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Human lymphocyte traffic assessed by indium-111 oxine labelling: clinical observations

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SUMMARY

Clinical studies using indium-111 oxine labelling of human peripheral blood lymphocytes are presented. Data from animal models of lymphocyte migration are compared with results found in healthy subjects and patients with malignant neoplasms. The physiological significance of bone marrow and liver localization on gamma camera imaging is discussed and the importance of considering the surface marker characteristics of the lymphocytes under study, when interpreting results, is emphasized. The possibility that the redistribution of lymphocytes within the body is a cause of the peripheral blood lymphopenia in patients with Hodgkin's disease and other malignancies is suggested, and the usefulness of indium-111 oxine labelling in clarifying this problem is proposed.

INTRODUCTION

In the first of two papers (Wagstaff *et al.*, 1981) we described a method whereby human lymphocytes could be labelled with indium-111 oxine and their migration followed *in vitro* after reinjection. We discussed the effects that physical handling and radiation from the isotope might have on the physiology of the labelled cells. It was concluded that the migratory properties would not be significantly impaired provided that certain constraints were applied. In the two normal subjects illustrated in the previous report it was shown that the changes in distribution that occurred following reinjection correlated well with data from a number of animal systems.

In this second paper we intend to present the results of initial clinical studies with the aim of discussing further some problems of interpretation that have arisen. We will also discuss the importance of lymphocyte recirculation in tumour immunology and illustrate the potential value of this technique for clinical investigation.

MATERIALS AND METHODS

The details of the separation, purification and labelling procedures have been presented previously (Wagstaff *et al.*, 1981). Likewise the methods for assessing the distribution of lymphocytes following reinjection have been described. Surface marker populations were characterized using the same methods as Garrett, Scarffe & Newton (1979).

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RESULTS

Patient details (Table 1). Three patients with chronic lymphocytic leukaemia (CLL) were investigated. One patient (J.P.) was shown to have infiltration of the liver by small lymphocytes on liver biopsy. Patient D.B. presented initially with generalized lymphadenopathy without blood involvement, and was diagnosed as well-differentiated diffuse lymphocytic lymphoma (WDDLL). A lymphocytosis subsequently developed. E.S. also presented with generalized lymphadenopathy again due to WDDLL. The patient with Hodgkin's disease had adenopathy of the mediastinal and lower cervical lymph nodes but no disease elsewhere. The patient with osteogenic sarcoma presented 3 years before the study with tumour involving the left tibia. He later developed massive abdominal metastatic disease (inset Fig. 2).

Lymphocyte separation, purification and cell labelling. The results are shown in Table 1 of the previous paper (Wagstaff *et al.*, 1981).

Lymphocyte surface characteristics (Table 1). The three patients with CLL had a circulating monoclone of B lymphocytes of between 48 and 90% of the peripheral blood lymphocytes. In patient D.B. with WDDLL, 48% of the peripheral blood lymphocytes were monoclonal B cells. The other patient (E.S.) had a lymphocytosis ($7{\cdot}15 \times 10^9$/l) with 52% T cells (E rosetting), 14% B cells (surface immunoglobulin). The κ to λ ratio was normal but 40% of the lymphocytes were positive for Ia antigen. The patient with Hodgkin's disease had a normal lymphocyte count with a normal surface marker distribution.

Blood disappearance curves (Fig. 1). A composite curve of three CLL patients and the patient with WDDLL is shown in Fig. 1. In contrast to the normal subjects there was a progressive removal of lymphocytes from the blood such that by 24 hr less than 10% of the injected lymphocytes remained in the blood volume. The other patient with WDDLL (E.S.) had a curve similar to the normal subject with an initial fall followed by a secondary rise and plateau.

Surface probe counts (Figs 2 and 3). The normal subject had a high count rate over the spleen at 4 hr which fell by 39% over the next 44 hr. At the same time the count rate over lymph node areas (neck and groin) rose. The liver count rate was the same at 4 and 24 hr but rose by 30·4% at 48 hr. The three patients with chronic lymphocytic leukaemia are represented by one patient. The features that were different from the normal subjects were the sustained high count rate over the spleen throughout the 48 hr. The count rates over lymph nodes rose over the 48 hr.

Patient D.B. (WDDLL) showed a different pattern of splenic count rate than the CLL and

Table 1. The activity of indium-111 labelled lymphocytes and the surface marker characteristics of the patients' peripheral blood lymphocytes

Patient	Activity per 10^8 lymphocytes (μCi)	Total activity (μCi)	E rosettes (%)	SIg (%)	Sκ (%)	Sλ (%)
Normal	41·1	369·0	65	20	2	11
Well-differentiated diffuse lymphocytic lymphoma (E.S.)	8·0	295·8	52	14	1	4
Well-differentiated diffuse lymphocytic lymphoma (D.B.)	25·3	416·8	28	48	7	35
Chronic lymphocytic leukaemia (J.P.)	11·7	233·0	21	68	50	1
Chronic lymphocytic leukaemia (H.L.)	41·7	369·0	16	62	61	5
Hodgkin's disease	40·0	180·5	77	18	7	1
Osteogenic sarcoma	17·7	195·0	n.d.	n.d.	n.d.	n.d.

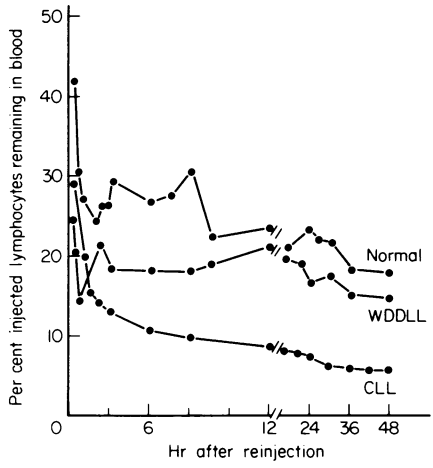

Fig. 1. Blood disappearance curves for indium-111 oxine-labelled lymphocytes. WDDLL = well-differentiated diffuse lymphocytic lymphoma (E.S.), CLL = chronic lymphocytic leukaemia ($n = 4$).

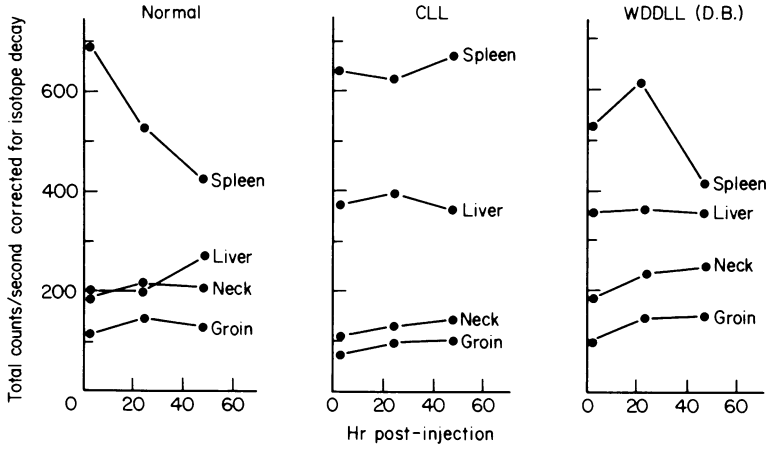

Fig. 2. Surface-probe counts, indium-111 oxine-labelled lymphocytes.

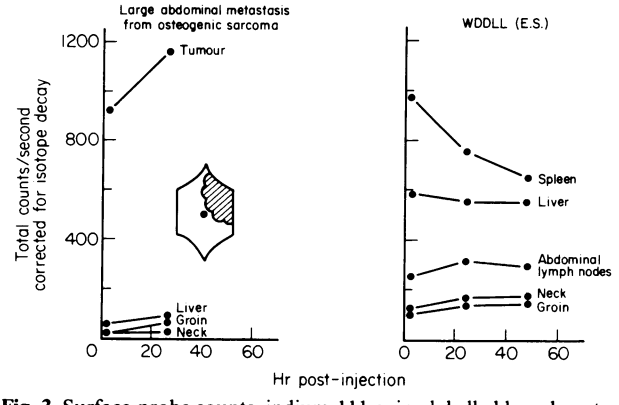

Fig. 3. Surface-probe counts, indium-111 oxine-labelled lymphocytes.

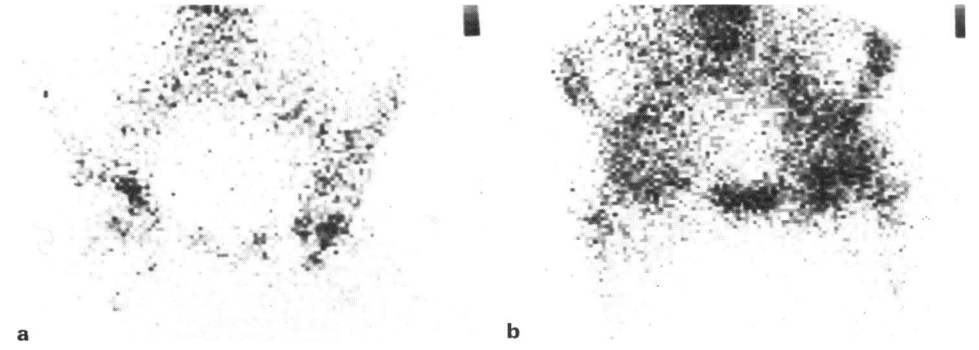

Fig. 4. Anteroposterior gamma camera pictures of the pelvic region obtained 24 hr after the injection of the indium-111 oxine-labelled lymphocytes. Pelvic bone marrow is visible as well as lymph nodes. (a) Normal lymph nodes, (b) lymphadenopathy due to well-differentiated diffuse lymphocytic lymphoma.

normals. There was a rise between 4 and 24 hr with a subsequent fall between 24 and 48 hr. The count rate over the lymph nodes rose during the 48 hr. E.S. (WDDLL) had a picture similar to the normal subjects with a fall in splenic count rate and an increase over lymph nodes. The subject with abdominal metastases from osteogenic sarcoma displayed very high count rates over the tumour at a time when the activity in the blood had fallen to 10% of the total injected.

Gamma camera imaging. The changes in distribution of labelled cells demonstrated by surface-probe counting were confirmed by the serial gamma camera pictures. At 4 hr it was unusual to see lymph node structures except where lymphadenopathy was marked (e.g. patient D.B. with WDDLL) but by 24 hr lymph nodes were clearly visible whether they were normal (Fig. 4a) or enlarged (Fig. 4b). Enlarged mediastinal lymph nodes were clearly seen in the patient with Hodgkin's disease (Fig. 5).

In the normal subject and in other patients with CLL, liver count density, although detectable, was much less than that over the spleen. In one patient with CLL, hepatomegaly had been shown to be due to infiltration by small lymphocytes. At 24 hr post-injection (Fig. 6) liver outlines were clearly seen in this patient and splenic activity was marked.

Prominent bone marrow outlines were seen from the earliest images obtained in both normal subjects (Fig. 4a) and those known to have marrow infiltration by WDDLL. The bone marrow was, however, more prominent in the latter patients.

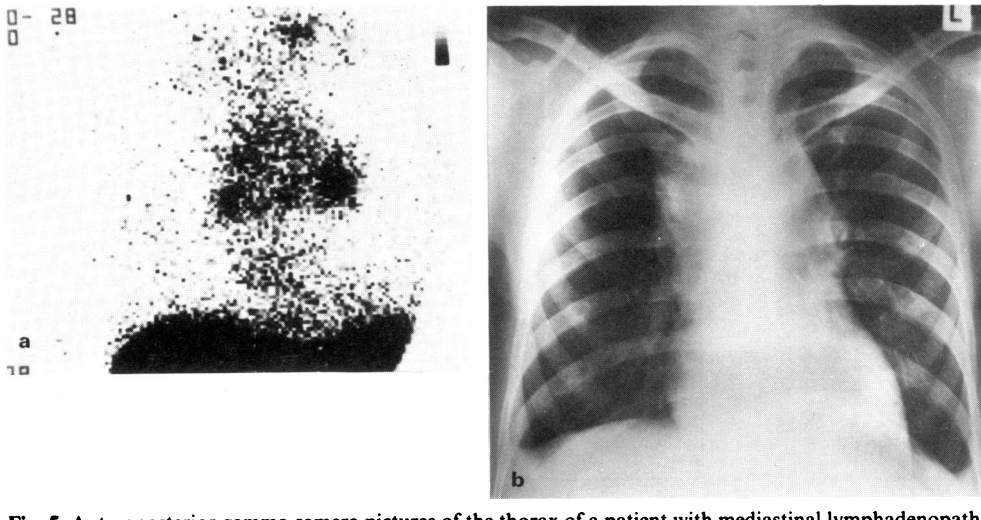

Fig. 5. Anteroposterior gamma camera pictures of the thorax of a patient with mediastinal lymphadenopathy due to Hodgkin's disease. A chest X-ray of the same patient is also shown demonstrating the lymphadenopathy.

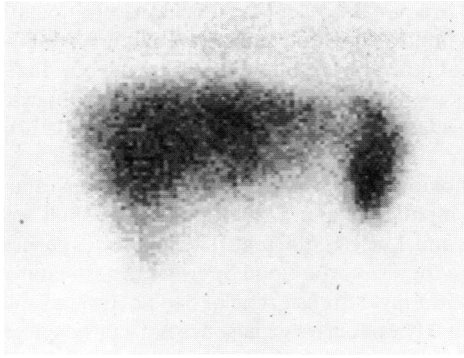

Fig. 6. An anteroposterior gamma camera picture 24 hr after the injection of indium-111 oxine-labelled lymphocytes in a patient with chronic lymphocytic leukaemia and lymphocytic liver infiltration. The prominent features are the marked splenic and liver activity.

DISCUSSION

Any new method designed to study the migration of cells *in vivo* must be assessed with regard to the possible effects that the technique might have on their normal physiology. Before results can be accepted it must also be shown that artefacts are not being interpreted as physiological events. In our previous report we discussed the possibility of damage to lymphocytes from handling procedures and intracellular radiation from the isotope. It was concluded that as long as certain restrictions were applied damage from these sources was likely to be minimal. However, certain features found in our early studies raise a number of problems which must be resolved.

Bone marrow outlines were seen in both normal subjects and those with disease (Fig. 4). In animals there is strong evidence that lymphocytes normally recirculate through bone marrow (Rannie & Donald, 1977; Rannie & Bell, 1979). The mean transit time of 3–4 hr is shorter than for the spleen. The bone marrow images obtained in these human studies could therefore have a physiological basis. However, it seemed possible that free indium-111 could be released into the circulation when damaged cells become disrupted *in vivo*. Indium-111 transferrin has been used as a bone marrow imaging agent and our studies following the injection of cell-free indium-111 oxine gave clear images of bone marrow (Wagstaff *et al.*, 1981). It was therefore possible that the bone marrow outlines seen in the labelled lymphocyte studies could result from cell-free indium-111. This was thought to be unlikely for two reasons. Firstly, very little cell-free indium-111 could be demonstrated in the plasma of patients injected with labelled damaged cells and bone marrow outlines were not seen in these patients. Secondly, Sparshott *et al.* (1981) have shown, by autoradiography, that after lymphocytes die the indium-111 becomes associated with static cells, probably macrophages, in the organs where cell death occurred and it is not released into the circulation. Therefore, marrow images seen in our studies probably do represent the traffic of lymphocytes from blood to bone marrow. The patient with extensive lymphomatous bone marrow infiltration had more prominent bone marrow outlines than normal suggesting increased migration of lymphocytes to marrow in this case.

In the three patients with chronic lymphocytic leukaemia and one with WDDLL (D.B.) there was a steady disappearance of labelled lymphocytes from the blood to levels much lower than in the normal subjects (Fig. 1). These results are at variance with other studies of lymphocytes in CLL and lymphoma (reviewed by Stryckmans, Debusscher & Collard, 1977) which have demonstrated a reduced rate of clearance from the blood. It could be argued that our labelling technique is damaging the more fragile CLL lymphocytes and the patterns described are due to removal of damaged cells by the macrophage system. This is not likely because in all cases, except one (J.P.), the pattern of distribution was completely different for labelled CLL lymphocytes than for labelled heat-damaged cells. In the patient J.P. with chronic lymphocytic leukaemia there is a possible

explanation for the marked liver uptake other than it being a reflection of cell damage. It was known that this patient's liver was infiltrated with lymphocytes and it is therefore possible that these cells could have been in a state of flux with the blood lymphocyte pool. Indeed, there is evidence from animal models that there is a physiological recirculation of lymphocytes through the liver which may reach 5–10% of the injected labelled cells (Rannie & Donald, 1977), and in CLL this could be exaggerated.

Circulating peripheral blood lymphocytes are heterogeneous and normally 50–80% are T lymphocytes, 20–50% B lymphocytes and a small percentage 'null' cells. In animal models the tempo of recirculation of B and T cells is different. T lymphocytes generally recirculate much more rapidly than B cells although they leave the blood by crossing post-capillary venules in lymph nodes at the same rate. T cells have a mean transit time of 5–6 hr through spleen and 16–18 hr through lymph nodes compared with B lymphocytes which have not left the spleen in significant numbers by 24 hr after reinjection and have a mean transit time of 30–36 hr through lymph nodes (reviewed by Ford, 1975). It is therefore clearly necessary to pay close attention to the nature of the lymphocyte suspension which is being studied as the results will vary depending on the proportions of B and T lymphocytes. This is emphasized by the two patients with WDDLL (E.S. and D.B.). The peripheral blood lymphocyte surface marker profiles were different in these two patients (Table 1). E.S. had twice as many E rosetting cells (T lymphocytes) than D.B. and the pattern of blood disappearance (Fig. 1) was similar to the normal subject. D.B., however, had a monoclonal expansion of the B cell subtype and here the blood disappearance curve (Fig. 1) had a pattern resembling other patients with CLL. These different curves could be explained on the basis of the T and B proportions studied. Since T cells migrate rapidly through the spleen, which is a major site of primary localization of lymphocytes, the rise and plateau seen in the lymphocyte blood clearance curves of the normal subject and patient E.S. could be due to the reappearance of labelled T cells in the blood that had passed through the spleen. The more continuous removal of labelled cells (predominantly B cells) in CLL and patient D.B. (WDDLL) suggests that even at 48 hr few labelled cells have returned to the blood after primary localization. This would be consistent with the more prolonged migration patterns of B lymphocytes found in animals. The surface-probe counts over the spleen support this view, with the count rate falling between 4 and 48 hr in both the normal and patient E.S. but being maintained in the CLL patients.

Lymphopenia without evidence of bone marrow failure is a frequent finding in cancer patients. Several workers have shown that in early Hodgkin's disease, when the spleen is not histologically involved, there is a decrease in the number of peripheral blood T lymphocytes and an increase in T cells in the spleen and lymph nodes (reviewed by Twomey & Rice, 1980). De Sousa *et al.* (1977) have suggested that this is due to a redistribution of lymphocytes from the intra- to extravascular compartments of the recirculating lymphocyte pool and have termed this 'ecotaxopathy'. Both Lavender *et al.* (1977) and ourselves (Fig. 5) have shown peripheral blood lymphocytes accumulating in nodes involved with Hodgkin's disease. Zatz, White & Goldstein (1974) demonstrated that lymphocyte trapping occurred in the primary tumours, regional lymph nodes and spleens of rats bearing Maloney sarcoma virus-induced tumours. In one of our subjects with metastatic osteogenic sarcoma, the surface-probe counts (Fig. 2) over an abdominal metastasis rose steadily. This was occurring at a time when the blood activity was falling and therefore suggested that lymphocytes were accumulating within the tumour. Labelling lymphocytes with indium-111 oxine would provide a means whereby the hypothesis that 'ecotaxopathy' is the cause of blood lymphopenia in some neoplastic diseases could be confirmed or refuted.

The preliminary studies have demonstrated that indium-111 oxine labelling of lymphocytes is a valid and promising technique for studying lymphocyte migration in a quantitative manner in man, both in health and disease.

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