The RNA Nucleotide Composition in Human Leukocytes from Normal and Leukemic Cases

By K. Kiss, G. Astaldi and R. Aibo

SOME OBSERVATIONS suggest that the RNA-nucleotide-composition of leukocytes from patients with leukemia differs from that of normal cells. Will et al.\(^1\) reported that the purine and pyrimidine base contents of total nucleic acids in acute leukemia and in normal bone marrow cells are different. This variation is not caused by any difference in the DNA-nucleotide-composition. In fact, the purine and pyrimidine-base-composition of the DNA in the leukocytes from both acute\(^2\) and chronic\(^3\) leukemic patients is the same as that in normal leukocytes. Gavosto and Pileri\(^4\) observed that (a) the ratio of thymine/total-nucleic-acid-bases is constant in both normal and leukemic cells, and (b) that the uracil/thymine ratio agrees with the RNA/DNA ratio in normal bone marrow cells, but these relationships do not occur in white cells from leukemic patients. These variations suggest that an alteration of the uracil metabolism occurs in the white cells of leukemic patients. A detailed quantitative and qualitative analysis of the pentosonucleic-acid-composition in normal and leukemic leukocytes has been made and our observations are reported at this time.

MATERIALS AND METHODS

The purine and pyrimidine base composition of leukocytes was determined in 13 leukemic patients, 6 with chronic lymphatic, 2 with acute, and 5 with chronic granulocytic leukemia (one of the latter was examined also during the terminal blastic crisis). Thirteen normal individuals constituted the control group; in six of these cases lymphocytes were obtained by filtering the leukocyte rich plasma through a cotton column, according to the technique of Rabinowicz\(^5\) with minor modifications. All patients with chronic leukemia, received no therapy for three months preceding the time of this investigation. The two patients with acute leukemia had not been treated before the RNA nucleotide determinations.

Preparation of leukocyte suspensions

The leukocytes were separated from the erythrocytes by dextran sedimentation. Twenty to 400 ml of heparinized blood were obtained from the antecubital vein and added to a 6% dextran solution in a proportion of 5 ml of dextran to 100 ml of blood. After 1 hour incubation at 4°C, the leukocyte rich plasma was removed and centrifuged at 600 × g for 10 minutes. The sedimented cells were suspended in 2 ml of ice cold 0.9% NaCl solution.

The erythrocytes and platelets were removed by the "hypotonic shock" technique. Six
ml of ice cold distilled water were added for 30 seconds, after which the isotonicity of the solution was reconstituted by adding 2 ml of ice cold 3.5% NaCl solution. This cell suspension was centrifuged at 600 × g for eight minutes and the layer of erythrocyte stroma was discarded. The remaining leukocytes were suspended in 5 ml of ice cold 0.9% NaCl solution. Two to 4 ml of this cell suspension were used for chemical analysis and the remainder for morphologic studies and cell counts. The leukocyte counts were made in triplicate using a Bürker chamber and counting 400 cells. The average of the three counts was used for each sample. Differentials were determined on May-Grünwald-Giemsa stained smears, examining 1,000 cells for each sample.

Lymphocytes were obtained by resuspension of the leukocyte concentrates from the dextran sedimentation in 5–8 ml of plasma from the same donor. This suspension of cells was then placed in a glass burette (36 × 1.2 cm) loosely packed with cotton. When the cells were absorbed by the cotton, which required only a few seconds, the burette was again filled with plasma and incubated at 37°C for 30 minutes. The plasma was removed and the burette again was filled with plasma at 37°C. The recovered plasma was centrifuged at 600 × g for 10 minutes. The lymphocytes and erythrocytes were suspended in 2 ml of an ice cold 0.9% solution of NaCl. The granulocytes and monocytes remained trapped in the cotton filter. The erythrocytes and platelets in this suspension were removed by the “hypotonic shock” technique previously described. Differential counts on smears of this cell suspension showed 86–98% lymphocytes (average 95±4%) in the white cell population.

Chemical Analysis

The cell suspension for chemical analysis was placed in a centrifuge tube of known weight. The cells were precipitated by adding a volume of ice cold 20% trichloracetic acid equal to the volume of the cell suspension. The tube containing this material was kept in an ice cold water-bath for 10 minutes; meanwhile, the precipitated cells were stirred several times with a glass-rod. The cells were centrifuged and washed twice in 10 ml of ice cold 10% trichloracetic acid.

The lipid extraction was begun with ice cold absolute ethanol containing 10% potassium acetate. The precipitate was suspended twice in a mixture of 3 volumes of absolute ethanol and 1 volume chloroform, then in a mixture of 3 volumes of absolute ethanol and 1 volume of ethyl ether and, lastly, only in ethyl ether. The precipitate was stirred frequently during each 10 minute period of washing and extraction. The centrifuge tube was kept in an ice cold water-bath during washing and the first lipid extraction.

The following steps in the lipid extraction were performed at room temperature: the lipid-free precipitate was dried for 20 hours at 37°C; the centrifuge tube with the nucleo-protein powder was weighed and the powder was calculated per 10⁶ leukocytes. Five to 20 mg of nucleo-protein powder was used to quantitatively determine the 4 mononucleotides in the RNA of 10⁶ leukocytes. The RNA hydrolysis in mononucleotides was performed in 0.25 ml of 0.3 N KOH solution at 37°C for 18 hours. After this hydrolysis, the solution was acidified with HClO₄ to pH 4.0. The nucleotides were separated from the protein DNA-KClO₄ precipitate by centrifugation and 2 washings. Acidification and washings were performed in an ice-cold water-bath and the centrifugation continued for a period of 1 minute at 1,000 × g. Chemical determinations of the DNA-content in the RNA hydrolysates were constantly negative, thus confirming that we were dealing with RNA-nucleotides.

The 4 mononucleotides of the RNA-hydrolisates were separated by the one-dimension ascending chromatography. Chromatograms of the hydrolysates were made for a period of nine hrs at room temperature on Whatman 3 MM paper with a solution composed of isobutyric acid and 0.5 M ammonium hydroxid (10:6, v/v). The dry chromatography paper was examined under ultraviolet illumination with a filter that removed most of the visible light. The dark ultraviolet-absorbing spots were outlined in pencil. These areas were cut and eluted in 4 ml of 1.0 M phosphate buffer at pH 7.0 and 37°C for 18–20 hrs. Pieces of control paper which had been chromatofied without any nucleotides were cut and eluted in the same manner. The solution obtained in this way were used as blanks.
RNA NUCLEOTIDE COMPOSITION IN HUMAN LEUKOCYTES

The absorption spectra of both the nucleotide solutions and the appropriate blanks were determined with a Beckman Model DU spectrophotometer. The amount of nucleo-protein powder in micro-mol/4 ml was determined per 10⁹ leukocytes by using the molecular weights of the different mononucleotides. The results are expressed in micrograms. Preliminary studies had shown that this method for extracting RNA, and for separating the four mononucleotides by chromatography, and for referring their values in micrograms to 10⁹ leukocytes was reproducible. However, in the present investigation duplicate determinations were made for each chemical analysis with agreements within a 10% limit. Statistical analyses, when necessary, were performed according to the “T” test.

RESULTS

1. The RNA-nucleotide contents and molar base ratios, as determined on the mixed leukocyte cell suspension from the control group, are given in Table 1. The four RNA-mononucleotides per 10⁹ leukocytes are recorded in micrograms for each case. The molar base ratios and the amount of mononucleotides are not influenced by the variations in the composition of leukocytes, i.e., by the proportion between polynuclears and mononuclears.

The values of the 4 RNA-mononucleotides per 10⁹ leukocytes are as follows: adenylic acid mcg 196±53; guanylic acid mcg 330±63; cytidylic acid mcg 288±53; and uridylic acid mcg 165±28. When the molarity of the adenylic acid is taken as 10.0 the molar bases ratios of nucleotides are: guanylic acid 16.0±0.6; cytidylic acid 15.6±0.8; uridylic acid 8.9±0.4. The ratio of 6-amino/6-keto acids is 1.02 and that of purines/pyrimidines 1.05.

2. The results obtained when testing the filtered leukocyte suspension, containing lymphocytes in the average percentage of 95±4, are given in Table 2. They show quantitative values of the four mononucleotides somewhat higher than those of the mixed leukocytes (Table 1). This difference is not statistically significant at the “T” test for unpaired experiments. The four mononucleotides for 10⁹ cells in the lymphocyte suspension were as follows: adenylic acid mcg 227±16; guanylic acid mcg 396±30; cytidylic acid mcg 343±31; and uridylic acid mcg 188±19. When the molarity of the adenylic acid is taken as 10.0, the guanylic acid is 16.6±0.6, the cytidylic acid 16.1±0.8, and the uridylic acid 8.8±0.4. The ratio of 6-amino/6-keto acids is 1.02 and that of purines/pyrimidines 1.06, respectively.

3. The results of the white blood cell suspensions obtained from chronic lymphocytic leukemia are shown in Table 3. The proportions of lymphocytes in these preparations ranged from 91–98% with an average of 95±2, i.e., with values very close to those obtained by filtrating the blood leukocytes from normal individuals as reported in Table 2. On the contrary, the quantitative values of the four RNA-nucleotides were much higher in chronic lymphocytic leukemia than in healthy persons. The average values of the mononucleotides in 10⁹ white cells from chronic lymphocytic leukemia were as follows: adenylic acid mcg 244±55; guanylic acid mcg 578±96; cytidylic acid mcg 505±87; and uridylic acid 299±47.

The statistical analysis shows that the differences between the quantitative values of mononucleotides in the lymphocytes from leukemic patients and those from healthy persons are highly significant (p<0.01). On the contrary, the ratio of the molar bases in the lymphocytes from leukemia patients is very...
Table 1.—Peripheral leukocytes from normal human blood

A. = Adenylie acid  G. = Guanylic acid
C. = Cytidylie acid  U. = Uridylic acid

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Qualitative composition of leukocytes</th>
<th>Nucleotides contents in pg. of 10⁶ leukocytes</th>
<th>Molar bases ratio of nucleotides (when &quot;A&quot; is taken as 10.0)</th>
<th>A+C</th>
<th>Purines</th>
<th>Pyrimidines</th>
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<td>264</td>
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<td>302</td>
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<td>249</td>
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<td>127</td>
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<tr>
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<td>68%</td>
<td>32%</td>
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<td>297</td>
<td>268</td>
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<td>5</td>
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<td>455</td>
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<td>324</td>
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<td>32%</td>
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<td>277</td>
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<td>± 63</td>
<td>± 53</td>
<td>± 28</td>
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</table>
similar to the ratio in the lymphocytes from normal individuals. In fact, taking the adenylic acid as 10.0, we have the following values: guanylic acid 16.0±0.6 in both leukemic patients and controls; cytidylic acid 15.6±0.4 and 16.1±0.8 in leukemic patients and controls; uridylic acid 9.2±0.3 and 8.8±0.4 in leukemic patients and controls. Finally, the ratio of 6-amino/6-keto acids is 1.01-1.02 and the ratio of purines/pyrimidines is 1.04 and 1.06 in leukemic patients and controls.

4. The results of the determinations carried out on the peripheral leukocytes from chronic granulocytic leukemia are recorded in Table 4/A. It is evident that the quantities of the four RNA-nucleotides considerably increase as the proportion of blast-cells increases. In the blastic crisis (No. 6 in Table 4), the following values were obtained for 10⁶ leukocytes: adenylic acid mcg 1,270; guanylic acid mcg 2,076; cytidylic acid mcg 1,802; and uridylic acid mcg 1,095. The differences between these values and those observed in control cases are statistically highly significant (p<0.01).

In spite of the quantitative increase of the four RNA-nucleotides in the peripheral leukocytes from chronic granulocytic leukemia over that of the controls, the molar base ratios were very similar. In fact, when the adenylic acid is considered as 10.0, the following ratios are obtained: guanylic acid 15.0±0.5 in leukemic patients and 16.0±0.6 in the controls; cytidylic acid 15.5±0.5 and 15.6±0.4, and uridylic acid 9.0±0.4 and 8.8±0.4, respectively, in chronic granulocytic leukemic patients and in the controls. Furthermore, the ratio of 6-amino/6-keto acids is 1.02-1.05 in chronic granulocytic leukemic patients and in the controls. This is the same relationship as observed when comparing the corresponding values in chronic lymphocytic leukemia with the normal.

5. The mononucleotides in peripheral blood leukocytes in two cases of acute granulocytic leukemia are shown in Table 4/B. In both, the mononucleotides are very high, probably because of the high proportions of blast forms in the peripheral blood. In the case with 32% blasts, the adenylic acid content in 10⁶ white cells was 590 mcg and the guanylic, cytidylic, uridylic acid contents, respectively, were 1,038, 849, and 526 mcg. In the case with 92% blasts, the corresponding values in the 10⁶ white cells were 1,345 mcg for the adenylic acid, 2,456 mcg for the guanylic, 1,930 mcg for the cytidylic, and 1,124 mcg for the uridylic acid. These data are very similar to those obtained in the terminal blastic crisis of a case of chronic granulocytic leukemia.

As far as the molar base ratios are concerned, in both cases of acute leukemia they are similar to the corresponding data obtained with the peripheral leukocytes in chronic granulocytic leukemia which are similar to the molar base ratios obtained with peripheral leukocytes of the control cases. The four RNA-mononucleotides in 10⁶ peripheral white cells varied markedly in the normal and leukemic patients, while the molar base ratios did not show any differences in the cases examined.

**DISCUSSION AND CONCLUSION**

Although origin, morphology, structure, metabolism and function of granulocytes and lymphocytes are quite different, we have not been able to show any significant difference in the RNA-nucleotide contents and in the molar base
Table 2—Peripheral lymphocytes from filtered normal human blood

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Nucleotides content in µg. of 10^9 lymphocytes</th>
<th>Molar base ratio (when “A” is taken as 10.0)</th>
<th>Pyrimidines</th>
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<tr>
<td></td>
<td>G/ C</td>
<td>A+G</td>
<td>C-U</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>G</td>
<td>A+C</td>
</tr>
<tr>
<td></td>
<td>Granulocytes</td>
<td>Lymphocytes</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>213</td>
<td>213</td>
<td>5.99</td>
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</tr>
<tr>
<td>6</td>
<td>219</td>
<td>219</td>
<td>5.90</td>
</tr>
<tr>
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<tr>
<td>S.D.</td>
<td>± 0.44</td>
<td>± 0.44</td>
<td>± 0.31</td>
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</tbody>
</table>

A = Adenyl acid
G = Guanilic acid
C = Cytidyl acid
U = Uridyl acid

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ratios between these two types of leukocytes in the control cases. Neverthe-
less, the nucleotide per cell in granulocytes and in lymphocytes does not mean
that the concentration of RNA in their cytoplasm is the same. In fact, the ab-
solute volume of cytoplasm, as well as the ratio of nucleus/cytoplasm in granulo-
cytes, is higher than it is in lymphocytes. These results indicate that the con-
centration of RNA in the cytoplasm of the lymphocytes is higher than it is in
the cytoplasm of the granulocytes.

The RNA-monomonucleotide content is significantly higher in a given number
of lymphocytes from chronic lymphocytic leukemia than in the same number
of lymphocytes from control cases. Again, a given number of peripheral leuko-
cytes from cases of chronic granulocytic leukemia has a RNA-monomonucleotide
content significantly higher than the same number of leukocytes from normal
individuals with a high percentage of granulocytes. The increase in RNA-mono-
nucleotide content in white blood cells from cases of granulocytic leukemia
parallels the degree of cell immaturity, reaching the highest values either
during the blastic crisis in cases of chronic granulocytic leukemia, or in cases
of acute granulocytic (myeloblastic) leukemia. On the contrary, the molar
base ratios are almost identical in the normal and leukemic cases, the chronic
granulocytic, the chronic lymphocytic, and the acute leukemia. In other
words, the white blood cells from leukemic patients, regardless of whether
they belong to the lymphocytic or granulocytic series, have a disturbance in
the RNA-metabolism which consists of an accumulation of the four mononu-
cleotides. Nevertheless, in these leukemic leukocytes, the proportion of the
four mononucleotides, the ratio of 6-amino/6-keto acids, and the ratio of
purines/pyrimidines are within the limits, as observed in the leukocytes from
normal individuals.

These data are in agreement with the results reported by Gross12,13 and by
Muller,14 using the spectrophotometric method. They showed an increase in
the RNA- and DNA-contents of the white cells from leukemic cases, confirm-
ing the earlier observations of Thorell.15 Will et al.,1 however, observed a sig-
nificant deviation between the mononucleotide base contents of the total
nucleic acid in bone marrow cells from normal persons and in the cells from
acute leukemic patients. Again, our results definitely agree with the observa-
tions that foetal, regenerating, and normal (adult) liver cells do contain dif-
ferent quantities of RNA, but that the molar base ratios of their RNA-com-
ponents remain the same whether the cells are normal, regenerating, or
foetal.16 The same behaviour has been observed in the case of dividing and
resting bacterial cells.17

Our results differ from the observations by Gavosto et al.4 They reported
that the ratio of uracil/thymine and RNA/DNA in leukemic cells differed from
that in normal leukocytes. We did not find any deviation in the proportion
of the examined pentosonucleotides. It is possible that such a difference is only
apparent, depending on the technique employed for the nucleic acid extrac-
tion. Gavosto et al.4 hydrolized the total nucleic acid in 12 N HClO₄ at 100° C
for 1 hour, but when the RNA-hydrolisis is not performed in mild alkali (i.e.,
in 0.3 KOH), part of the cytosine is converted into uracil. This conversion
may be higher when the RNA/DNA ratio is high, such as in white cells from
Table 3.—Peripheral lymphocytes from Chronic Lymphocytic Leukemia

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Qualitative composition of leukocytes</th>
<th>Nucleotides contents in μg of 10⁶ leukocytes</th>
<th>Molar bases ratio of nucleotides (when &quot;A&quot; is taken as 10.0)</th>
<th>A+C</th>
<th>Purines G+U Pyrimidines</th>
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<td>96%</td>
<td>327</td>
<td>563</td>
<td>478</td>
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<tr>
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<td>622</td>
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RNA NUCLEOTIDE COMPOSITION IN HUMAN LEUKOCYTES

Table 4.—A Peripheral leukocytes from Chronic Granulocytic Leukemia

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Table 4.—B Peripheral leukocytes from Acute Leukemia

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<td>6%</td>
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<td>849</td>
<td>526</td>
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<td>2</td>
<td>92%</td>
<td>6%</td>
<td>2%</td>
<td>—</td>
<td>1345</td>
<td>2436</td>
<td>1930</td>
<td>1124</td>
<td>10.0</td>
<td>17.3</td>
<td>15.4</td>
<td>8.9</td>
<td>0.96</td>
</tr>
</tbody>
</table>

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cases of leukemia. Therefore, the difference observed in the ratio of uracil/thymine and RNA/DNA in normal and leukemic leukocytes could be explained by the partial conversion of cytosine into uracil during the extraction procedure.

The observation that the proportion among the four mononucleotides in white blood cells from different forms of human leukemia are not altered in respect to the proportion of the same mononucleotides in the corresponding cells from control cases does not support the viral theory for human leukemia. In fact, it has been shown that the viruses which cause leukemia in experimental animals are RNA-containing viruses and that the purine and pyrimidine base composition in the cells infected with such viruses differs from the composition of normal cells since it becomes similar to the virus-RNA. On the contrary, we have not been able to show in man any difference in the nucleotide base composition between normal and leukemic white blood cells.

In any case, our data cannot be taken as evidence against the virus theory of human leukemia. In fact, (1) it is not proven that the hypothetical etiologic virus of human leukemia has a RNA-base-composition significantly different from the cellular RNA-composition; (2) the viral RNA-synthesis in leukemic white cells could be insufficiently higher over the cellular RNA-synthesis to be detectable by the method we have used; (3) the white cells of human leukemia could not carry viruses, but they could be just an end-product of a viral infection. These possibilities suggest that our results cannot be taken as evidence against the virus as causative agent of human leukemia, although they do show nothing in favour of the viral etiology of human leukemia.

**SUMMARY**

The RNA-nucleotide composition of leukocytes from normal and leukemic patients has been analyzed. The amount of the four mononucleotides per 10^6 leukocytes is significantly increased in patients with either chronic lymphocytic or chronic granulocytic leukemia when compared with leukocytes from normal subjects. High values have been observed in acute granulocytic leukemia and during blastic crisis occurring in cases of chronic granulocytic leukemia. This suggests that the mononucleotide increase may be correlated with the number of blast cells. The molar base composition of the 4 mononucleotides and the ratio of 6-keto/6-amino acids and that of purine/pyrimidine bases in all leukemic cells examined were similar to the ratios occurring in normal leukocytes.

The significance of these findings, relative to the theory of viral origin of human leukemia, is discussed.

**SUMMARIO IN INTERLINGUA**

Esseva analysate le composition de nucleotidas de acido ribonucleic ab subjectos normal e leucemic. Le quantitates del quatro mononucleotidas es augmentate significativamente (exprimite per 10^6 leucocytos) in patfentes con chronic leucemia lymphocytic o granulocytic in comparation con subjectos normal. Alte valores esseva observate in acute leucemia granulocytic e durante crises blastic in casos de chronic leucemia granulocytic. Isto suggestiona que le augmento del mononucleotidas es possihilemente relazionate con le numeros del cellulas blastic. Le composition del bases molar in le quatro mononucleotidas e le propor-
IINA
RNA NUCLEOTIDE COMPOSITION IN HUMAN LEUKOCYTES
717
tiones de acidos 6-keto/6-amino e de bases purinic/pyrimidinic in omne le leucocytos exame
nitate esseva simile al proportiones que occurre in leucocytos normal.

Le signification de iste constatationes es commentate con respecto al theoria del origine
virusal de leucemia human.

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The RNA Nucleotide Composition in Human Leukocytes from Normal and Leukemic Cases

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