Increased Blood Clearance Rate of Indium-111 Oxine-Labeled Autologous CD4+ Blood Cells in Untreated Patients With Hodgkin's Disease

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Untreated patients with Hodgkin's disease (HD) have a blood T-lymphocytopenia mainly caused by a reduction of the CD4+ subset. Indirect support for a sequestration of T cells in the spleen and tumor-involved lymphoid tissue has accumulated. To test the hypothesis that the blood CD4 T-lymphocytopenia in patients with HD is caused by an altered lymphocyte traffic, 12 untreated HD patients and five in complete clinical remission (CCR) were studied. Blood lymphocytes were collected by leukapheresis and gradient centrifugation, and were further purified by an adherence step. The cells were labeled with indium-111 oxine and reinfused intravenously into the patient. The radioactivity of CD4+ and CD8+ blood lymphocytes separated by immunoabsorption was measured from serial blood samples. CD4+ cells were eliminated more rapidly in untreated patients than patients in CCR. Repeated γ camera imaging after autotransfusion of indium-111 oxine labeled cells demonstrated an accumulation of radioactivity in tumor-involved tissue of untreated patients. These findings support the concept of an enhanced elimination of CD4+ cells in patients with active HD that may contribute to the observed blood T-lymphocytopenia and may reflect a biologic response to the tumor.

THE MAJORITY of untreated patients with Hodgkin's disease (HD), including those with limited disease, exhibit impaired cell-mediated immunity and a reduced number of circulating T cells as well as alterations in the relative proportion of phenotypically and functionally distinct T-cell subsets.[1-4] The T-lymphocytopenia is mainly attributed to a lack of CD4+ cells.[4,5] Decreased in vitro synthesis of interleukin-2 (IL-2) and interferon-γ has also been reported in both untreated and treated patients with HD.[6,7] A persisting functional immune defect has been documented in long-term survivors after successful therapy. T-lymphocytopenia is also a characteristic of patients in continuous unmaintained complete remission, in particular after radiotherapy, although the lymphocyte count seems to recover at a slow rate.[7]

Although T-lymphocytopenia is present in all clinical stages, it tends to be more pronounced in patients with advanced disease. Moreover, patients with B symptoms usually have lower lymphocyte counts than asymptomatic cases, possibly reflecting the lower proportion of stage III/IV disease among the latter.[8,9] Most studies have not demonstrated any association between blood lymphocyte counts and histopathology, age, or sex. It may be concluded that HD patients not exposed to cytoreductive treatment display a decrease in total and relative CD4+ cell counts that is associated with the extent of disease.[10]

The mechanisms of the T-lymphocytopenia and of the T-cell deficiency in HD are unknown, but may be due to decreased production, increased destruction, or maldistribution of lymphocytes among lymphoid compartments. Consequently, an altered lymphocyte traffic may contribute to the T-lymphocytopenia and to a relative change in blood T-cell subsets. Indirect support for the sequestration of T cells in the spleen and tumor-involved lymphoid tissues has accumulated.[11,12] Thus far, the information from previous studies of lymphocyte traffic in HD has been limited by either the use of indirect methods[13] or has not been given consideration to the T-cell subpopulations involved.[13-15]

In this report we have used indium-111 labeled autologous blood lymphocytes to study the elimination of CD4+ and CD8+ cells from the blood in patients with HD. The results unequivocally show selective increase in the elimination rate of the CD4+ cells in patients with active HD.

PATIENTS AND METHODS

Patients

The patient series include 7 women and 9 men with a median age of 36 years (range 16 to 65 years). Patient characteristics and clinical data are presented in Table 1. One patient (no. 9) was studied before and after successful chemotherapy. There were 12 previously untreated patients and five patients in complete clinical remission (CCR). Patients who had undergone splenectomy or who received radiotherapy to the spleen were excluded from the study, as were severely ill patients requiring prompt institution of therapy. Clinical staging followed the Ann Arbor recommendations,[16] and histopathology was subclassified according to the Rye nomenclature.[17] In one patient (no. 2), the diagnosis of HD was established after cytologic examination of tumor tissue (for further details on clinical procedures see references 18 and 19). The control series of blood lymphocyte subset studies consisted of 108 healthy hospital staff members with a median age of 32 years (range 18 to 74 years). The study was approved by the Ethics Committee at Karolinska Institute and the Isotope Committee at Karolinska Hospital, Stockholm, Sweden. All patients gave informed consent.

Methods

The various methodologic steps are summarized in Fig 1.

Preparation, tracing, and imaging of indium-111 oxine-labeled cells. An IBM 2997 cell separator (Cobe Laboratories, Lakewood, CO) was used for the harvest of lymphocyte-enriched leukocytes from the patients. Cannulas were inserted into each of the cubital or femoral veins. The blood was anti-coagulated with acid citrate dextrose. The lymphocyte-enriched buffy coat, which was collected

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Table 1. Demographic and Clinical Data of Patients

No.	Symbol*	Sex	Age (y)	Clinical Stage at Diagnosis	Histopathology†	Disease Status (duration of CCR; treatment)‡
1	◈	F	18	IVB	NS	CCR (2; CT + RT)
2	⊠	F	20	IIB	UC	CCR (3; CT)
3	●	F	27	IA	LP	UT
4	■	F	43	IIB	LP	UT
5	△	M	46	IVB	MC	UT
6	♦	M	64	IVB	LD	UT
7	▼	F	20	IA	MC	UT
8	◘	F	40	IA	NS	UT
9a	◑	M	40	IIIA	MC	UT
9b	◑	M	41	IIIA	MC	CCR (23;CT)
10	□	M	30	IVB	NS	UT
11	▽	M	55	IVB	NS	UT
12	△	M	65	IB	NS	UT
13	○	M	16	IIIA	MC	UT
14	◊	F	36	IIA	NS	UT
15	×	M	26	IA	MC	CCR (14; RT)
16	+	M	31	IIB	LP	CCR (16; CT + RT)

*Symbols refer to Table 3 and Figs 2 through 4.

†LP, lymphocyte predominance; NS, nodular sclerosis; MC, mixed cellularity; LD, lymphocyte depletion; UC, unclassified.

‡CCR, complete clinical remission (duration in months); CT, chemotherapy; RT, radiotherapy; UT, untreated.

from about 5 L of whole blood, was diluted in 100 mL Hanks' salt solution without calcium, magnesium, or phenol red (HSS, pH 6.5). An IBM 2991, Model I Blood Cell Processor (Cobe Laboratories) was used to purify the lymphocytes. At 600*g* the buffy coat was layered over the 150 mL Lymphoprep (Nycomed AS, Oslo, Norway; 50 mL/min). The system was rinsed with 40 mL HSS. After centrifugation for 25 minutes the cells in the lymphocyte-rich interphase layer were harvested and washed three times with HSS containing 5% human albumin; the first wash at 600*g* for 75 seconds, the second at 400*g* for 60 seconds, and the third at 340*g* for 60 seconds. The lymphocyte-rich pellet was resuspended in HSS with albumin and the cells were counted. To purge the suspension from monocytes and granulocytes, 10^8 cells in 30 mL balanced salt solution (BSS) with 10% autologous fresh plasma were added to plastic bottles (600 mL/175 cm^2; Nunc Intermed, Roskilde, Denmark). The cells were incubated at 37°C for 1 hour. After gentle shaking the nonadherent cells were removed. The bottles were rinsed once with BSS and nonadherent cells were pooled. Cell aggregates were removed by sedimentation for 2 minutes and the nonaggregated cells in the supernatant were counted. After centrifugation of the supernatant at 400*g* for 10 minutes, the cells were resuspended in 5 mL BSS with 5 MBq indium-111 oxine per 10^9 cells. After incubation for 15 minutes, 15 mL BSS and 5 mL autologous plasma were added. After centrifugation at 400*g* for 10 minutes, the supernatant was removed and the cells were resuspended in 10 mL saline and counted. The radioactivity was measured and a sample was collected for T-cell subset separation (see below). The procedure was performed under strict sterile conditions.

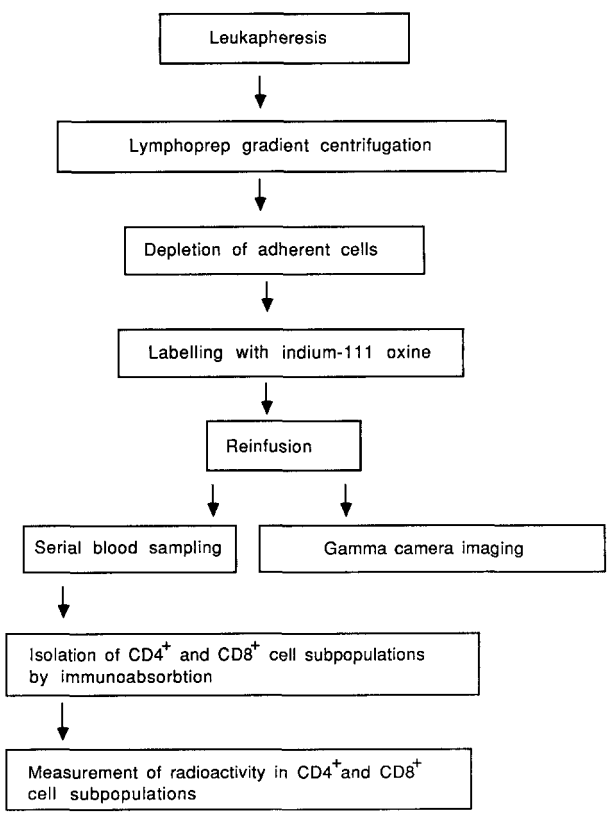

Fig 1. Methodologic study design.

Reinfusion of cells. The labeled cell suspension was infused intravenously over 1 to 2 minutes.

Serial blood sampling. Two to four blood samples were drawn from each patient at different intervals.

Purification of lymphocytes for marker studies. Lymphocytes were enriched by Ficoll-Isopaque (Pharmacia, Uppsala, Sweden) gradient centrifugation of heparinized venous blood. After incubation with iron powder, phagocytic cells were removed by a magnet.[20] The lymphocyte suspensions contained more than 98% viable lymphocytes as assessed by trypan blue exclusion test.

Lymphocyte surface markers. T cells and subsets were identified using monoclonal antibodies (MoAbs) OKT3 (CD3), OKT4 (CD4), and OKT8 (CD8) (Ortho Pharmaceutical Corp, Raritan, NJ) in indirect immunofluorescence using a fluorescein-conjugated absorbed goat anti-mouse IgG (Meloy Lab, Springfield, VA) in the second step as described previously.[21] Two hundred to 400 cells were counted using a Leitz Dialux 20 B fluorescence microscope (Ernst

Leitz, GMBH, Wetzlar, West Germany) with epi-illumination in ordinary light and UV light at 1,000 magnification.

T-cell subset separation. For determination of radioactivity in the $CD4^+$ and $CD8^+$ cell subsets, respectively, the following technique was used in the first seven experiments. Lymphocytes were separated into T and non-T cells by a rosetting technique. Sixty to 80×10^6 lymphocytes were suspended in 5 mL of RPMI 1640 (Flow Laboratories, Irvine, Ayrshire, Scotland) with 10 mmol/L HEPES buffer and 20% fetal calf serum (FCS; Flow Laboratories) and mixed with 1 mL of 10% neuraminidase (Sigma Chemical Co, St Louis, MO) treated sheep red blood cells (SRBC) in 100% FCS. The cells were incubated at 37°C for 15 minutes, centrifuged for 5 minutes at 150*g*, and then incubated for 2 hours on ice. The pellet was then gently resuspended and layered onto a Ficoll-Isopaque gradient. After centrifugation for 20 minutes at 600*g*, unrosetted cells (non–T-cell fraction) were removed from the interphase layer and washed twice in 0.15 mol/L Hanks Tris (HT) buffer. The pellet (T-cell fraction) was resuspended in 5 mL of RPMI with 10% FCS and incubated at 37°C for 15 minutes and agitated on a Vortex. After centrifugation for 6 minutes at 600*g*, SRBCs were lysed after the addition of 1 mL of distilled water and 0.5 mL of HT containing 10% FCS. The cell suspension was agitated on a Vortex (Scientific Industries, Bohemia, NY) for 20 to 30 seconds and 8 mL of HT was added. The cells were washed twice in HT.

Polyvalent rabbit anti-mouse immunoglobulin G (IgG; Dako/AS, Copenhagen, Denmark) was purified on cyanogen bromide-activated Sepharose (Pharmacia) coated with normal mouse IgG diluted in trypsin buffer without Ca^{2+} and Mg^{2+}. Two milliliters of the anti-mouse IgG (12.25 g of protein/mL) was added to plastic Petri dishes (Falcon 3002; Becton Dickinson, Oxnard, CA). After gentle agitation for 1 hour at room temperature, the dish was rinsed twice with trypsin buffer. Two milliliters of Protein A (50 μg/mL) (Pharmacia), diluted in trypsin buffer, was then added to each dish followed by gentle agitation for 1 hour at room temperature. The dish was rinsed twice with trypsin buffer, and 2 mL of trypsin buffer with 20% FCS were added to each dish. Lymphocytes, 15×10^6, (T-cell fraction) in 0.1 mL of RPMI 1640 with 5% FCS and 0.1 mL of OKT4 or OKT8 MoAbs, respectively (diluted 1:100), were incubated on ice for 1 hour and washed three times with trypsin buffer containing 5% FCS. The cells were suspended in 2 mL of typsin buffer with 20% FCS and poured onto the coated dishes. The cells were allowed to settle during one centrifugation (5 minutes at 150*g*). The nonbound cells were collected after gentle tilting of the dish. The dishes were washed twice with RPMI 1640 containing 5% FCS. The nonbound cells were washed three times in HSS. Lymphocyte subpopulations were characterized and enumerated by indirect IFL using MoAbs (see above). The radioactivity of all samples from each patient was measured in a γ counter (Wallac, Turku, Finland) on the same occasion.

In the last 10 experiments the following technique was used for cell separation and radioactivity measurement of indium-111 oxine-labeled T-cell subsets.

Lymphocytes were isolated as described above. MoAbs OKT4 or OKT8 were mixed with the cells, respectively (50 $\mu g/10^6$ cells). The cells were incubated for 30 minutes on ice and washed twice. Dynabeads M450 sheep anti-mouse IgG (Dynal AS, Oslo, Norway), 7.5 μL, were added to 10^6 cells and incubated for 30 minutes on ice. Cells bound to the beads were removed by a magnet and resuspended in 2 mL of phosphate-buffered saline. The relative proportion of $CD4^+$ and $CD8^+$ cells was analyzed using Simultest (Becton Dickinson) and flow-cytometry (FACScan; Becton Dickinson). The radioactivity was determined as described above.

Calculation of blood lymphocyte radioactivity. The following formula was used to define the proportion of remaining blood lymphocyte radioactivity: $(A \times B \times C)/(D \times E \times F)$, where A = radioactivity (mean cpm/10^6 $CD4^+$ or $CD8^+$ cells); B = estimated number of circulating lymphocytes in the blood (calculated using an arbitrary blood volume of 5 L); C = relative proportion of $CD4^+$ or $CD8^+$ cells; D = relative proportion of $CD4^+$ or $CD8^+$ cells of the infused cells; E = radioactivity (mean cpm/10^6 given cells); and F = number of infused cells. $A \times B \times C$ = radioactivity of the T-cell subset in the blood; $D \times E \times F$ = radioactivity of the infused T-cell subset.

RESULTS

The median number of autotransfused labeled cells was 1.0×10^9 (range 0.2 to 2.6×10^9). The cell suspensions contained 90% lymphocytes (median; range 83% to 95%) and the viability (trypan blue exclusion test) was greater than 95% in all tested samples. The labeling efficiency of the radionuclide was 83% (median; range 79% to 90%) and total radioactivity infused was 6.3 MBq (median; range 1.9 to 12.3 MBq). Radioactivity per 10^8 cells varied between 0.3 MBq (7.1 μCi) and 1.1 MBq (29.1 μCi; median 0.7 MBq, 18.6 μCi).

The total blood lymphocyte count as well as total $CD4^+$ and $CD8^+$ cell counts were significantly lower in the patient group as compared with controls ($P < .01$; Student's unpaired *t*-test; Table 2). There was no statistical difference in total lymphocyte counts or subsets between untreated patients and patients in complete remission ($P > .05$; Mann-Whitney, nonparametric test). However, the proportion of $CD4^+$ cells was lower in patients in clinical remission ($P < .05$, Mann-Whitney, nonparametric test). The proportion of the $CD8^+$ cells did not differ between the two patient groups (Tables 2 and 3).

Between the first and last test after infusion of the autologous lymphocyte suspension, a decrease of the radio-labeled $CD4^+$ cell fraction was recorded. This decrease was 54% (median) in untreated patients, but only 8% in CCR patients ($P < .01$; Wilcoxon-Mann-Whitney test; Fig 2, Table 3). The corresponding values for the radio-labeled $CD8^+$ cells were 23% and 28%, respectively ($P > .05$; Fig 3, Table 3). As a consequence, the ratio between the residual radio-labeled $CD4^+$ and $CD8^+$ cell fractions in peripheral

Table 2. Lymphocyte Subsets in Patients and Controls

	Total Cell Counts × 10^{-9}/L Median (range)			Percentage Median (range)	
	Total Lymphocytes	$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
All patients (n = 17)	0.79 (0.23-1.92)	0.20 (0.06-0.86)	0.24 (0.05-0.92)	25 (13-53)	29 (15-60)
Untreated patients (n = 12)	0.76 (0.23-1.78)	0.22 (0.06-0.86)	0.21 (0.05-0.92)	30 (20-53)	28 (15-52)
CCR (n = 5)	0.86 (0.44-1.92)	0.15 (0.07-0.50)	0.29 (0.08-0.59)	17 (13-26)	33 (19-60)
Controls (n = 108)	1.85 (0.38-4.8)	0.90 (0.14-3.02)	0.47 (0.10-1.75)	45 (12-72)	23 (9-49)

Table 3. Blood Lymphocyte Counts and Change in Blood T-Cell Subset Bound Radioactivity

Patient No.	Symbol†	Blood Lymphocyte Counts* Total $\times 10^{-9}$/L	$CD4^+$ Cells $\times 10^{-9}$/L	%	$CD8^+$ Cells $\times 10^{-9}$/L	%	Relative Change in Blood T-Subset Bound Radioactivity $CD4^+$ %	$CD8^+$ %
1	◈	0.44	0.07	17	0.08	19	+36	−50
2	⊠	0.68	0.13	19	0.39	57	−8	−23
3	●	0.69	0.17	24	0.23	33	−45	−23
4	■	1.63	0.86	53	0.72	26	−74	−51
5	△	0.23	0.07	30	0.05	23	−56	−35
6	♦	0.99	0.53	53	0.33	33	−87	−84
7	▼	1.51	0.73	48	0.35	23	−82	−73
8	◘	0.24	0.06	25	0.07	28	−58	+13
9	◑	0.63	0.16	25	0.29	45	−54	−51
9b	◑	0.98	0.13	13	0.59	60	−10	−28
10	□	0.48	0.10	20	0.12	26	+13	+41
11	▽	0.45	0.09	20	0.12	26	−31	+31
12	△	1.78	0.43	24	0.92	52	−29	−16
13	○	1.74	0.43	25	0.36	21	−30	+14
14	◊	0.88	0.27	30	0.13	15	−29	−9
15	×	0.83	0.11	13	0.19	23	+71	+51
16	+	1.92	0.50	26	0.52	27	−65	−82

*As determined at the first blood sampling after autotransfusion.

†Symbols refer to Table 1 and Figs 2 through 4.

blood decreased in all previously untreated patients, while increased ratios were observed in the five patients in CCR ($P < .001$, Wilcoxon-Mann-Whitney test; Fig 4). No association between the elimination rate of either T-cell subset and clinical variables such as stage, age, histopathology, or sex was observed (Tables 1 and 3). The elimination rate of $CD4^+$ cells was positively correlated with both relative and total number of blood $CD4^+$ cells ($P < .001$ and $P < .01$, respectively; simple regression analysis), but not to total lymphocyte or total or relative $CD8^+$ cell count (Tables 1 and 3). A similar relationship between the elimination rate of $CD8^+$ and the relative or total numbers of the same T-cell subset was not found.

DISCUSSION

Among the various mechanisms that have been proposed to show the blood lymphocyte defect in HD patients, few account both for the persistent T-lymphocytopenia and the functional T-cell impairment. One attractive possibility to explain the major features of the immunodeficiency in blood is a decrease in responder or regulator blood T cells caused by increased destruction, decreased production, or maldistribution. The blood T-lymphocytopenia of untreated HD patients is mainly caused by a decrease of $CD4^+$ cells.[4,5] T-lymphocytopenia is seen in patients with localized disease but is more marked in patients with a large tumor burden.[4,10]

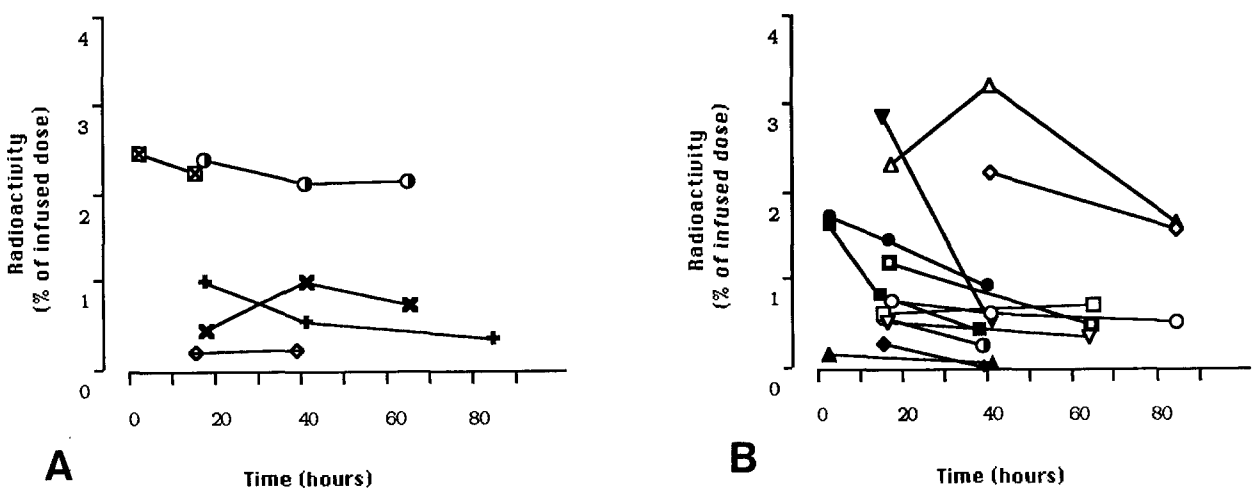

Fig 2. Remaining radioactivity in blood, bound to labeled and autotransfused blood $CD4^+$ cells, expressed as the percentage of the total infused dose in relation to time after infusion (for symbols see Tables 1 and 3). (A) Patients in CCR; (B) untreated patients.

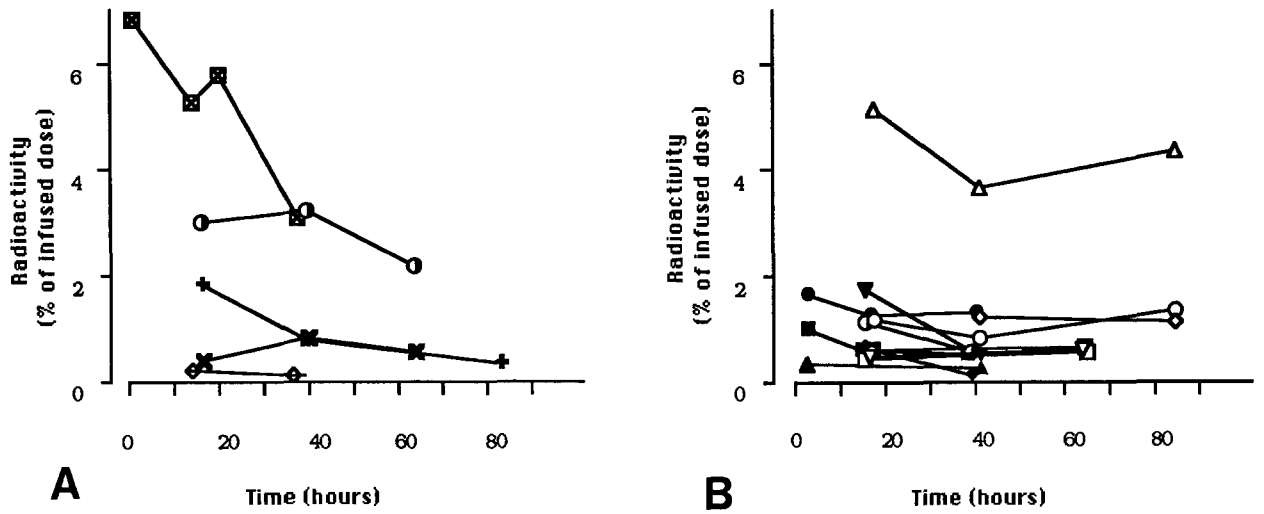

Fig 3. Remaining radioactivity in blood, bound to labeled and autotransfused blood CD8$^+$ cells, expressed as the percentage of the total infused dose in relation to time after infusion (for symbols see Tables 1 and 3). (A) Patients in CCR; (B) untreated patients.

In support of the hypothesis of an altered distribution of T cells are studies showing increased proportions of T lymphocytes, and especially CD4$^+$ cells, in tumor-involved tissue and spleens.[10] The increased mitogenic responsiveness of splenic lymphocytes, which has been observed,[22-25] and the strong inverse correlation between spleen weight and blood lymphocyte DNA synthesis induced by mitogens in HD patients with splenic involvement indirectly suggests a sequestration of T cells in the spleen.[26,27]

Recirculating lymphocytes traffic continuously between the blood and the lymph. Although the cells circulate freely in the blood stream, they seem to seek out those blood vessels that provide entry into specific lymphoid tissues.[28] The high endothelial cell venules (HEV) is the first barrier for the lymphocytes on their way from the blood into the tissue. Lymphocytes bind through the receptor to specific HEV structures, and that is the first step in the movement across the capillary wall.[29] Only recently Freedman et al[30] demonstrated that activated T cells and B lymphocytes in particular exhibit preferential adherence to the germinal center of the lymphoid follicle.

To explain the altered distribution of T-cell subsets in HD, a failure of T-cell migration and recirculation with a preferential accumulation of T cells within the spleen and tumor-involved lymph nodes (ecotaxopathy) has been suggested.[12] A lectin-binding galactose structure (HD lectin) on the surface of cultured HD cells as well as in HD involved tissue has been characterized.[31] This HD lectin binding structure functions as a receptor for T lymphocytes and is capable of mediating stimulation of the agglutinated lymphocytes. It

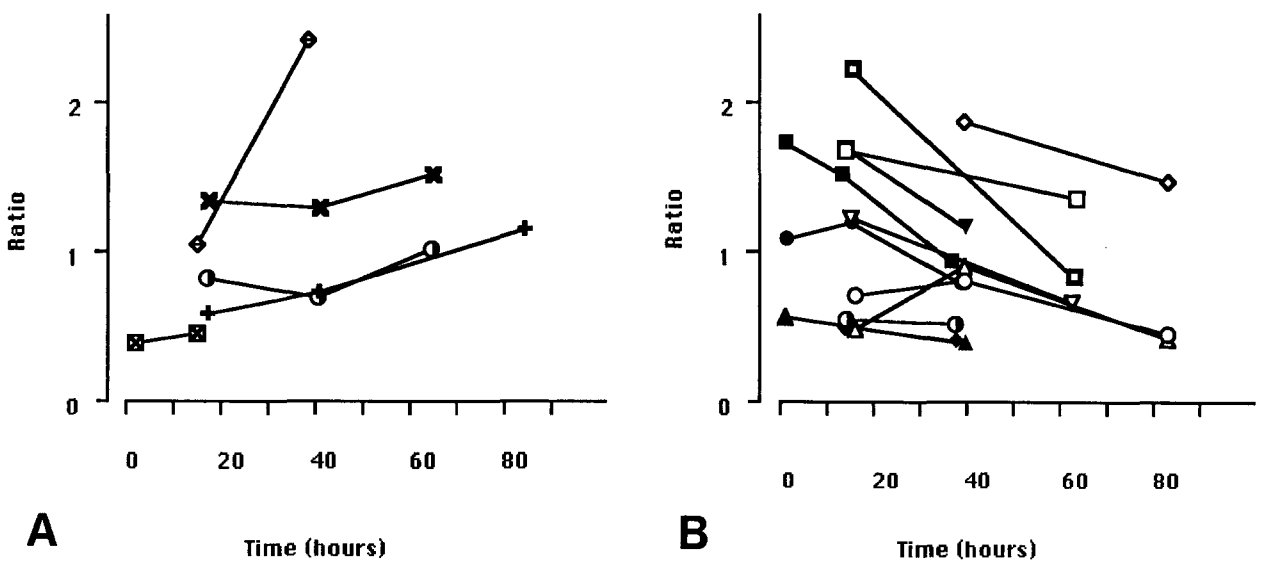

Fig 4. Ratio between the fraction of remaining radioactivity in blood, bound to labeled and autotransfused blood CD4$^+$ and CD8$^+$ cells, in relation to time after infusion (for symbols see Tables 1 and 3). (A) Patients in CCR; (B) untreated patients.

was postulated that the interaction between these two cell types results in mutual activation and stimulation of HD cell growth and rosette formation between Reed-Sternberg cells and, mainly, $CD4^+$ lymphocytes. Such a mechanism is compatible with an increased depletion of $CD4^+$ cells from the blood and the suggested sequestration of $CD4^+$ cells in HD tumor-involved tissue. Moreover, IL-2 production has been reported to be impaired in HD patients and might be a consequence of a diminished CD4 cell pool, which is the main producer of IL-2.

The understanding of lymphocyte migration has, to a great extent, been derived from studies using indium-111 oxine-labeled cells.[14,15] Alternative radionuclides such as ^{51}Cr have definite shortcomings with respect to labeling efficiency, poor γ camera imaging characteristics, long half-life (28 days), and dissociation of the radionuclide from lymphocytes. However, with the introduction of indium-111 oxine as an alternative labeling procedure, progress within this field has been made in humans.[32] Using this technique, further evidence for lymphocyte trapping has been demonstrated in HD. An increased accumulation of labeled blood lymphocytes was seen in tumor-involved lymph nodes.[14,33]

The functional integrity of indium-111 oxine-labeled lymphocytes has been questioned,[34] but the consensus is that a labeling dose of 3.7 MBq (100 μCi)/10^8 cells or less does not seem to influence lymphocyte kinetics and has been proposed for short-term lymphocyte migration studies.[35] However, even at this dose there appears to be an increased frequency of chromosomal aberrations in indium-111 oxine-labeled lymphocytes, and the risk for secondary lymphoid malignancies must be considered.[34] Nevertheless it should be stressed that the significance of chromosomal aberrations is largely unknown and the risk for tumor induction appears to be extremely small.[35] In the present study the median radioactivity per 10^8 cells did not exceed 1.1 MBq (29.1 μCi) in any patient.

The hypothesis of this study was that $CD4^+$ cells would be eliminated faster from the blood stream in patients with untreated HD as compared with cured patients. This would support the notion of a sequestration of $CD4^+$ blood cells into tumor-involved nodes. The results of the study unequivocally confirm a faster elimination of $CD4^+$ cells from the blood in patients with active disease compared with patients in CCR. Furthermore, the labeled $CD4^+$ cells decreased more rapidly than the $CD8^+$ cells in the untreated patients. There were no differences in clinical stage, histopathology, sex, age, number of autotransfused labeled cells, or radioactivity bound to infused cells between the two patient groups. No difference was seen in total lymphocytes or subset cell numbers between untreated patients and patients in CCR, but the proportion of $CD4^+$ cells was lower in CCR patients. The low lymphocyte and subset counts in CCR patients is explained by the effect of combination chemotherapy and/or radiotherapy also seen in patients with diseases other than HD.[7,10] Ideally, comparisons should be made using healthy volunteers, but such an approach has not been accepted from an ethical standpoint at our institution. In untreated patients of this study with a faster elimination of $CD4^+$ cells from the blood we have also observed, by γ camera imaging, an accumulation of radionuclide in enlarged lymph nodes.[18] However, such a pattern was not seen in CCR patients. The fast elimination of $CD4^+$ cells from blood of patients with active HD compared with patients in CCR, and an accumulation of radioactivity in tumor-involved lymph nodes strongly support the hypothesis of a sequestration of $CD4^+$ lymphocytes in tumor-involved tissues. The existence of tumor-specific cytotoxic T lymphocytes in human tumors[36] are most interesting for future cancer therapy. Such tumor-specific T lymphocytes might reside within the $CD4^+$ cell population. Another potential effective treatment of HD might be the use of indium-114m, a long half-life β-emitting radionuclide that can be chelated with oxine in the same way as indium-111 and labeled to autologous lymphocytes.[37] The cells migrate normally and a selective internal irradiation of lymphoid tissue might be obtained.[38] Treatment of HD with indium-114m–labeled autologous cells could possibly be more sensitive and effective if purified $CD4^+$ cells were used.

This is the first demonstration of in vivo lymphocyte maldistribution and homing to tumor-involved sites as a cause of immunodeficiency. The importance of these results lies not only in the demonstration of lymphocyte kinetics as a tool in clinical immunologic studies, but also in the potential use of such a technique in diagnosis and further treatment.

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