METHYLATION OF DNA CYTOSINE IN LYMPHOCYTES FROM HEALTHY PERSONS AND PATIENTS WITH CHRONIC LYMPHATIC LEUKEMIA

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When lymphocytes were incubated with L-methionine-(methyl-C^{14}) for 18 h the methyl group was effectively utilized not only for methylation of cytosine but also for the de novo biosynthesis of the pyrimidine and purine bases of DNA. Twice as much label was incorporated into cytoplasm as into thymine in the healthy human lymphocytes, but 2.4 times as much label was incorporated into thymine as into cytosine in lymphocytes from patients with chronic lymphatic leukemia. The ratio between the specific activity of 5-methylcytosine and the specific activity of cytosine was four times greater in the patients with lymphatic leukemia than in the healthy persons.

The existence of a connection between the methylation of DNA and the functional activity of the genes has frequently been postulated [3, 11, 12]. Lymphocytes of patients with chronic lymphatic leukemia have a functional defect, for their blast transformation reaction to various mitogens is depressed and delayed by comparison with healthy human lymphocytes.

It is possible that the functional defect of the lymphocytes in chronic lymphatic leukemia may be attributed to the character of methylation of the DNA cytosine, and the investigation described below was carried out to investigate this problem.

EXPERIMENTAL METHOD

Experiments were carried out on lymphocytes isolated [4] from heparinized peripheral blood of healthy human subjects and on lymphocytes from patients with chronic lymphatic leukemia. The isolated cells were

TABLE 1. Incorporation of L-Methionine-(Methyl-C^{14}) into DNA Bases of Lymphocytes from Healthy Human Subjects and Patients with Chronic Lymphatic Leukemia (M ± m)

Sources of lymphocytes	Activity of bases (in %)					$\frac{T}{C}$	$\frac{5MC \times 20}{C}$
	guanine	adenine	thymine	cytosine	5MC		
Donors (4)	12,5±2,5	14,2±3,3	15,3±1,4	39,6±5,5	18,4±1,5	0,42±0,07	9,8±1,2
Patients: group 1 (4)	22,6±6,6	11,5±2,0	25,1±1,7	11,8±2,8	29,0±6,4	2,7±0,56	50,2±4,3
group 2 (3)	5,6±3,3	6,1±3,3	41,4±8,4	20,6±1,0	26,2±1,2	2,1±0,49	25,6±1,3

Note. Number of donors and patients in groups shown in parentheses.

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suspended in Eagle's incubation medium with the addition of inactivated group AB (IV) serum, antibiotics, and glutamine. To 1 ml incubation medium containing $20 \cdot 10^6$ cells 2.5 μCi L-methionine-(methyl-C^{14}) was added. Incubation proceeded at 37°C for 18 h. The DNA was isolated and purified by the usual methods [7]. Hydrolysis of DNA to bases was carried out in 85% formic acid for 1 h at 170°C. The resulting bases were fractionated by one-dimensional chromatography on FN12 paper (East Germany) in a system of isopropanol–concentrated HCl–water (65:17:18) for 24-26 h, using 5-methylcytosine (5MC) as the reference substance. The radioactivity of the bases was measured on a liquid scintillation counter (Nuclear Chicago Mark I).

EXPERIMENTAL RESULTS

The results in Table 1 show that the C^{14}-methyl group of methionine-C^{14} is effectively utilized not only for methylation of the DNA cytosine of lymphocytes both from healthy persons and from patients with chronic lymphatic leukemia, but also for the de novo biosynthesis of the pyrimidine and purine bases of DNA. More than twice as much label was found to be incorporated into cytosine as into thymine in the healthy human lymphocytes. In lymphocytes of patients with chronic lymphatic leukemia, on the other hand, on the average 2.5 times more label was incorporated into thymine than into cytosine. This considerable incorporation of label into DNA cytosine of the healthy subjects is evidence that the C^{14}-methyl group is utilized chiefly in the synthesis of the pyrimidine ring through the synthesis of carbamoyl phosphate or aspartate.

Approximately 5% of the cytosine in the DNA of mammalian cells undergoes enzymic methylation with the participation of DNA-methylase and of S-adenosylmethionine as the donor of methyl groups. Consequently, in the experiments in which methionine-(methyl-C^{14}) was used incorporation of the label into 5MC of the lymphocyte DNA took place, first, at the nucleotide level during de novo biosynthesis of deoxycytidine triphosphate, and second, at the polynucleotide level during the specific methylation of cytosine by DNA-methylases, so that the percentage of methylation of cytosine could not be determined. However, since 5MC is formed from cytosine already existing as a component of the DNA, the ratio between the specific activity of 5MC and the specific activity of cytosine (Activity of 5MC × 20/Activity of C) gives some idea of the fraction of the radioactivity incorporated into 5MC during the DNA-methylase reaction and enables the level of methylation of DNA cytosine in the lymphocytes of healthy persons and of patients with chronic lymphatic leukemia to be compared, despite differences in the incorporation of methionine-(methyl-C^{14}) into DNA cytosine during de novo biosynthesis. It will be clear from Table 1 that the ratio (5MC × 20/C) in healthy human lymphocytes is about 10 while the patients with chronic lymphatic leukemia can be divided into two groups with reference to this index. Comparison of the results with the clinical picture of the disease showed that in patients for whom the mean value of this ratio was 50 had well-marked evidence of progressive leukemia: an increased leukocyte count, enlargement of the peripheral lymph glands, liver, and spleen, a high count of lymphopoietic cells in the bone marrow. On the other hand, in the patients whose mean value of the ratio was 25.6, no evidence of progression of the leukemia was found.

Healthy human lymphocytes and lymphocytes from patients with chronic lymphatic leukemia are resting cells, but certain DNA fractions are synthesized and methylated for reasons other than division of lymphocytes [1,2]. These fractions may include metabolic DNA [9] and mitochondrial and cytoplasmic DNA [8].

The results of these experiments agree with earlier observations [5, 10] made on peripheral blood leukocytes of healthy persons and patients with chronic lymphatic leukemia. The increased level of methylation of certain DNA fractions in the lymphocytes of patients with chronic lymphatic leukemia may be the chief cause of their functional defect, for the distribution of 5MC in the DNA chain is known not to be random in character and the appearance of 5MC in new sequences could lead to changes in the functional state of the corresponding genes [6].

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