CONCISE REPORT

Reduced Tocopherol Content of B Cells From Patients With Chronic Lymphocytic Leukemia

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The tocopherol content of lymphocytes, erythrocytes, and plasma from patients with chronic lymphocytic leukemia (CLL), hairy cell leukemia (HCL), and normal subjects was measured by a sensitive high performance liquid chromatographic method. Lymphocytes from patients with CLL had lower values of tocopherol (1.7 ± 1.0 $\mu g/10^9$ cells) than lymphocytes from normal subjects (3.8 ± 0.7 $\mu g/10^9$ cells). Mononuclear cells from patients with HCL had an increased tocopherol content of 6.2 ± 1.0 $\mu g/10^9$ cells. Subfractionation of the lymphocytes from patients with CLL into T- and B-cell subgroups showed that the tocopherol content of T cells was the same as in normal subjects (4.1 ± 0.5 $\mu g/10^9$ cells versus 3.5 ± 1.2), but that the tocopherol content of the B cells was markedly reduced compared to normals (2.6 ± 1.0 versus 6.0 ± 1.3).

TOCOPHEROL FUNCTIONS as an antioxidant protecting unsaturated fatty acids from oxidation. In the plasma membrane, tocopherol and cholesterol are thought to be intercalated between the phospholipid fatty acids within the lipid bilayer.[1] Since several studies have shown a decreased cholesterol content in lymphocytes from patients with chronic lymphocytic leukemia (CLL) and high levels of cholesterol in hairy cell leukemia (HCL),[2,3] we decided to measure the tocopherol levels in these cells to determine whether they paralleled the cholesterol content. The results of this analysis were compared to the tocopherol levels in T- and B-cell subpopulations obtained from normal subjects and patients with CLL.

MATERIALS AND METHODS

The diagnosis and staging of CLL was established by standard criteria in 9 patients with CLL and 3 patients with HCL. None of the patients had received chemotherapy or vitamin E supplementation. Only normal subjects not taking vitamin E were chosen for the study. All patients and normal subjects gave informed consent, according to the provisions of the Helsinki conference.

The procedure for isolating lymphocytes and the T- and B-cell subpopulations from normal subjects and patients with CLL have been described previously.[4] In brief, heparinized blood was centrifuged on a Ficoll-Hypaque gradient, and the mononuclear cell layer was depleted of monocytes either by adherence to Falcon plastic cultures flasks or by centrifugation through a Percoll gradient.[5] The resulting lymphocyte preparations generally contained less than 2% monocytes and less than 10% platelets. T- and B-lymphocyte subpopulations were determined by standard rosetting techniques using neuraminidase-treated sheep erythrocytes for T cells and complement-coated sheep erythrocytes for B cells. The T cells were purified after rosette formation by centrifugation through Ficoll-Hypaque, and B cells were isolated by pouring the T-cell-poor suspension over antibody-coated [anti-F(ab)$_2$] plastic dishes, with attachment of the B cells and their subsequent release in incubation with human gamma-globulin. The final preparation had 58%–89% B cells and 87%–99% T cells in normal subjects and 90%–97% B cells and 52%–83% T cells in CLL preparations. Cells were counted in a Coulter Counter.

The method for the analysis of the tocopherol content of the cells and plasma has been reported.[6] High performance liquid chromatography (HPLC) of organic solvent extracts of the cells was used to isolate the tocopherol, which was quantitated by the measurement of fluorescence intensity using an excitation wavelength of 205 nm with an emission filter at 340 nm. Replicate values varied by less than 5%.

RESULTS AND DISCUSSION

The tocopherol concentration in lymphocytes, erythrocytes, and plasma from 5 normal subjects, 9 patients with CLL, and 3 patients with HCL are shown in Table 1. The lymphocytes from normal subjects had a tocopherol concentration of 3.8 ± 0.7 $\mu g/10^9$ cells, while the mean value for lymphocytes from patients with CLL (1.7 ± 1.0 $\mu g/10^9$ cells) was significantly lower ($p < 0.001$). Cells from patients with HCL contained 6.2 ± 1.0 $\mu g/10^9$ cells, which was significantly higher than normal lymphocytes ($p < 0.002$). Neither the erythrocytes nor the plasma content of tocopherol was statistically different from the three groups. While the lymphocytes from 3 patients with stage 0 disease had a tocopherol level within the normal range, and the lowest levels were found in patients with stage III and IV disease, the small number of patients precludes any definitive comment on an association between stage of disease and tocopherol content.

Since the percentage of B lymphocytes is increased above normal in CLL, we compared the tocopherol

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Supported in part by grants from the National Institutes of Health, (CA11655), U.S. Public Health Service, Hoffmann-LaRoche. L.F.L. is a Scholar of the Leukemia Society of America, Inc.

Submitted April 13, 1983; accepted July 20, 1983.

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0006-4971/84/6301-0028$01.00/0

Table 1. Tocopherol Concentration of Lymphocytes, Erythrocytes, and Plasma From Patients With Chronic Lymphocytic Leukemia, With Hairy Cell Leukemia, and From Normal Subjects

Subjects	Stage	Percent B Cells	Lymphocytes ($\mu g/10^9$ Cells)	Plasma ($\mu g/ml$)	RBC ($\mu g/10^{10}$ Cells)
Chronic lymphocytic leukemia					
H.H.	0	65	3.0	8.5	1.5
W.P.	0	72	3.4	11.0	2.3
S.M.	0	82	2.4	9.1	1.9
S.S.	I	n.d.	1.6	14.2	2.2
L.R.	II	92	1.0	10.3	1.8
M.W.	II	84	1.2	15.8	2.0
C.G.	III	91	1.4	n.d.	1.3
J.G.	III	86	0.6	10.2	1.2
B.H.	IV	84	0.9	7.8	1.3
Mean ± SD (n = 9)			1.7 ± 1.0	10.9 ± 2.8	2.2 ± 0.4
Hairy cell leukemia					
H.H.			5.1	9.8	1.2
A.S.			6.6	9.3	3.2
P.P.			7.0	7.4	1.2
Mean ± SD (n = 3)			6.2 ± 1.0	8.8 ± 1.3	1.9 ± 1.2
Normal (n = 6)			3.8 ± 0.7	8.8 ± 3.9	1.4 ± 0.2

Test of significance, Student's t test.
Normal lymphocytes versus CLL lymphocytes, $p < 0.001$.
n.d. = not determined.

content of CLL B cells to normal B cells and CLL T cells to normal T cells. The tocopherol content of CLL T cells was not different from that in normal subjects (Table 2). In contrast, the mean value of B cells in normal subjects (6.0 ± 1.3 $\mu g/10^9$ cells) was significantly greater than in CLL B lymphocytes (2.6 ± 1.0 $\mu g/10^9$ cells) ($p < 0.002$, Student's t test), despite the fact that 3 of the 4 patients studied had stage 0 disease. Normal B cells were also significantly higher in tocopherol content than normal T cells (6.0 ± 1.3 versus 3.5 ± 1.2 $\mu g/10^9$ cells) ($p < 0.001$).

The studies presented demonstrate that the B-cell subclass of lymphocytes from patients with CLL have a decreased level of tocopherol compared to normal B cells; the tocopherol content of T cells, plasma, and erythrocytes from CLL patients was not different from those of normal subjects. That there is an abnormality in the tocopherol content of B cells from CLL patients is a finding consistent with the monoclonal expansion of B cells in this disorder. While the explanation for the decreased tocopherol content of these cells is not apparent, it is not an unexpected finding in view of the decreased cholesterol content of these cells.

The cause of the decrease in tocopherol content of CLL B-cells is unclear. Normal rates of cholesterol synthesis have been reported in freshly isolated lym-

Table 2. Reduced Tocopherol Content of B Cells From Patients With Chronic Lymphocytic Leukemia as Compared to Those From Normal Subjects

Subject	Unfractionated Lymphocytes ($\mu g/10^9$ Cells)	T Cells ($\mu g/10^9$ Cells)	B Cells ($\mu g/10^9$ Cells)	Plasma ($\mu g/ml$)	RBC ($\mu g/10^{10}$ Cells)
Chronic lymphocytic leukemia					
H.H.	3.0	3.9	3.3	8.5	1.5
S.M.	2.4	4.2	2.0	9.1	1.9
C.G.	1.4	4.7	1.6	n.d.	1.3
C.P.	3.4	3.5	3.6	11.0	2.3
Mean ± SD		4.1 ± 0.5	2.6 ± 1.0	9.5 ± 1.3	1.8 ± 0.4
Normal		2.5	4.2	6.9	1.2
		5.0	8.1	n.d.	n.d.
		3.0	5.2	n.d.	n.d.
		3.0	5.7	8.3	1.4
		4.9	6.5	8.0	1.2
		2.3	6.2	7.0	1.1
Mean ± SD		3.5 ± 1.2	6.0 ± 1.3	7.6 ± 0.7	1.2 ± 0.1

Test of significance, Student's t test.
CLL-B versus normal B, $p < 0.002$.
Normal B versus normal T, $p < 0.001$.
n.d. = not determined.

phocytes from patients with CLL.[7] Since tocopherol is an exogenously supplied vitamin, differences in cellular uptake might be considered. Tocopherol, like cholesterol, is transported primarily in low-density lipoproteins (LDL). The uptake of cholesterol by cells is regulated via the specific high-affinity LDL receptor mechanism.[8] The mean rate of receptor-mediated uptake and degradation of ^{125}I-LDL in CLL lymphocytes is within the normal range,[7] suggesting that decreased LDL uptake is not responsible for the decreased cholesterol and tocopherol contents. Studies of the tocopherol exchange between plasma and lymphocytes are needed for a more definitive answer. Finally, the possibility that surface shedding occurs in CLL, which would account for the decrease in these membrane component lipids,[9] must be considered.

Tocopherol and cholesterol are intercalated between the fatty acids of the phospholipids in the plasma membrane lipid bilayer.[1,10] Tocopherol has a well recognized antioxidant function.[11] The decreased concentration in CLL B lymphocytes may also reflect an augmented oxidative metabolism in these cells. Support for this suggestion is provided by the finding of glutathione instability in CLL lymphocytes during in vitro incubation.[12] While the significance of the depressed content of tocopherol in B cells in CLL remains to be explored, it is possible that the lower tocopherol content of CLL B cells provides an opportunity for a therapeutic approach using oxidant-generating drugs or other free-radical-producing systems delivered via monoclonal antibodies directed to CLL lymphocytes.

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