Leukapheresis Therapy of Chronic Lymphocytic Leukemia

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The effects of repeated leukapheresis with the IBM Blood Cell Separator were assessed in 13 patients with chronic lymphocytic leukemia (CLL). Five patients had from 14 to 25 leukaphereses at intervals ranging from 7 to 33 days. The median total number of lymphocytes removed was 18.0×10^{11} (range 11.9×10^{11}–26.6×10^{11}). Two of these patients had a decrease in lymphocyte count and in lymphadenopathy. One of the above and eight additional patients had intensive leukapheresis therapy consisting of five procedures per week for 1–4 wk. The median total number of lymphocytes removed from these patients was 11.34×10^{11} (range 3.30×10^{11}–47.4×10^{11}). Eight of these patients had a decrease in lymphocyte count, five in spleen size, eight in lymph node size, seven in bone marrow infiltration, and one in liver size. Lymphocyte counts before beginning leukapheresis therapy ranged from 20,500 to 684,000 (median 70,900). The median lymphocyte count on completion of treatment was 19,200 (range 6,590–40,000). After leukapheresis therapy, the median doubling time of the lymphocyte count was 71 days (range 29–118 days). In addition to demonstrating a significant proliferative component in CLL, the objective regression of disease in ten of the 13 patients suggests that leukapheresis is a potentially useful treatment for CLL.

THE DEVELOPMENT OF A SAFE and efficient blood cell separator has made available a new method for the removal of large numbers of peripheral blood leukocytes from normal subjects and patients with leukemia.[1] Preliminary studies demonstrated that more than 10^{11} leukocytes could be obtained from patients with chronic lymphocytic leukemia (CLL) during a single 3–4 hr procedure.[2] The present investigation was undertaken to assess the effectiveness of repeated leukaphereses as remission induction and maintenance therapy for patients with CLL, and to study the effects of lymphocyte depletion by leukapheresis on the clinical, hematologic, immunologic, and kinetic features of CLL.

MATERIALS AND METHODS

Experimental Design

Thirteen patients with CLL were chosen for leukapheresis therapy. For the initial five patients (intermittent leukapheresis group), the frequency of leukapheresis therapy was

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adjusted to maintain a stable clinical and hematologic status. Prior to leukapheresis therapy, these patients had increasing disease as determined by increasing leukocyte count, decreasing hematocrit, decreasing platelet count, or increasing organomegaly. Later, one of the above and eight additional patients with increasing disease (intensive leukapheresis group) had intensive leukapheresis therapy consisting of five procedures per week for 2–4 wk in an attempt to induce a partial regression of their disease.

Patient Selection

The diagnosis of CLL was based on a persistent peripheral blood lymphocytosis of more than 15,000/cu mm and a bone marrow containing more than 50% lymphocytes and less than 10% blasts. The median duration of disease from the time of diagnosis to the beginning of leukapheresis was 3 yr (range: 1 mo–6 yr). Seven of the patients had had prior chemotherapy with chlorambucil, and one had been treated with cyclophosphamide. Only two patients, however, had been treated within the 6 mo preceding leukapheresis therapy. Two patients, A. N. and J. C. M. (see below), received glucocorticosteroids during leukapheresis therapy. Written informed consent was obtained from each patient.

Leukapheresis Procedures

The leukaphereses were done using the IBM Blood Cell Separator, Model 2990 (International Business Machines, Inc., Endicott, N.Y., as described previously.[1-3]) Continuous flow of blood through the centrifuge was maintained at a rate of 30–50 ml/min. The centrifuge was operated at 950 rpm (65 *g*). For anticoagulation, acid-citrate-dextrose (ACD) solution, NIH formula A, was added to the blood at the junction of the input line, and the Teflon cannula inserted in one antecubital vein.

Hematologic Investigations

The peripheral blood counts and the counts on the buffy coat were done by standard hematologic techniques.

Bone marrow was aspirated from each patient before the start of leukapheresis therapy. The intensive leukapheresis patients had bone marrow aspirations at weekly intervals during the treatment period and at least once during the follow-up period. Differential counts were made on each bone marrow specimen, the cellularity estimated, and the absolute infiltrate calculated.[4]

Immunologic Studies

Delayed hypersensitivity was assessed by intradermal testing.[3,5] The amount of induration after 48 hr was measured for two perpendicular diameters, and the mean of these measurements was recorded. The antigens used for skin testing included Dermatophytin (1:10 dilution) (Hollister-Stier Laboratories, Dallas, Tex.), Dermatophytin "0" (1:10 dilution) (Hollister-Stier), Monilia Mixture (1:10 dilution) (Hollister-Stier), Streptokinase-Streptodornase (Sk-Sd) (Varidase, Lederle Laboratories, Pearl River, N.Y.), Histoplasmin (Parke, Davis and Co., Detroit, Mich.), and intermediate strength purified protein derivative (PPD) (Parke, Davis).

In vitro lymphocyte blastogenesis was assessed by measuring the incorporation of ^{3}H-thymidine into the DNA of cultured lymphocytes. The techniques of lymphocyte culture and measurement of ^{3}H-thymidine incorporation have been described previously.[5] A set of cultures for each individual consisted of an unstimulated control culture in duplicate and stimulated cultures each containing one of the following: 0.05 ml PHA (Phytohemagglutinin-M, Difco Laboratories), 0.1 ml SLO (Streptolysin-O, Difco), and 0.1 ml Streptokinase-Streptodornase (Varidase, Lederle Laboratories). The cultures were incubated for 7 days at 37°C in 5% carbon dioxide in air. On the seventh day of culture, the cultures were pulse labeled for 3 hr with 2 μCi ^{3}H-thymidine (specific activity 1.9 Ci/mmole; Schwarz BioResearch, Orangeburg, N.Y.), and the incorporation of isotope in the acid-insoluble fraction of the lymphocytes determined by liquid scintillation counting. ^{3}H-thymidine incorporation was expressed as cpm/10^{6} lymphocytes. The isotope incorporation of the

appropriate unstimulated control was subtracted from that of the stimulated cultures to determine the net ^{3}H-thymidine incorporation.

RESULTS

The mean numbers of leukocytes and lymphocytes collected during a single leukapheresis per liter of blood processed were 1.27×10^{10} (range 0.24×10^{10} –12.1×10^{10}) and 1.25×10^{10} (range 0.20×10^{10}–12.0×10^{10}), respectively. A direct linear relationship existed between the number of lymphocytes collected by leukapheresis per liter of blood processed and the lymphocyte count before leukapheresis (correlation coefficient, $r = + 0.93 \pm 0.01$). This relationship was expressed by the equation, $y = 0.31 \times 10^{10} + 2.2 \times 10^5 x$, where y was the number of lymphocytes collected per liter of blood processed and x was the absolute lymphocyte count per cu mm before leukapheresis. A similar relationship was found between the number of lymphocytes collected by leukapheresis corrected for the level of the circulating lymphocyte count and the volume of blood processed during a procedure (correlation coefficient $r = + 0.62 \pm 0.05$). The regression equation calculated for this relationship was $y = (3.4 + 2.2x)\ 10^9$, with y the number of lymphocytes collected per 10,000 circulating lymphocytes and x the volume of blood processed in liters during a procedure. Variation in the rate of blood flow through the centrifuge from 30 to 50 ml/min did not alter the magnitude or efficiency of the cell collection.

The 13 patients underwent 240 leukapheresis procedures (Table 1). Patient J. C. was maintained on intermittent leukapheresis therapy for 127 days and later was treated by intensive leukapheresis when there was an increase in the activity of her disease. Patients O. H. and W. S. each had two series of intensive leukapheresis separated by 146 days and 32 days, respectively. The longer procedures of the intensive leukapheresis group of patients were accompanied by increased removal of platelets and increased blood loss. The number of cells collected and the efficiency with which the leukocytes were collected were similar for both groups of patients. The leukaphereses were not associated with significant morbidity, and most of the patients were treated as out-patients.

Two patients in the intermittent leukapheresis group had objective evidence of regression of their disease during treatment (Table 2). Patient G. M. had a decrease in the peripheral leukocyte count and lymphadenopathy. His disease remained stable for nearly 6 mo after stopping leukapheresis therapy. The patient J. C. had a similar decline in leukocyte count and a decrease in spleen size. The lymphocyte count of this patient increased after cessation of leukapheresis with a doubling time of 60 days. By 95 days postleukapheresis there was increased lymphadenopathy and the spleen was palpable again. The other three patients had evidence of slow progression of disease during the period of leukapheresis.

Eight of the nine patients in the intensive leukapheresis group had regression of their disease during leukapheresis (Table 3). Eight patients had a decrease in lymphadenopathy, and for six of these the decrease was greater than 50%. There was a decrease in splenomegaly in five patients and a decrease in hepa-

Table 1. Summary of Leukocyte Collections From Patients With Chronic Lymphocytic Leukemia

Patient	No. Procedures	Duration of Therapy (Days)	Frequency of Leukapheresis (Days)	Mean Volume Blood Processed/ Procedure (Liters)	Total Leukocytes Collected × 10^{11}	Efficiency Leukocyte Collection (%)*	Total Lymphocytes Collected × 10^{11}	Efficiency Lymphocyte Collection (%)*	Mean Platelet Loss (%) per Procedure†	Mean Blood Loss (ml)‡
Intermittent Leukapheresis										
R.H.	19	340	18.8	5.5	17.34	26.2	26.60	27.0	10.2	30.0
L.B.	25	171	7.0	5.0	16.75	27.6	15.51	29.1	27.1	16.3
G.M.	21§	354	16.8	5.4	18.23	27.0	18.01	29.4	14.9	24.7
J.C. (1)	16	127	7.9	5.4	19.25	36.2	18.91	38.1	14.7	20.0
C.M.	17	566	33.3	5.7	12.32	25.1	11.94	29.3	13.3	13.4
Intensive Leukapheresis										
A.N.	12	34	2.8	8.1	13.16	24.9	12.45	28.2	17.4	12.8
E.M.	15	26	1.7	7.8	11.64	28.7	11.34	33.3	23.5	19.8
J.C. (2)	22	31	1.4	7.7	16.98	37.9	16.38	45.8	20.8	23.7
V.M.	9	28	3.1	6.4	6.13	37.2	5.96	39.7	27.0	49.7
O.H. (1)	20	28	1.4	8.7	8.58	31.5	8.39	40.9	23.0	46.2
O.H. (2)	5	5	1.0	9.4	3.33	32.0	3.30	39.4	13.9	34.4
L.T.	21	30	1.4	12.7	47.88	27.4	47.37	31.9	38.3	93.9
J.M.	13	16	1.2	13.0	16.50	38.8	15.76	51.1	18.7	61.9
J.C.M.	12	19	1.6	14.4	11.80	26.6	11.59	28.6	8.5	45.0
W.S. (1)	4	4	1.0	10.0	3.22	29.0	3.11	31.0	22.7	72.5
W.S. (2)	9	11	1.2	10.0	3.62	28.5	3.90	29.1	11.8	50.7

*Number of leukocytes (lymphocytes) collected × 100/average of leukocyte (lymphocyte) count before and after leukapheresis × volume blood processed (1) × $10.^{3}$

†Number platelets collected × 100/platelet count preleukapheresis × 10^{3} × blood volume (1).

‡Volume red blood cells in buffy coat collection.

§Median values underlined.

Numbers in parentheses indicate number of studies.

Table 2. Clinical Changes During Itermittent Leukapheresis Therapy of Patients With Chronic Lymphocytic Leukemia

Patient	Time of Study	Hemato-crit (%)	Leukocyte Count	Absolute Lymphocyte Count	Platelet Count	Spleen	Liver	Lymph Nodes	Remarks
R.H. F, 72 yr	Before*	28	65,700	57,800	303,000	6 cm below L.C.M.‡	8 cm below R.C.M.§	No change	Increasing easy Tiring and Lethargy
	After†	29	137,000	128,000	134,000	8 cm below L.C.M.	6 cm below R.C.M.		
L.B. F, 71 yr	Before	35	45,000	42,600	61,000	2 cm below L.C.M.	2 cm below R.C.M.	Increase in 6 of 6	Antiglobulin test became positive
	After	32	89,700	87,700	54,000	No change	No change		
G.M. M, 55 yr	Before	38	227,300	220,000	228,000	7 cm below L.C.M.	Not palpable	Greater than 50% decrease of 3 of 4	Improved general well-being
	After	48	41,000	35,600	143,000	No change	Not palpable		
J.C. (1) F, 68 yr	Before	34	165,000	165,000	213,000	1 cm below L.C.M.	Not palpable	No change	Asymptomatic
		35	57,000	45,600	227,000	Not palpable	Not palpable		
C.M. M, 54 yr	Before	49	35,000	38,700	164,900	Not palpable	Not palpable	Increase in 4 of 6	Asymptomatic
	After	46	71,300	68,400	163,000	Not palpable	Not palpable		

*Before series of leukapheresis.
†On completion of series of leukapheresis.
‡L.C.M., Left Costal Margin.
§R.C.M., Right Costal Margin.

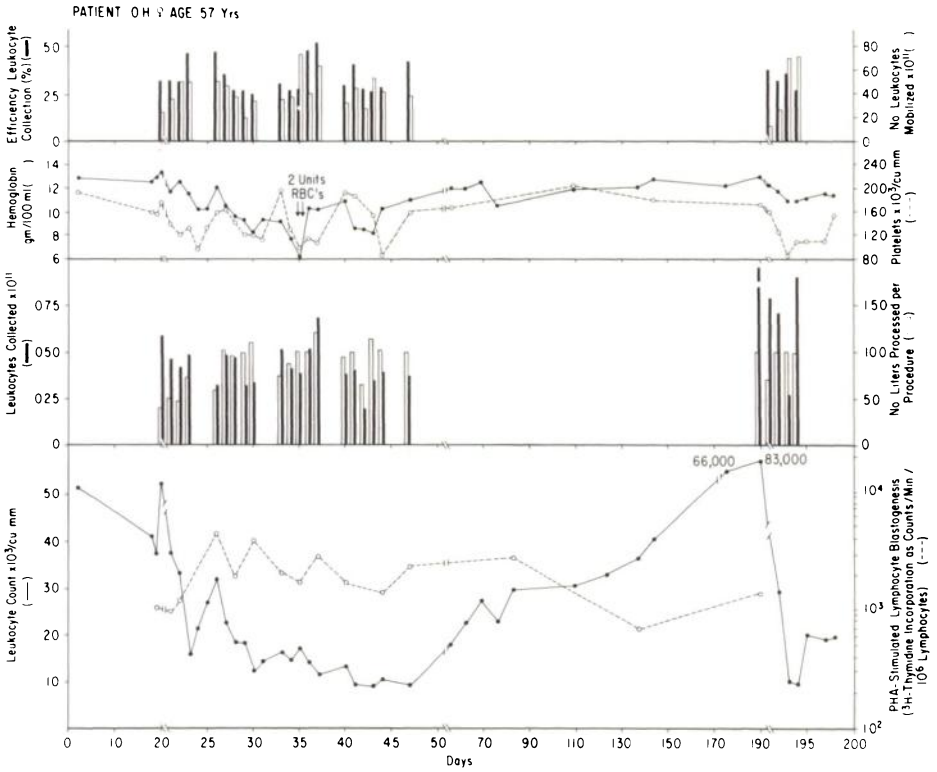

Fig. 1. Rate of decline of lymphocyte count during intensive leukapheresis therapy, and rate of increase of lymphocyte count after leukapheresis. Middle line indicates the mean, and shaded area equals 1 SO.

tomegaly in one. Seven patients had a decrease in the lymphocyte infiltration of the bone marrow ranging from 7% to 25% (median 11%). The absolute lymphocyte infiltration of the bone marrow decreased in two patients. Five patients had subjective improvement, including four with improved general well-being and one with decreased hypersensitivity to mosquito bites. The median time required for patients showing a hematologic or clinical response during the leukaphereses to return to their preleukapheresis status was 106 days and ranged from 54 to 141 days.

Ten of the 11 series of intensive leukapheresis resulted in a decrease in the total leukocyte and absolute lymphocyte count (Table 3). The median reduction in the lymphocyte count by the end of these ten series of leukapheresis was 76.5% (range 57%–97%). The first four to five leukaphereses resulted in a rapid decrease in the lymphocyte count of these patients (Fig. 1). The lymphocyte count decreased at a slower rate during the remaining leukaphereses.

In the first week after completion of the series of leukaphereses, the lymphocyte count increased rapidly. Thereafter, the lymphocyte count rose more slowly. The median doubling time of the lymphocyte count during the latter period was 71 days in the six patients evaluated. The typical hematologic changes during intensive leukapheresis are illustrated in Fig. 2 for a patient who had two series of intensive leukapheresis.

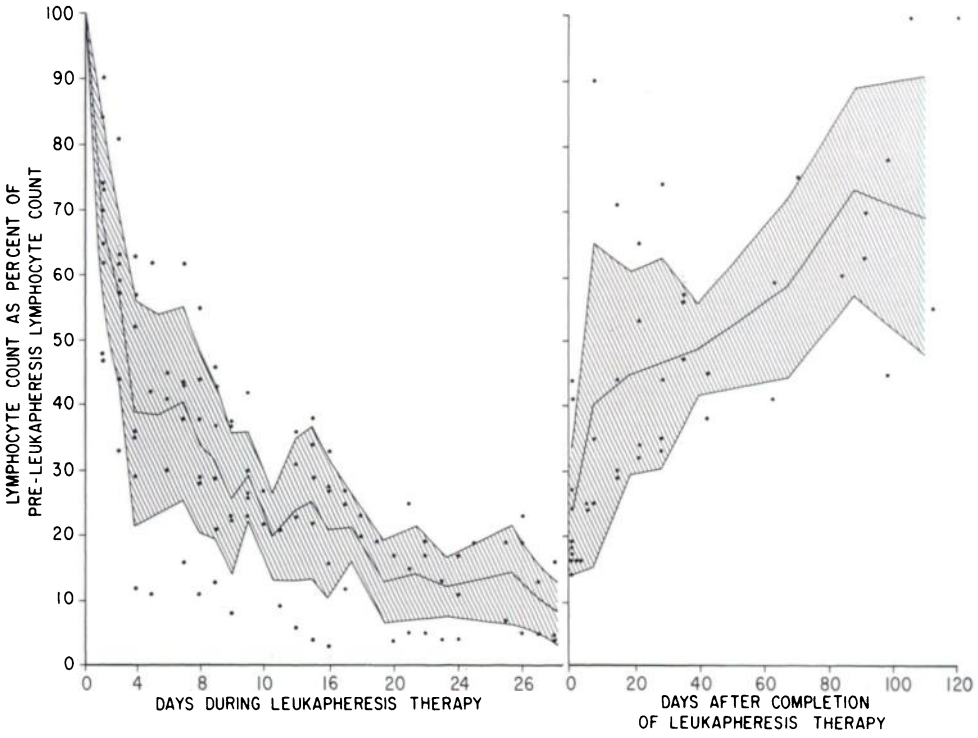

Fig. 2. Summary of clinical course of patient O.H. who was treated with intensive leukapheresis therapy on two occasions. Changes in leukocyte count and other parameters observed in this patient were typical of patients having intensive leukapheresis.

One patient (J. C. M.) received prednisone for 8 days during intensive leukapheresis therapy with a transient increase in his lymphocyte count, the number of lymphocytes collected, and the number of cells mobilized during leukapheresis. Despite the removal of 11.6×10^{11} lymphocytes, the leukocyte count at the end of the series of leukapheresis was not decreased from the pre-leukapheresis level of 22,400 (Table 3). The increased mobilization of lymphocytes in this patient during prednisone administration is in contrast to the relatively constant rate of lymphocyte mobilization in the other patients (Fig. 2).

The effects of intensive leukapheresis on in vitro lymphocyte blastogenesis were studied in nine patients (Table 4). PHA-stimulated lymphocyte blast transformation increased in six, remained unchanged in one, and decreased in two patients. The SLO- and SK-SD-stimulated lymphocyte blastogenic responses followed similar patterns, although the increase in ^{3}H-thymidine incorporation were less.

The in vitro blastogenic responses of the lymphocytes of patients having intensive leukapheresis were corrected for the effect of the total number of lymphocytes per ml of blood by multiplying the ^{3}H-thymidine incorporation in cpm/10^6 lymphocytes by the absolute lymphocyte count per ml on that day (Table 4). The PHA-stimulated blastogenic response per ml of blood decreased

Table 3. Clinical Changes During Intensive Leukapheresis Therapy of Patients With Chronic Lymphocytic Leukemia

Patient	Study Time of	Hemato-crit (%)	Platelet Count	Leukocyte Count	Absolute Lymphocyte Count	% Reduction Lymphocyte Count	Doubling Time of Lymphocyte Count (days)	Spleen	Liver	Lymph Nodes
A.N. M, 55 yr	Before*	29	60,000	96,700	92,000			6 cm below R.C.M.§	Not palpable	Greater than 50% decrease
	After†	23	38,000	43,000	40,000	57	–	Not palpable	3 cm below R.C.M.	of 3 of 5
E.M.	Before	38	150,000	112,400	106,000			Not palpable	Not palpable	Less than 50%
F, 67 yr	After	37	128,000	16,000	14,200	93	66	Not palpable	Not palpable	decrease of 2 of 3
V.M. F, 58 yr	Before	34	115,000	71,600	70,900			1 cm below L.C.M.‡	3 cm below R.C.M.	Less than 50% decrease of
	After	34	85,200	29,300	29,000	59	29	No change	No change	1 of 2
J.C. (2) F, 68 yr	Before	37	158,000	123,000	120,300			2 cm below L.C.M.	Not palpable	Greater than 50% decrease
	After	32	144,000	19,000	15,200	87	54	Not palpable	Not palpable	of 4 of 4
O.H. (1) F, 57 yr	Before	44	180,000	52,400	48,700			1 cm below L.C.M.	Not palpable	Greater than 50% decrease
	After	38	156,000	9,400	6,590	86	118	Not palpable	Not palpable	of 6 of 6
O.H. (2)	Before	40	174,000	83,000	78,020			1 cm below L.C.M.	Not palpable	No change in 6 nodes
	After	33	106,000	20,700	19,200	75	–	No change	Not palpable	

L.T. M, 54 yr	Before	39	144,000	698,000	684,000			8 cm below L.C.M.	16 cm below R.C.M.	Greater than 50% decrease of 1 of 4
	After	30	90,000	26,800	22,000	97	–	6 cm below L.C.M..	4 cm below R.C.M.	
J.M. F, 43 yr	Before	37	153,000	67,000	66,300			6 cm below L.C.M.	Not palpable	Greater than 50% decrease
	After	28	168,000	18,100	15,200	77	74	5 cm below L.C.M.	Not palpable	of 2 of 4
J.C.M.	Before	45	245,000	22,400	20,500			Not palpable	Not palpable	No change in
M, 52 yr	After	35	238,000	21,900	20,000	2	–	Not palpable	Not palpable	4 nodes
W.S. (1)	Before	26	188,000	59,000	57,800			Not palpable	Not palpable	No change in
M, 58 yr	After	30	124,000	20,800	19,600	66	–	Not palpable	Not palpable	6 nodes
W.S. (2)	Before	29	230,000	32,800	31,800			Not palpable	Not palpable	Greater than
	After	28	158,000	10,000	7,700	76	86	Not palpable	Not palpable	50% decrease of 2 of 4

*Before series of leukapheresis.
†On completion of series of leukapheresis.
‡Left Costal Margin.
§Right Costal Margin.

Table 4. Effects of Intensive Leukapheresis Therapy on PHA-Stimulated In Vitro Lymphocyte Blastogenesis

	Median PHA-Stimulated Lymphocyte Blastogenic Responses			
	CPM/10^6 Lymphocytes		CPM/ml Blood ($\times 10^5$)*	
Patient	Before	During	Before	During
E.M.	11,335 (1)†	*22,333* (11)	110 (1)	83 (11)
V.M.	13,368 (1)	10,507 (5)	95 (1)	36 (5)
J.C. (2)	*11,468* (1)‡	16,126 (13)	132 (3)	*44* (12)
O.H. (1)	10,804 (1)	20,152 (9)	40 (1)	37 (9)
L.T.	438 (1)	24,313 (8)	15 (1)	64 (10)
J.M.	12,261 (1)	23,527 (6)	*82* (1)	73 (6)
J.C.M.§	16,041 (1)	7,534 (9)	35 (1)	26 (8)
W.S. (1)	29,632 (1)	29,398 (1)	174 (1)	63 (1)
W.S. (2)	10,820 (1)	23,924 (4)	35 (1)	43 (3)
Normal range	23,300-128,000		56-192	

*Product cpm/10^6 lymphocytes × absolute lymphocyte count per ml.
†Numbers in parenthesis indicate number of studies.
‡Middle values italicized.
§On prednisone.

in seven and increased in two patients. Similar but less marked changes were observed for the SLO- and SK-SD-stimulated lymphocyte blastogenic responses.

The percentage of positive delayed hypersensitivity responses to common antigens remained unchanged during leukapheresis in four and decreased in three of the patients evaluated before and after therapy. The positive skin tests after therapy were smaller than the pretreatment responses. The median decrease was 34% (range 18%–48%).

DISCUSSION

This study has demonstrated that leukapheresis with the Blood Cell Separator is a useful new technique for the study and treatment of patients with CLL. Serial leukapheresis induced objective partial remissions lasting over 3 mo. This therapy was not associated with significant thrombocytopenia or other morbidity and did not require hospitalization. In contrast to chemotherapy or radiation therapy, leukapheresis can be employed intermittently to maintain patients for many months in a clinically stable condition without the development of drug resistance or permanent bone marrow damage.[6-8]

Direct relationships between the level of the lymphocyte count and the volume of blood processed and the number of lymphocytes collected indicate that both methods for increasing the mobilization of lymphocytes from the tissues and longer procedures may increase the effectiveness of leukapheresis in patients with CLL. Administration of glucocorticosteroids to one patient having intensive leukapheresis therapy was associated with an increase in the lymphocyte count, an increase in the number of cells collected, and an increase in the number of lymphocytes mobilized from lymphoid tissues. The use of steroids with leukapheresis warrants further investigation.[9,10] Red blood cell

agglutinating agents, such as hydroxyethyl starch, have increased the yield of granulocytes collected from normal persons.[11] Application of this technique also may improve the efficiency of leukapheresis of patients with CLL.

Intensive leukapheresis therapy may be compared with the effects of extra corporeal irradiation of the blood (ECIB) on CLL.[12-16] Sixteen of 18 patients treated with ECIB had a decrease in lymphocyte count that persisted from 1 to over 12 mo (median approximately 5 mo).[12-16] Decrease in spleen size, lymphocytic infiltration of the bone marrow, or decreased lymph node size has been recorded for six of these patients. However, 14 of the patients were treated with glucocorticosteroids or had other therapy both during and after ECIB. In contrast, the remissions of CLL obtained with intensive leukapheresis were not associated with the administration of glucocorticosteroids or other treatment. In addition, these remissions were associated regularly with objective decreases in tissue levels of leukemic cells.

During leukapheresis the lymphocyte counts tended to decrease rapidly during the first four to six leukaphereses and then decrease slowly. A similar pattern of change in the peripheral lymphocyte count has been observed during ECIB.[16] This suggests that mobilization of lymphocytes from the tissues was slow or that some populations of lymphocytes do not have ready access to the circulation.[17,18] On the other hand, an equilibrium may have been established during the series of leukapheresis between the rate of removal of lymphocytes and the rate of proliferation of lymphocytes and their entry into the blood.[17]

Investigations of the cell kinetics of CLL have suggested that the fraction of proliferating cells is small.[19,20] The doubling times of the lymphocyte count after intensive leukapheresis and ECIB may measure either the rate of tumor cell accumulation or the rate of entry of leukemic cells into the blood from areas relatively inaccessible to the circulation. The concurrent increase in spleen and lymph node sizes suggest that the former may be the case. Further investigation of the proliferative fraction of CLL is indicated as cycle-sensitive chemotherapeutic agents may have a role in the chemotherapy of CLL either alone or in conjunction with alkylating agents or leukapheresis.[21]

The studies on delayed hypersensitivity and in vitro lymphocyte blastogenesis again have illustrated an immunologic defect in patients with CLL.[22,23] The absolute number of mitogen- and antigen-reactive lymphocytes in the blood decreased during leukapheresis therapy, suggesting that responsive and, presumably, normal lymphocytes were removed along with the leukemic cells during the leukapheresis. However, the increase in lymphocyte blastogenesis per 10^6 lymphocytes during leukapheresis indicates that normal lymphocytes were being generated more rapidly than leukemic lymphocytes, entered the circulation more rapidly, or were removed less rapidly than the leukemic cells. This is in keeping with the concept that there are several populations of cells in CLL.[17,24]

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