5 *</td>
<td>3.6 ± 0.2</td>
<td>4.3 ± 0.3</td>
<td>4.2 ± 0.5 **</td>
</tr>
<tr>
<td>Total nucleotides</td>
<td>99.4 ± 11.4 **</td>
<td>142.5 ± 4.3</td>
<td>111.1 ± 3.4 **</td>
<td>139.5 ± 6.3</td>
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<tr>
<td>Purine:pyrimidine ratio</td>
<td>6.0 ± 0.4 **</td>
<td>10.7 ± 0.3</td>
<td>3.6 ± 0.2 **</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>Adenine:guanine ratio</td>
<td>2.0 ± 0.2</td>
<td>3.5 ± 0.1</td>
<td>3.0 ± 0.3</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Uracil:cytosine ratio</td>
<td>3.6 ± 0.4 *</td>
<td>4.3 ± 0.2</td>
<td>2.3 ± 0.2</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>UDPNAG:UDPG ratio</td>
<td>2.4 ± 0.5 *</td>
<td>1.1 ± 0.1</td>
<td>2.1 ± 0.3</td>
<td>3.3 ± 0.2 **</td>
</tr>
<tr>
<td>UDP sugars</td>
<td>8.1 ± 0.5 **</td>
<td>5.3 ± 0.2</td>
<td>11.8 ± 0.8</td>
<td>12.9 ± 1.3</td>
</tr>
<tr>
<td>Total nucleotides (pmol/10⁶ cells)</td>
<td>1504 ± 134 ± 69</td>
<td>2033 ± 69</td>
<td>4297 ± 66</td>
<td>2998 ± 173</td>
</tr>
<tr>
<td>Mean cell volume (top channel no.)</td>
<td>15 ± 1</td>
<td>14 ± 1</td>
<td>40 ± 3</td>
<td>21 ± 1</td>
</tr>
<tr>
<td>Mean protein content (µg/10⁶ cells)</td>
<td>71 ± 5</td>
<td>70 ± 3</td>
<td>140 ± 8</td>
<td>76 ± 5</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.005.

UDPNAG, UDP-N-acetylglucosamine and/or UDP-N-acetylgalactosamine; UDPG, UDP glucose and/or UDP galactose.

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Fig. 1. Relative concentrations of the various nucleotide species in CML cells (C), neutrophils (N), exponentially growing HL-60 cells (E), and HL-60 cells cultured for 6 days without medium refreshment in the absence (−) or presence (+) of DMSO (1.25%, v/v). Concentrations are calculated as a percentage of the total nucleotide content (sum of adenine, guanine, uridine, and cytidine nucleotides and UDP sugars). PU/PY, purine:pyrimidine nucleotide ratio; A/G, adenine:guanine nucleotide ratio; U/C, uracil:cytosine nucleotide ratio; UDPNAG/UDPG, ratio of UDP-N-acetyhexosamines over UDP hexoses.

Fig. 2. Elutriation pattern of HL-60 cells. At a constant medium flow of 17 ml/min, the rotor speed was step-wise diminished from 3000–1600 rpm, yielding 15 fractions. Mean ± SD (bars) of three experiments with a recovery of 70 ± 2%. □ viable cells; □ nonviable cells; cell volume, peak of the size distribution on the Coulter Channelizer.

showed that fractions 7–10 contained cells mainly in G1 phase, that fractions 11–13 were enriched with cells in S phase, and that fractions 14 and 15 contained mainly cells in G2 + M. Nucleotide analysis of unfraccionated HL-60 cells and of the cells in fractions 7–15 showed an increasing nucleotide content, correlating with the increase in cell volume (Fig. 3) and protein content (not shown). The composition of all nucleotide pools was similar in all analyzed cell fractions (only the purine:pyrimidine ratio is shown in Fig. 3). Thus, the imbalance in the ribonucleotide pool of HL-60 cells is not associated with a specific cell cycle phase of growing cells.

Differentiation Studies

Cell Morphology and Function. Next, the relationship between the imbalance in the nucleotide pool and the maturation stage and/or the transformed character of the HL-60 cells was investigated. HL-60 cells were induced to myeloid differentiation with DMSO (1.25%, v/v). Because differentiation leads to cessation of proliferation, nonproliferating HL-60 cells (cells
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in stationary phase) were also studied to distinguish between changes associated with cell proliferation and those associated with maturation. For the differentiation experiments the HL-60 cells were diluted from stationary phase cultures 24 h (day -1) before addition of DMSO. The cell number increased only minimally during these 24 h, but exponential growth was observed from day 0–day 3, both in the absence and presence of DMSO (Fig. 4). DMSO induced a decrease in the mean cell volume within 24 h, whereas in the absence of DMSO the mean cell volume started to decrease only after 48 h (Fig. 4). During exponential growth the increase in cell number was slightly but significantly faster in the presence of DMSO. The viability of the HL-60 cells started to decrease, both in the presence and absence of DMSO, after 3 days in culture without medium refreshment. After 6 days without medium refreshment, the viability of the cells under both conditions was still >85%. At that time both induced and noninduced cells were found to be predominantly in G1 and/or G1-early S phase. The cell concentration decreased from 1.5 x 10⁶ cells/ml after 3 days to 1.0 x 10⁶ cells/ml after 6 days.

Differentiation of HL-60 cells in the presence of DMSO was proven by the ability of these cells to show an oxidative burst after stimulation with PMA plus STZ, with a maximum response after 6 days (Table 2). The morphology of the DMSO-treated HL-60 cells changed from promyelocytic to myelocytic after 6 days, with already a few band-form and polymorphic nuclei. After prolonged culture (10 days) in the presence of DMSO, the percentage of cells with band-form or polymorphic nuclei increased, and the cells could be stimulated to oxygen consumption with either PMA or STZ. Immunological typing of DMSO-induced HL-60 cells showed the appearance of some relatively mature markers on the cell membrane of 40–60% of the cells; this was not observed with noninduced resting or proliferating HL-60 cells. These markers, recognized by the McAbs CLB-LFA1/1, B2.12, and 63D3, are not specific for the myeloid or monocytic lineage. McAb B4.3 reacted with 100% of induced and noninduced (resting and proliferating) HL-60 cells, whereas for the other McAbs that were used (see “Materials and Methods”) no significant reaction was found with the different types of HL-60 cells.

**Nucleotide Measurements**

The total nucleotide concentration per unit of cell volume of both induced and noninduced HL-60 cells increased during culture without medium refreshment, probably reflecting the increase in cells in G0 phase (Fig. 5). Because of the low cell number and the low viability, no samples were analyzed after day 6. The most pronounced changes compared to exponentially growing HL-60 cells were increases in the adenine nucleotides, both absolute and relative to the total nucleotide content (Fig. 1), in the absolute amount of guanine nucleotides (Table 1) and in the purine:pyrimidine ratio (Table 1).

The kinetics of the changes in the relative concentrations are

![Fig. 3. Nucleotide content (sum of adenine, guanine, uracil, and cytosine nucleotides) (*) and ratio of purine:pyrimidine nucleotides (■) of fractionated and unfractionated HL-60 cells. Mean value of three experiments. ■, □, Values for unfractionated cells. nr, number.](image)

![Fig. 4. Cell growth in the presence or absence of DMSO (1.25%, v/v). Representative experiment from a series of five with 10% variation. □, viable cells; □, nonviable cells. Cell volume, peak of the size distribution on the Coulter Channelizer.](image)

![Fig. 5. Concentrations of the total intracellular nucleotides in HL-60 cells cultured in the absence (*) or presence (■) of DMSO (1.25%, v/v). Points, average of five paired experiments ± SE (bars). The value on day 2 (•) was obtained from the experiments with exponentially (exp.) growing cells shown in Table 1. * significant difference (P < 0.0005) between values for induced and noninduced cultures. Statistical analysis was performed with Student’s t test for grouped values with paired variance estimate.](image)

### Table 2 Induction of HL-60 cell differentiation by DMSO

<table>
<thead>
<tr>
<th></th>
<th>With DMSO</th>
<th>Without DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 3</td>
</tr>
<tr>
<td><strong>O₂ consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At rest</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>With PMA + STZ</td>
<td>1.3</td>
<td>3.4</td>
</tr>
<tr>
<td><strong>NBT reduction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At rest</td>
<td>0–2</td>
<td>0–2</td>
</tr>
<tr>
<td>With PMA</td>
<td>0–2</td>
<td>30–40</td>
</tr>
</tbody>
</table>

**Materials and Methods**

Statistical analysis was performed with Student’s t test for grouped values with paired variance estimate.
cultured in the absence (•) or presence (○) of DMSO (1.25%, v/v). Points, average of five paired experiments ± SE (bars). Values on day 2 (○) were obtained from the experiments with exponentially (exp.) growing cells shown in Table 1. Pu/Py, purine/pyrimidine nucleotide ratio; A/G, adenine:guanine nucleotide ratio; U/C, uracil:cytosine nucleotide ratio; *, significant difference between values for induced and noninduced cultures. Statistical analysis performed with Student's t test for grouped values with paired variance estimate.

The experiments with exponentially growing (exp.) cells already 1 day after addition of DMSO (Fig. 6). In the presence of DMSO, these values were significantly different from the undifferentiated and exponentially growing cells already 1 day after addition of DMSO (Fig. 7).

DISCUSSION

Nucleotide Pools and Cell Cycle Phase. The elutriation centrifugation experiments showed that the size of the nucleotide pools in (proliferating) HL-60 cells correlates closely with the cell size (Fig. 3). Therefore, we have expressed all nucleotide values per unit of cell size. Although we did not find differences in nucleotide contents between HL-60 cells in different cell cycle stages (G1, S phase, G2, or M), we did find changes when these cells were put into maintenance culture without medium refreshment (“arrested growth”), accompanied by an increase in cells in G0.

Nucleotide Pools and Proliferation. Proliferating HL-60 cells contain a decreased amount of adenine and guanine nucleotides and an increased amount of uracil and cytosine nucleotides in comparison to growth-arrested HL-60 cells (Table 1). This changed composition of the ribonucleotide pool in proliferating cells might be explained by the increased requirement for the various nucleotides as precursors for RNA and DNA synthesis and for cellular regulation processes associated with growth. Chou et al. (23) have described an obligatory increase in the concentration of UTP for the activation of quiescent 3T3 cells to DNA synthesis.

Our results with HL-60 cells showed that proliferation is associated with low concentrations of UDP-N-acetylhexosamines (Fig. 7). Krug et al. (24) have described for human colon cancer cells (HT29) an association of the antiproliferative effect of d-glucosamine (25) with an increase in the concentration of UDP-N-acetylhexosamines. d-Glucosamine might therefore be useful as part of the chemotherapeutic regimen in myeloid leukemia.

Nucleotide Pools and Differentiation. Treatment with DMSO induces exponentially growing HL-60 cells to mature into myelocytic cells. After 6 days with DMSO, the concentration and composition of the nucleotide pools, except for the uracil and cytosine nucleotides, are more in agreement with those of mature neutrophils than before the initiation of maturation (Table 1). The composition of the UDP sugars (UDP-N-acetylgalactosamine and/or UDP-N-acetylglucosamine; UDP glucose and/or UDP galactose ratio) in the differentiated HL-60 cells on day 6 is also more in agreement with that of mature neutrophils than that of noninduced HL-60 cells, although the concentration of these sugars is still higher (Table 1).

During differentiation of HL-60 cells a decreased glycosylation of membrane proteins has been described (26), which might be related to changes in the metabolism and intracellular pools of UDP and other nucleotide sugars. For example, changes in activities of glycosyltransferases during differentiation have been described for HL-60 cells by Liu et al. (27); especially, the increased activity of N-acetylglucosaminyl transferase might be responsible for the changes in the UDP sugar composition detected during differentiation of these cells (Table 1). The decreased glycosylation associated with differentiation of HL-60 cells seems to be a prerequisite for differentiation, because inhibitors of glycosylation also induce HL-60 cells to differentiate (28, 29). Changed activities of nucleotide-sugar hydrolyzing enzymes in transformed cells, as described by Sela et al. (30) for embryonic cells of the golden hamster, might also be
Nucleotides in Myeloblasts

Table 3 Changes in ribonucleotide pattern due to myelocyte proliferation, immaturity, or transformation

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Proliferation (exponentially growing vs. arrested HL-60 cells)</th>
<th>Immaturity (arrested vs. DMSO-induced HL-60 cells)</th>
<th>Transformation (DMSO-induced HL-60 cells vs. mature neutrophils)</th>
<th>CML myeloblasts vs. mature neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenine nucleotides</td>
<td>↓†</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Guanine nucleotides</td>
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<td>Uracil nucleotides</td>
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<td>Total nucleotides</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>% adenine nucleotides</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>% guanine nucleotides</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>% uracil nucleotides</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>% cytosine nucleotides</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>% UDPNAG</td>
<td>↓†</td>
<td>↓†</td>
<td>↓†</td>
<td>↓†</td>
</tr>
<tr>
<td>% UDPG</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>% UDP sugars</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Ratio of purine:pyrimidine</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Ratio of adenine:guanine</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Ratio of uracil:cytosine</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Ratio of UDPNAG:UDPG</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
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</tr>
</tbody>
</table>

* UDPNAG, UDP-N-acetylglucosamine and/or UDP-N-acetylgalactosamine; UDPG, UDP glucose and/or UDP galactose.

Responsibilities for changes in the UDP sugar content.

Taken together, the results concerning the changes in the concentrations of the UDP sugars during proliferation and differentiation suggest a physiological role for the UDP sugars in the regulation of growth and differentiation.

Nucleotide Deviations Specific for Myelocyte Transformation.

Thus, for changes in the nucleotide pools concurring with myelocyte proliferation, we have compared the values found in exponentially growing HL-60 cells with those found in HL-60 cells under arrested growth. For changes in the nucleotide pools concurring with myelocyte maturation, we have compared the values found in HL-60 cells under arrested growth without and with DMSO, and for changes in the nucleotide pools concurring with myelocyte transformation, we have compared the values found in DMSO-induced HL-60 cells with those found in mature neutrophils, assuming that the state of transformation is maintained during cell differentiation. The results are schematically summarized in Table 3. For comparison, the differences between CML myeloblasts and mature neutrophils have been added to Table 3.

Transformation to the malignant state is associated with a decrease in the absolute amounts of adenine and guanine nucleotides. However, such changes are also found in proliferating and/or immature cells and therefore are not specific for the transformation process. Transformation is also accompanied by a strong increase in uracil and cytosine nucleotides, but this was observed only to a minor extent in CML myeloblasts. Perhaps mature neutrophils have undergone additional changes in their nucleotide composition in comparison with DMSO-induced HL-60 cells, resulting in additional decreases in uracil and cytidine nucleotides.

The changes observed in the fractional amounts of the various nucleotides in CML myeloblasts are completely similar to those observed in proliferating HL-60 cells and thus probably due to the low number of cells in G0 but not related to the immature character of CML cells (Table 3). However, because cell transformation is accompanied by opposing effects on the levels of purine and pyrimidine nucleotides, the ratio of purine:pyrimidine nucleotides is strongly decreased in DMSO-induced HL-60 cells as well as in CML myeloblasts in comparison to mature neutrophils. This effect may be specific for myelocyte transformation, because it is not observed as a result of immaturity and only to a minor extent as a result of proliferation (Tables 1 and 3). Similarly, the ratio of uracil:cytosine nucleotides is decreased in DMSO-induced HL-60 cells and CML myeloblasts in comparison to mature neutrophils, but hardly in noninduced HL-60 cells (Tables 1 and 3). Thus, also the decrease in the ratio of uracil:cytosine nucleotides may be specific for myelocyte transformation.

The increase in absolute and relative amounts of UDP-N-acetylhexosamines and in the percentage of total UDP sugars found in CML myeloblasts in comparison to mature neutrophils may be due to either the immature or the transformed character of the CML cells or to both (Table 3). However, the strong increase in the ratio of UDP-N-acetylhexosamines:UDP-hexoses in the CML blasts must be a consequence of immaturity, because this phenomenon was not induced by transformation or proliferation (Table 3). Transformation and immaturity have opposing effects on the fraction of UDP-hexoses, perhaps resulting in similar values for this parameter in CML myeloblasts and mature neutrophils.

In conclusion, we consider certain deviations in the ribonucleotide pools of CML myeloblasts as specific changes caused by the transformed character of these cells. These deviations are the decreased ratios of purine:pyrimidine nucleotides and uracil:cytosine nucleotides. The increase in UDP-N-acetylhexosamines and in total UDP sugars may also be (partly) caused by the transformation process. All other deviations from the nucleotide pattern found in mature neutrophils can be explained by the state of proliferation and/or immaturity of the CML myeloblasts.

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Imbalance in the Nucleotide Pools of Myeloid Leukemia Cells and HL-60 Cells: Correlation with Cell-Cycle Phase, Proliferation, Differentiation, and Transformation

Dirk de Korte, Willem A. Haverkort, Martin de Boer, et al.


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