Distribution and Content of Nuclear and Cellular RNA among Cell Populations of Acute Lymphoblastic and Nonlymphoblastic Leukemia

Alexander J. Walle

ABSTRACT

The RNA content of intact cells and isolated nuclei of normal human lymphocytes and mononuclear cell populations (containing at least 50% blasts) from patients with acute lymphoblastic leukemia (ALL) and acute non-lymphoblastic leukemia (ANLL) was measured by flow cytometry based on metachromatic red luminescence of acridine orange-stained cells. Relative nuclear RNA (n-RNA) and cellular RNA (c-RNA) content was estimated in relation to luminescence of RNase-treated nuclei, which served as a standard. The mean values (±SE) of n-RNA were 22.6 ± 3.2, 25.8 ± 3.2, and 51.5 ± 6.1 arbitrary units for normal lymphocytes, ALL, and ANLL cell populations, respectively. The mean values for c-RNA were 51.3 ± 5.2, 71.9 ± 11.3, and 128 ± 13.4 for the same cell populations, respectively. The differences between normal lymphocytes or ALL and ANLL cell populations were statistically significant (t-test, with respect to both n- and c-RNA), while differences between normal and ALL populations were not. The proportions of n-RNA versus c-RNA were similar within all three types of cell populations. The intercellular variabilities with respect to n- and c-RNA among the G0/G1 cell populations of all three types of cells were characterized by the coefficient of variation (CV) of the mean RNA and the third moment about the mean (MOM3). CV and MOM3 of c-RNA were significantly different between all three types of cell populations, whereas CV and MOM3 of n-RNA showed significant differences between control and leukemia cells. Thus, mean RNA, CV, and MOM3 of RNA on the cellular and nuclear levels of G0/G1 cells discriminate normal lymphocytes, ALL lymphoblasts, and ANLL blast cells from each other fully on statistically significant levels.

INTRODUCTION

There are significant differences in total cellular RNA content between various types of leukemia. In particular, the RNA content of individual blast cells of ANLL often is markedly higher than that of the cells of ALL (1). In those studies total cellular RNA was measured, which mostly reflects the content of ribosomal or polysomal RNA and thus the translational potential of the cell. Nuclear RNA, however, is closely related to the rate of DNA transcription, RNA processing, and RNA transport. This parameter may thus be a very sensitive marker of changes in genome transcription and may react more rapidly to any metabolic change which affects transcription than does total cellular RNA content. We describe presently the RNA content of isolated nuclei of normal lymphocytes, ALL, and ANLL cell populations. Because various species of RNA with different turnover rates are present in nuclei and cytoplasm, respectively, it is of interest to compare cells with respect to their nuclear versus cellular RNA content as well. The nucleoli are very prominent and more frequent in blast cells of ANLL than ALL; this morphological feature points toward possible differences in n-RNA.

MATERIALS AND METHODS

Heparinized venous blood (n = 13) and bone marrow aspirate (n = 4) samples obtained from previously untreated patients with ANLL (n = 8) and with ALL (n = 9) were depleted of erythrocytes and mature myeloid cells by a Ficoll gradient centrifugation method (2). Only samples containing more than 50% blast cells according to morphological evaluation were processed further. The nuclei were isolated in 1.5% citric acid, as described in detail (3), from aliquots containing 1 × 10^7 cells.

The efficiency of nuclear isolation was controlled by phase contrast microscopy. Either whole cells or isolated nuclei were stained with acridine orange using a procedure designed to differentially stain DNA versus RNA (4–6). The green fluorescence (F530) and red luminescence (F600) of so stained cells or nuclei reflecting stainability of DNA and RNA, respectively, was measured by flow cytometry using an FC200 flow cytometer (Ortho, Westwood, MA), as described (4). Incubation of nuclei with RNase A (Worthington Biochemical Corp., Freehold, NJ; 10^3 units per ml) resulted in marked loss of F600. The remaining non-specific F600 [R, which represents 20–50% of total red luminescence of nuclei, depending on cell type, and is due to DNA-derived spectrum overlap (7)] served as the control base line value to which the RNase-specific red luminescence component was related. Thus, the RNase-sensitive portion of F600 of isolated nuclei or whole cells of the G0/G1 cells that cell cycle is expressed as arbitrary units of F600 representing the relative amount of RNA (Equation A). G0/G1 cells (nuclei) were gated on green fluorescence (DNA content) (8, 9), and their red luminescence was estimated. Thus, the data presented refer only to G0/G1 cell populations. Because RNA content varies during the cell cycle (9), especially during progression through S and G2 phases, measurements of G0/G1 cells therefore are less affected by the cell cycle distribution than are measurements of the whole population.

The computer-generated histograms of red luminescence distributions of c-F600, n-F600, and R of each measurement were evaluated with respect to the mean channel number (mean RNA), the standard error of the mean, the percentage of standard deviation (CV), and the third moment about the mean as a test of skewness. We calculated the mean c-RNA from the mean channel number of c-F600 and R of each individual patient sample as follows:

\[
\frac{(c-F600 - R)}{R} \times 100 = c-RNA AU
\]
NUCLEAR RNA OF LEUKEMIC BLAST CELLS

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 15)</th>
<th>ALL (n = 9)</th>
<th>ANLL (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNA (AU)</td>
<td>22.58 ± 3.16</td>
<td>25.80 ± 3.23</td>
<td>51.48 ± 6.10</td>
</tr>
<tr>
<td>CV (%)</td>
<td>7.38 ± 0.33</td>
<td>11.81 ± 1.12</td>
<td>12.91 ± 1.02</td>
</tr>
<tr>
<td>MOM3</td>
<td>22.41 ± 4.14</td>
<td>122.23 ± 60.48</td>
<td>149.00 ± 51.86</td>
</tr>
<tr>
<td>RNA (AU)</td>
<td>51.25 ± 5.24</td>
<td>71.93 ± 11.29</td>
<td>128.12 ± 13.38</td>
</tr>
<tr>
<td>CV (%)</td>
<td>15.28 ± 0.43</td>
<td>21.91 ± 1.86</td>
<td>28.66 ± 2.82</td>
</tr>
<tr>
<td>MOM3</td>
<td>165.00 ± 26.83</td>
<td>539.92 ± 87.37</td>
<td>1294.80 ± 302.87</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Table 2

Comparisons of RNA parameters of nuclei and whole cells between and within groups of diseases

P-values for comparisons between groups (ALL, ANLL, and control) and P-values for comparisons of cellular and nuclear RNA within groups are listed. The compared mean values are those of Table 1. All probabilities are smaller than the indicated P-values.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CONT</th>
<th>ALL</th>
<th>ANLL</th>
<th>ALL vs. CONT</th>
<th>ANLL vs. CONT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean RNA</td>
<td>CONT 0.000</td>
<td>ALL vs. CONT 0.007</td>
<td>ANLL vs. CONT 0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean CV</td>
<td>CONT 0.001</td>
<td>ALL vs. CONT 0.001</td>
<td>ANLL vs. CONT 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean MOM3</td>
<td>CONT 0.001</td>
<td>ALL vs. CONT 0.001</td>
<td>ANLL vs. CONT 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* c, cellular RNA; n, nuclear RNA; CONT, normal control lymphocytes; NS, not significant.

RESULTS

Nuclear RNA represents less than one-half of the total RNA content of the measured cells, both in the two leukemia groups and in the control lymphocytes (Table 1). The whole cells and isolated nuclei of patients with ANLL contain, on an average, twice as much RNA as do the representative cells or nuclei of patients with ALL. The differences in mean n- and c-RNA content between both groups of leukemic cells as well as between ANLL and control cells are statistically significant (Table 2).

Chart 1 shows RNA profiles of cells and nuclei of cases of ALL (68% blast cells) and APL (90% blast cells) as a type of ANLL. The G0/i cells of this APL cell population are heterogeneous, displaying two distinct populations, whereas the ALL cell population shows a monophasic pattern. The n-RNA distribution in the APL population is not as homogenous as is that of the ALL population. This heterogeneity in c-RNA patterns was often found in ANLL.

Analysis of the RNA histograms of the G0/i cell populations of all three groups of cell types revealed that the shapes of these histograms carried group-specific significance. On the cellular level, the mean values of the CV (Table 1) and MOM3 (Table 1) of the F600 histograms were significantly different between all three groups of cells (Table 2). On the nuclear level, CV and MOM3 (Table 1) of control lymphocytes were significantly different from those of ALL and ANLL cell populations, but CV and MOM3 were not significantly different between ALL and ANLL (Table 2). The shapes of the c-F600 histograms are significantly different from those of the n-F600 histograms within each of the three types of cells.

DISCUSSION

We report here that blast cell populations of the G0/i phase of the cell cycle from patients with ALL as compared to ANLL differ significantly with respect to RNA content of both isolated nuclei and whole cells. Differences in total c-RNA have been described previously (1, 9). It should be emphasized that the actual differences between normal lymphocytes and the blast cell populations are even higher than are those estimated presently, be-
cause the statistical analysis of the ALL and ANLL groups is based on RNA values which include mixed populations, which may contain up to almost 50% non-leukemic cells.

Also, the intercellular distribution of RNA among G0/G1 cells of normal, ALL, and ANLL cell populations appears to be characteristic of each type of cell in so far as each type of population had its definite pattern of heterogeneity. Thus, the RNA content of control cells had a comparatively tight distribution (CV = 15.28%) and a small degree of skewness (MOM3 = 165.00), whereas the distributions of RNA in ALL (CV = 21.91%; MOM3 = 539.92) and ANLL (CV = 28.66%; MOM3 = 1294.80) were significantly more heterogeneous (Tables 1 and 2). These differences in the population heterogeneity hold also for n-RNA when comparing ALL and ANLL nuclei with control nuclei. Thus, the mean c-RNA and the mean n-RNA content and the MOM3 of the cellular (and partially nuclear) RNA content all seem to constitute features characteristic of each type of cell population. The biological relevance of n-RNA is evident when one considers that n-RNA is a reflection of transcription activity of the cell. It is related to cell kinetic activity. Clinical relevance, on the other hand, is apparent since many drugs, especially intercalators, affect nuclei through DNA transcription as well as transport and processing of n-RNA. Also, mean RNA values and RNA histograms differ in various situations; they are different in plateau and exponential phase growth in vitro. In vivo, mean n-RNA and c-RNA, as well as MOM3, differ at first presentation of leukemia and shortly after induction chemotherapy. Therefore, these parameters may be used as biologically relevant features for diagnostic purposes or for studies monitoring chemotherapeutic effects of specific drugs in vitro and in vivo.

An increasing body of evidence indicates that there is a correlation between RNA content of individual cells and their rate of proliferation (for reviews, see Refs. 10 and 11). It was observed recently that the rates of cells’ traversal through the cell cycle are highly correlated with their RNA content (12, 13). Also, during exponential unperturbed growth, the transition of cells from the G1 to S-phase of the cell cycle seems to be associated with the attainment of a threshold amount of RNA. In leukemic cell populations threshold amounts of RNA appear to be different in ALL and ANLL, which would indicate tissue-specific differences between cell types. Since, however, S-phase cells were infrequent in some of the investigated specimens, an exact quantitation of the different threshold amounts of RNA was not possible in this study.

It is evident from the P-values (Table 2) that the discrimination of ALL from ANLL cells is more pronounced on the basis of mean n-RNA measurements than on c-RNA measurements. However, ALL cell populations cannot be differentiated from control cell populations with reasonable reliability on the basis of both c-RNA and n-RNA mean content alone. Therefore, F600 distribution histogram analysis provides more information for discriminating cell populations than mean RNA values alone.

The high degree of heterogeneity of n-RNA and c-RNA distribution in G0/G1 cells of ANLL may be of importance for the relatively high failure rates of currently used chemotherapeutic drug regimens to induce complete remissions and to maintain long disease-free survival times (14) as compared to ALL (15, 16), in which disease the n-RNA and c-RNA distribution of G0/G1 cell populations is significantly more uniform. In both ALL and ANLL, n-RNA is expected to be a more sensitive parameter for estimating DNA-directed drug effects in vivo and in vitro than is c-RNA, due to its close relationship with transcription and the very active intranuclear RNA metabolism.

The high SE value (Table 1) of the mean c-RNA of the ANLL group may reflect various cell subpopulations which might perhaps be further identified by flow cytometric determination of RNA content in concert with cell surface markers. A similar heterogeneity among the ANLL group in terms of functional properties like in vitro growth characteristics was reviewed by Zighelboim and Gale (14). It is possible that both proliferative potential and cell sensitivities toward drugs may be modulated by differences in the metabolism of n-RNA.

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* A. J. Walle, unpublished observations.
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