CLINICAL RESEARCH

Dynamic studies of lymphocytes labelled with indium-111 during and after treatment with monoclonal anti-idiotype antibody in advanced B cell lymphoma

E M RANKIN, A HEKMAN, M R HARDEMAN, C A HOEFNAGEL

Abstract

The migration pattern of lymphocytes labelled with indium-111 was followed in a patient with B cell non-Hodgkin's lymphoma treated with a murine monoclonal anti-idiotype antibody. During the early phase of continuous infusion of antibody rapid fluxes of labelled lymphocytes into and out of the blood were seen. Dynamic scanning showed immediate uptake in the lungs; thereafter activity decreased in the lungs and increased in the liver. Studies of labelled and unlabelled cells in the circulation showed that treatment resulted in the removal of lymphocytes from the blood which was repopulated from an extravascular compartment. Tumour cells were shown to be cleared from the blood by the reticuloendothelial system in the liver.

Indium-111 should be used circumspectly because it may cause chromosomal damage in labelled cells, but it is clearly useful as a radiolabel for following the migration pathways of lymphocytes in vivo.

Introduction

The migratory properties of cells in the blood may be studied by tagging the cells with a radiolabel in vitro and tracing the patterns of homing and recirculation of the labelled cells after reinjection.

The Netherlands Cancer Institute, Antoni van Leeuwenhoekhuis, 1066 CX Amsterdam, The Netherlands

E M RANKIN, MRCP, research fellow of the European Organisation for Research of Treatment of Cancer, division of immunology

A HEKMAN, PHD, biochemist, division of immunology

C A HOEFNAGEL, MD, consultant in nuclear medicine

Department of Internal Medicine, University of Amsterdam, Amsterdam

M R HARDEMAN, PHD, clinical chemist

Correspondence to: Dr A Hekman.

The fate of radiolabelled lymphocytes has been studied in vivo in normal subjects,[1] in patients with lymphoma,[2] and in patients with chronic lymphocytic leukaemia.[3-6] The earlier studies of lymphocyte kinetics used tritiated thymidine,[3 4] cytidine labelled with tritium,[5 6] or chromium-51 (as sodium chromate (^{51}Cr))[6] as the radiolabel.

The survival in the blood of reinjected tumour cells labelled with ^{51}Cr and coated in vitro with monoclonal antibody Ab89 (which is directed against an antigen associated with lymphoma) was examined by Nadler *et al.*[7] Dillman *et al* used chromium-51 as a radiolabel to follow the effect of administration in vivo of monoclonal antibody T101 in two patients with chronic lymphocytic leukaemia.[8] Whereas chromium-51 is a useful tool for following the kinetics of blood lymphocytes, indium-111 may be used to follow the circulation and distribution of labelled lymphocytes by gammacamera imaging. Several workers have shown that indium-111 oxine is a reliable radioactive label for studies in vivo of lymphocyte traffic.[9 10] Miller *et al* used lymphocytes labelled with indium-111 oxine to study the effect of a monoclonal antibody (L17F12) directed against a normal T cell differentiation antigen in a patient with T cell leukaemia.[11]

Here we report on a patient with advanced B cell non-Hodgkin's lymphoma whom we treated with infusion of monoclonal anti-idiotype antibody. Autologous lymphocytes were reinjected, after labelling with indium-111, as infusion was started, and the changes in the migratory patterns of the malignant cells during treatment were followed.

Patient and method

A 71 year old woman with advanced, diffuse, poorly differentiated lymphocytic non-Hodgkin's lymphoma had widespread lymphadenopathy, bulk abdominal disease, ascites, and affected blood and bone marrow. The spleen had been removed 10 months before treatment began. She was treated with a monoclonal antibody directed against the immunoglobulin idiotype that was expressed on the malignant tumour cells (Rankin and Hekman, submitted for publication). The idiotype (the determinants of the immunoglobulin unique to each normal or malignant B cell clone) can be regarded as a tumour specific antigen and a possible target for immunotherapy.[12] She was given a total of 3800 mg of the anti-idiotype antibody (designated T2) in our treat-

ment sessions over two months. In the present study the antibody was given as a continuous infusion at a rate of 75 mg/hour for two hours followed by 20 mg/hour for 40 hours.

Autologous lymphocytes, of which more than 80% were tumour cells, were reinjected after labelling with indium-111, and the redistribution was assessed on two occasions with serial blood sampling and gammacamera imaging: on the first the migration pattern of 4×10^8 labelled lymphocytes was followed; on the second we studied the fate of 6×10^8 labelled lymphocytes injected simultaneously with the start of infusion of monoclonal anti-idiotype antibody.

The blood lymphocyte count was $18{\cdot}8 \times 10^9$/l when the first study was performed and $20{\cdot}7 \times 10^9$/l when the effect of treatment was assessed. More than 80% of the lymphocytes in the circulation were malignant B cells, as indicated by their reaction with the anti-idiotype antibody. Despite the presence of a large tumour burden, free idiotypic immunoglobulin was not detectable in the serum by enzyme immunoassay or inhibition of immunofluorescence.

Antibody—The methods of production, characterisation, and isolation of the antibody T2 have been described in detail elsewhere.[13] T2 is a murine IgG2a immunoglobulin that binds specifically with the idiotype expressed on the malignant B cells of our patient. It does not react with normal cells. The antibody is cytotoxic in vitro with rabbit complement but not with human complement. Incubation of the tumour cells with T2 for up to 24 hours did not modulate the antigen as detected by immunofluorescence.

Labelling with indium-111 oxine—Lymphocytes were isolated from defibrinated peripheral blood by centrifugation over Ficoll-metrizoate (Lymphoprep, Nyegaard, Oslo), washed twice, and resuspended in 2 ml phosphate buffered physiological saline, pH 7·4. Viability of the cells, measured by trypan blue exclusion, was 95%. Labelling was performed with 1·48 MBq (40 μCi) indium-111 oxine (Byk Mallinckrodt, the Netherlands) per 10^8 lymphocytes. Labelling efficiency measured after 15 minutes' incubation at room temperature was 90%. The total injected activity varied from 5·2 to 8·3 MBq (142-224 μCi).

Serial blood sampling—Samples of blood of 3 ml were taken from a peripheral vein at regular intervals after reinjection of the cells. On the first occasion whole blood was counted. On the second the sample was centrifuged at 1400 *g* and the plasma and cell pellet were separately counted. All the samples were stored at 4°C and counted at the end of the sampling period in a gammacounter.

Gammacamera imaging—Scintigrams were made with a double headed gammacamera with a large field of view (Siemens Rota 2 75ZLC) fitted with parallel hole and medium energy collimators and connected to an on line computer system (MDS A2). We used a dual window setting over the energy peaks of 171 and 245 kiloelectron volts. Digital scintigrams were recorded in a 256×256 matrix. After intravenous injection of autologous lymphocytes labelled with 5·2 MBq (141 μCi) indium-111, static anterior view scintigrams were made at intervals of three minutes, 30 minutes, one hour 45 minutes, 18 hours, and 120 hours. At the onset of infusion of antibody 8·3 MBq (224 μCi) autologous lymphocytes labelled with indium-111 were injected, and dynamic acquisition of thorax, liver, and upper abdomen was performed during the first 30 minutes. Thereafter static anterior and posterior view scintigrams, collecting counts over 15 minutes, were recorded simultaneously at intervals of 30 minutes and two, 18, 24, 44, and 49 hours.

Results

Blood disappearance curves—Figure 1 shows the rate of disappearance of the lymphocytes labelled with indium-111 oxine reinjected at a time when the patient was not receiving treatment. Initially, activity fell rapidly until 12 hours, when a secondary rise occurred followed by a more gradual decrease. Activity was still detectable at 118 hours. The disappearance curve for the period of treatment (fig 2) was very different. Rapid fluxes of cells into and out of the circulation occurred during the first six hours, after which activity stayed constant and low with a minor rise corresponding to the end of the infusion of the antibody. In contrast, counts in the plasma varied little, indicating that the cells were not being destroyed intravascularly. These fluxes in the circulation did not parallel the changes in the total number of lymphocytes in the blood (fig 3). These fell from $20{\cdot}7 \times 10^9$/l to a nadir at 3·5 hours of 14×10^9/l, gradually rising over the next few hours to reach $20{\cdot}6 \times 10^9$/l at 12 hours and $28{\cdot}1 \times 10^9$/l at 140 hours.

Gammacamera images—Figure 4 shows some of the gammacamera images obtained when the patient was not being treated. At 30 minutes after reinjection of lymphocytes the uptake in the liver was already appreciable, that in the lungs much less. By 18 hours the uptake by bone marrow in the pelvic girdle could be seen clearly and the retroperitoneal mass of nodes below the liver was delineated. Figure 5 shows dynamic scans taken during the first 30 minutes of infusion of antibody, and figure 6 shows serial scans taken at intervals thereafter. Infusion of the antibody through a cannula in the right superior vena cava began at the same time as the labelled lymphocytes were reinjected into the right arm. Considerable lung activity occurred at one minute. This decreased thereafter as the liver activity increased. By 18 minutes three hot spots were visible in the thorax; these corresponded to: the site of insertion of the long line; a right pleural effusion; and a mass of tumour measuring 5×6 cm in the left breast. At two hours the tumour in the mediastinum was visible, and by 18 hours uptake by bone marrow was apparent and activity in the retroperitoneal nodes could be seen.

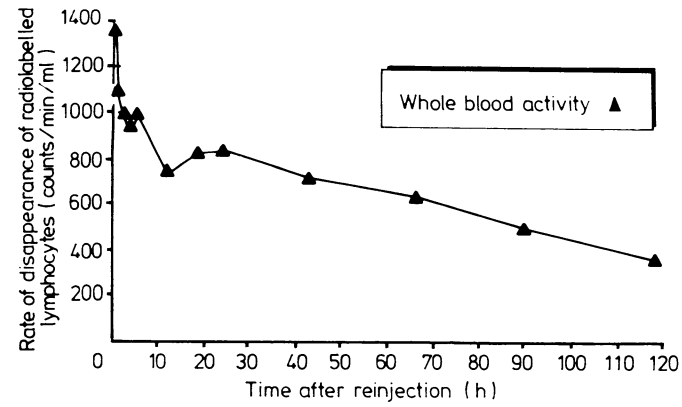

FIG 1—Blood disappearance curve for lymphocytes labelled with indium-111 reinjected at a time when the patient was not receiving treatment.

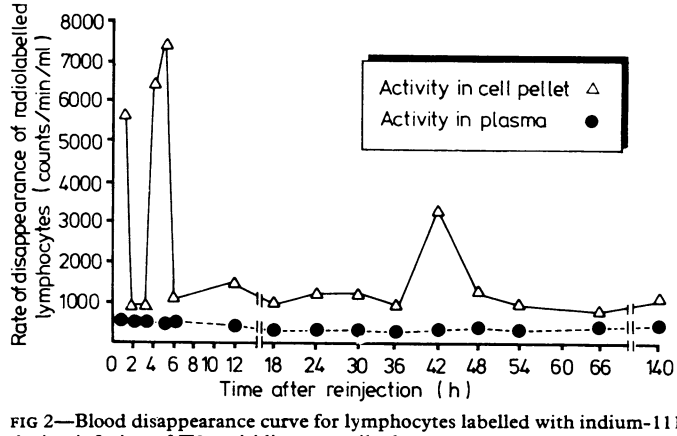

FIG 2—Blood disappearance curve for lymphocytes labelled with indium-111 during infusion of T2 anti-idiotype antibody.

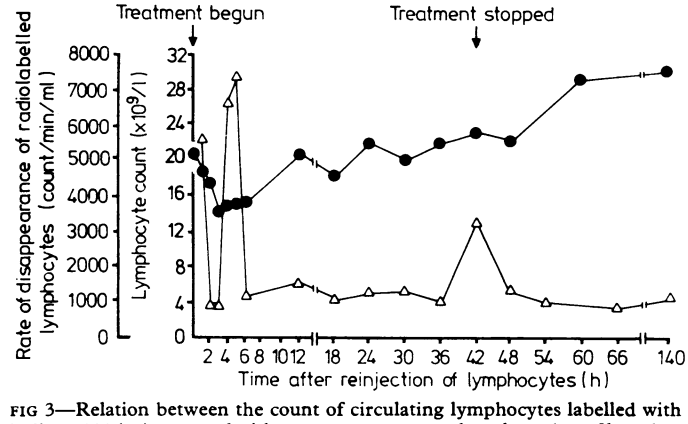

FIG 3—Relation between the count of circulating lymphocytes labelled with indium-111 (△), counted with a gammacounter, and total number of lymphocytes (●), counted with a Coulter counter, in blood during treatment with T2 anti-idiotype antibody.

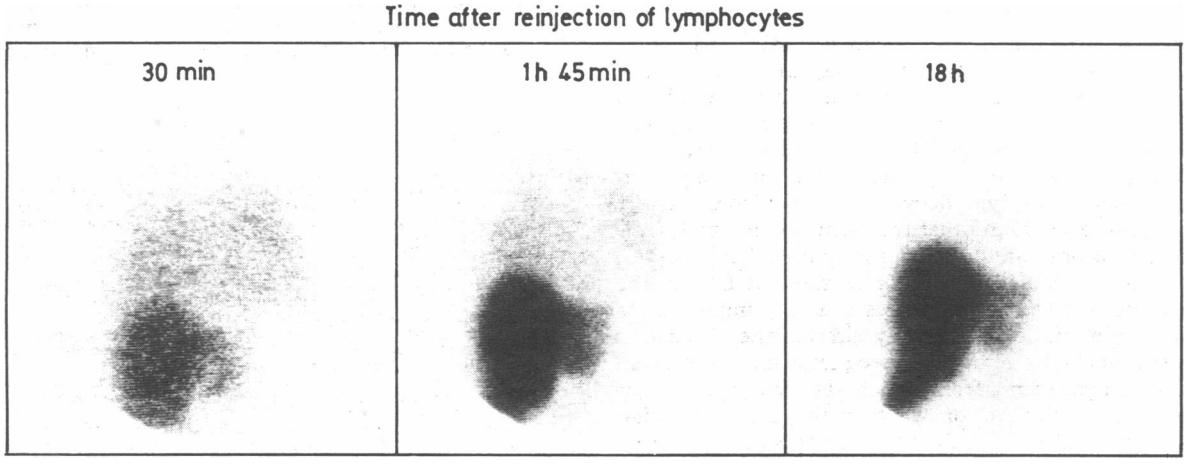

FIG 4—Serial gammacamera scans taken when not receiving treatment, after reinjection of indium-111 labelled autologous lymphocytes.

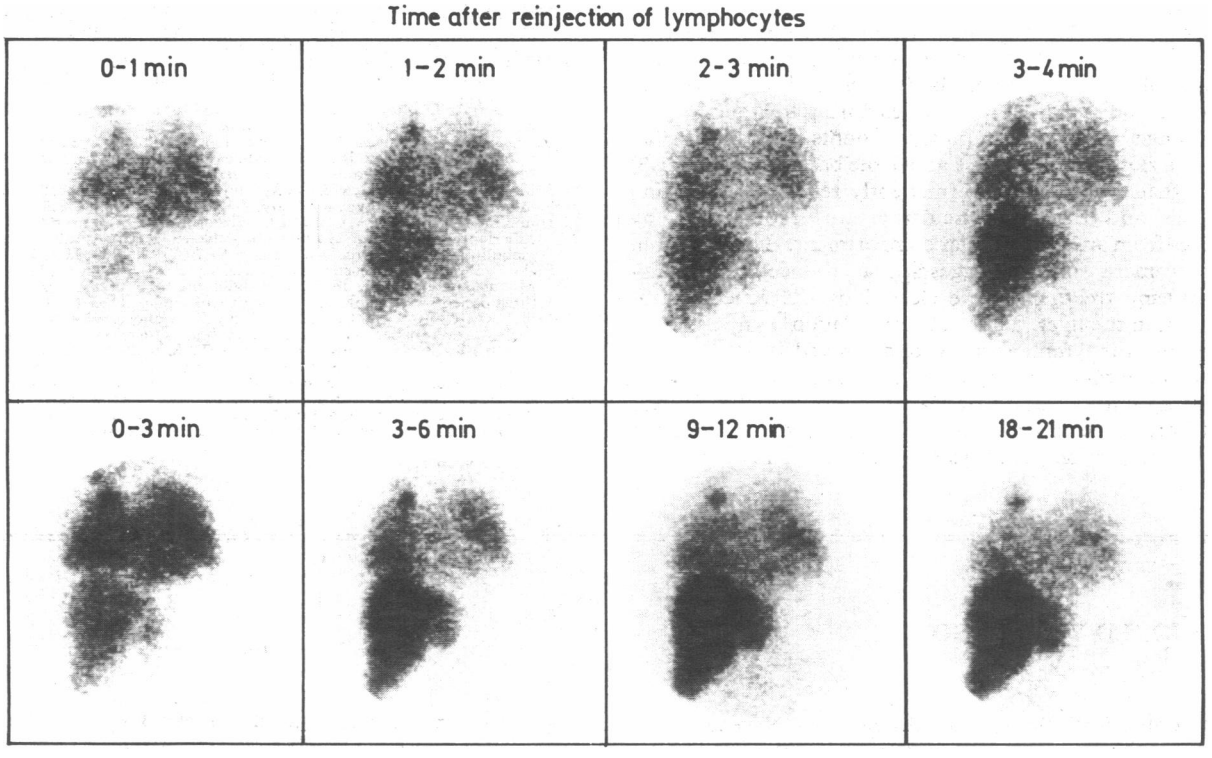

FIG 5—Dynamic gammacamera scans taken after reinjection of autologous lymphocytes labelled with indium-111 during 30 minutes of infusion with antibody. Injection and start of infusion were simultaneous.

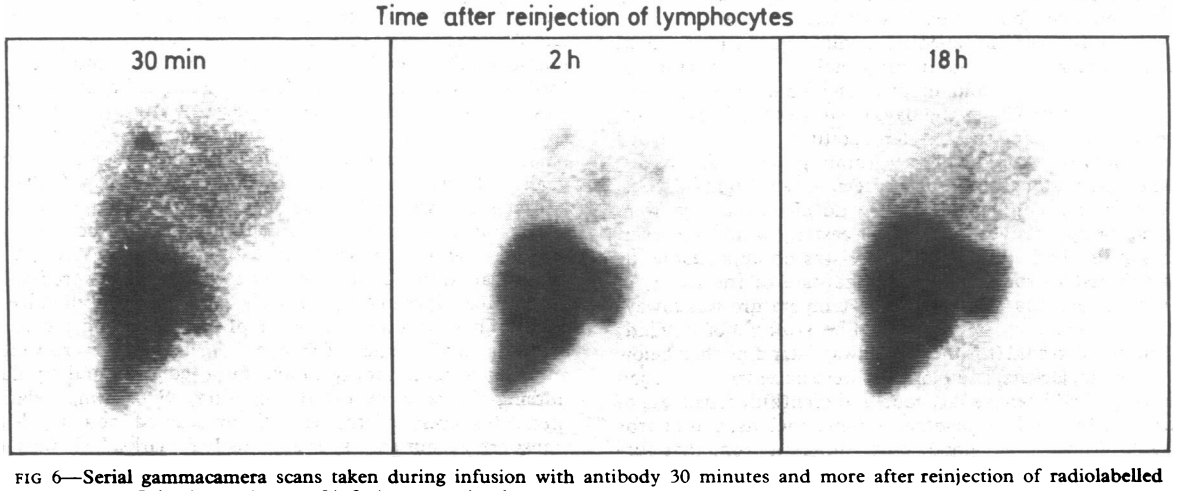

FIG 6—Serial gammacamera scans taken during infusion with antibody 30 minutes and more after reinjection of radiolabelled lymphocytes. Injection and start of infusion were simultaneous.

Discussion

We found that treatment with a monoclonal anti-idiotype antibody, which was specific for the malignant tumour cells, resulted in a transient fall in the tumour cells in the circulation (Rankin and Hekman, submitted for publication). Only one report has previously been published of the use in vivo of a monoclonal anti-idiotype antibody, and in that case the patient did not have malignant lymphocytes in the blood.[14] Other workers have, however, found that treatment in vivo with antibodies directed against antigens on the tumour lymphocytes, including idiotype, resulted in rapid but transient falls in the circulating tumour cells.[6 7 10 15-18] It is crucially important to know whether the antibody is merely altering the circulation pathways of the cells by temporarily sequestering them in an extravascular compartment, from which they emerge near or after the end of treatment, or whether the cells are being removed altogether. If radiolabelled tumour cells are injected at the time antibody treatment begins their fate can be followed; if cells returning when the cell count rebounds during treatment do not carry the radiolabel they are new cells and not those that disappeared.

In the study reported here the patterns of lymphocyte circulation in the blood of our patient were radically altered during the infusion of anti-idiotype antibody. The lack of correlation between the blood disappearance curves during treatment (fig 2) and the changes in blood lymphocyte counts—a transient fall followed by a slow inexorable rise above the baseline count (fig 3)—suggests that the antibody permanently removed circulating tumour cells and that the circulation was repopulated by lymphocytes from an extravascular compartment. Evidence from the gammacamera images suggests that the cells that disappeared during treatment were cleared by the reticuloendothelial system in the liver. Thus the recirculation pathways were altered because the lymphocytes were destroyed.

Although indium-111 must be used circumspectly as it may cause chromosomal damage in labelled cells,[19] the studies described here serve to show its usefulness as a radiolabel for following the migration pathways of lymphocytes in vivo.

We thank Dr R Somers and Dr W W ten Bokkel Huinink for allowing us to study their patient; and the staff of the clinical research unit of the Netherlands Cancer Institute for their care of the patient during the study.

References

1 Wagstaff J, Gibson C, Thatcher N, *et al.* A method for following human lymphocyte traffic using indium-111 oxine labelling. *Clin Exp Immunol* 1981;**43**:435-42.
2 Wagstaff J, Gibson C, Thatcher N, Ford WL, Sharma H, Crowther D. Human lymphocyte traffic assessed by indium-111 oxine labelling: clinical observations. *Clin Exp Immunol* 1981;**43**:443-9.
3 Zimmerman TS, Godwin HA, Perry S. Studies of leukocyte kinetics in chronic lymphocytic leukaemia. *Blood* 1968;**31**:277-91.
4 Theml H, Trepel F, Schick P, Kaboth W, Begemann H. Kinetics of lymphocytes in chronic lymphocytic leukaemia: studies using continuous ^{3}H-thymidine infusion in two patients. *Blood* 1973;**42**:623-36.
5 Manaster J, Fruhling J, Stryckmans P. Kinetics of lymphocytes in chronic lymphocytic leukaemia. I. Equilibrium between blood and a readily accessible pool. *Blood* 1973;**41**:425-38.
6 Scott JL, McMillan R, Marino JV, Davidson JG. Leukocyte labelling with ^{51}chromium. IV. The kinetics of chronic lymphocytic leukaemia lymphocytes. *Blood* 1973;**41**:155-62.
7 Nadler LM, Stashenko P, Hardy R, *et al.* Serotherapy of a patient with a monoclonal antibody directed against a lymphoma associated antigen. *Cancer Res* 1980;**40**:3147-54.
8 Dillman RO, Shawler DL, Sobol RE, *et al.* Murine monoclonal antibody therapy in two patients with chronic lymphocyte leukaemia. *Blood* 1982;**59**:1036-45.
9 Lavender JP, Goldman JM, Arnot RN, Thakur ML. Kinetics of indium-111 labelled lymphocytes in normal subjects and patients with Hodgkin's disease. *Br Med J* 1977;ii:797-9.
10 Wagstaff J, Gibson C, Thatcher N, Crowther D. The migratory properties of indium-111 oxine labelled lymphocytes in patients with chronic lymphocytic leukaemia. *Br J Haematol* 1981;**49**:283-91.
11 Miller RA, Maloney DG, McKillop J, Levy R. In vivo effects of murine hybridoma monoclonal antibody in a patient with T-cell leukaemia. *Blood* 1981;**58**:78-86.
12 Stevenson GT, Stevenson FK. Antibody to a molecularly-defined antigen confined to a tumour cell surface. *Nature* 1975;**254**:714-6.
13 Rankin EM, Hekman A. Mouse monoclonal antibodies against the idiotype of human B-cell non-Hodgkin lymphomas: production, characterization and use to monitor the progress of disease. *Eur J Immunol* (in press).
14 Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B cell lymphoma with monoclonal anti-idiotype antibody. *N Engl J Med* 1982;**306**:517-22.
15 Hamblin TJ, Ahad AKA, Gordon J, Stevenson FK, Stevenson GT. Preliminary experience in treating lymphocytic leukaemia with antibody to immunoglobulin idiotypes on the cell surface. *Br J Cancer* 1980;**42**:495-502.
16 Ball ED, Bernier GM, Cornwell GG, McIntyre OR, O'Donnell JF, Fanger MW. Monoclonal antibodies to myeloid differentiation antigens: in vivo studies of three patients with acute myelogenous leukaemia. *Blood* 1983;**59**:1203-10.
17 Miller RA, Oseroff AR, Stratle PT, Levy R. Monoclonal antibody therapeutic trials in seven patients with T cell lymphoma. *Blood* 1983;**62**:988-95.
18 Macbeth FR, Stevenson FK, Stevenson GT. Anti-idiotype antibody therapy of patients with non-Hodgkin's lymphoma. Paper presented at second European conference on clinical oncology and cancer nursing, Amsterdam, 1983. p 75.
19 Ten Berge RJM, Natarajan AT, Hardeman MR, Van Royen EA, Schellekens PThA. Labelling with indium-111 has detrimental effects on human lymphocytes: concise communication. *J Nucl Med* 1983;**24**:615-20.

(*Accepted 8 August 1984*)

ONE HUNDRED YEARS AGO

Another correspondent, writing from Assouan, on November 3rd, informs us that the expedition for the relief of General Gordon and of Khartoum, was, at that date, assembling at Assouan and Wady Halfa; and that, as steamers and trains were daily becoming disabled, it would be some time, he expected, before an advance to the front could be accomplished. Meanwhile, the following cheering notes respecting climate, food, prevailing diseases, and the sanitary aspects and surroundings of the expedition, will be read with interest. As regards *climate*, there had been, during October, a combination of hot days and comparatively cold nights. The Fahrenheit thermometer ranged from 93° or 95° in the day, down to 65°, or even 60°, at night; though, as a rule, the day-temperature seldom exceeded 89°. There were very heavy night-dews, saturating the men's clothes; but, at this early period of the campaign, few, except night-sentries, were exposed to it. So long as the north wind blew, the days were not felt to be hot or oppressive; southerly or easterly winds, however, made the days sultry and stifling; but there was no appreciable illness to be attributed to the weather. At the date of the letter, in November, the climate was delightful. The temperature was always under 80° in the shade by day, with a fine strong north wind; whilst the nights were so cold (the mercury always standing then below 60°) that flannel vests, jackets, and blankets, were necessary. The food issued to the troops had been, so far, good and plentiful, consisting of fresh beef, frequently supplied, potatoes, onions, melons, and gourds in abundance. The bread was good, quite different from that furnished to the troops during the campaign in 1882. The men could buy quantities of eggs, milk, poultry, dates and other fruits; and this remark was applicable to all the stations from Alexandria to Assouan. There were a number of cases of enteric fever of a severe type; asthenic, with high temperature. These existed chiefly among the young soldiers, mere lads, in some of the infantry regiments. Dysentery of an ordinary type prevailed, though not to a great extent. Cases of sunstroke were very rare; there had been a few instances amongst officers who had previously suffered from it, and whose military zeal had led them to return to Egypt from England too soon. Diarrhoea was mild and harmless, and prevailed among fresh arrivals. There were a few cases of mild conjunctivitis. Syphilis was very prevalent; the villages were "saturated" with it, and the troops were consequently restricted from entering them, except "on pass." All kinds of *sanitary precautions* had been adopted to preserve the health of the men. Camp-filters, large vessels ("chatties"), and pocket-filters were in use by the troops. Bathing every morning was, if possible, accomplished; flannel shirts and flannel belts were worn; "goggles" and green veils could be used, if required; blankets and great-coats were supplied for use at night; tents were issued; and the dry earth latrines in deep trenches were situated well to leeward of the camp. There was an abundance of medicines and medical comforts, even for small parties of troops. The hospitals were well equipped, and had a good supply of mosquito-curtains and fly-flaps. A few nursing-sisters were up the country. Everything relating to the medical success of the expedition seemed now to depend upon transport. As our correspondent justly remarks, "Medical men cannot work without appliances." (*British Medical Journal* 1884;ii:1034.)