Discussion

Of the 100 patients, 88 had *S typhi* in their blood or bone marrow, or both. This confirms our observation that *S typhi* is a common cause of fever of unknown origin in Karachi. The remaining 12 patients whose blood or bone marrow cultures did not grow any organism recovered without any antibiotic treatment. This suggests that these patients probably had some viral infection(s).

In the patients with *S typhi* infection (typhoid fever) the organism was isolated from the bone marrow of all of them. The organism was also isolated from the blood of 58 (66%) patients. This means that bone marrow culture could confirm a diagnosis of typhoid fever in patients whose blood cultures are negative.

One of the reasons for blood cultures being negative and bone marrow being positive in typhoid fever may be antibiotic treatment before collection of blood for culture. An injudicious or suboptimal antibiotic regimen may diminish or eliminate the organism from blood, but *S typhi*, being an intracellular organism, persists in the reticuloendothelial system including bone marrow.⁷ The intracellular location of bacteria protects them from conventional chemotherapeutic measures.^{8,9}

We recommend that when typhoid fever is suspected blood for culture must be taken before antibiotics are given. In the case of patients who have already received antibiotics and whose blood cultures do not show any bacterial growth after three to four days of incubation, bone marrow should be cultured.

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Recovery of CD3+ and CD5- lymphocyte subpopulation after autologous bone marrow transplantation and chemotherapy

P J McKay, A McLaren, L M Andrews, N P Lucie

Abstract

Although most circulating T cells in normal subjects express both CD3 and CD5 antigens on the cell surface, a small number lack the CD5 antigen. Recipients of allogeneic bone marrow transplants develop increased numbers of CD3+ CD5- cells, particularly those who develop graft versus host disease (GVHD). This CD3+ CD5- population may rise transiently in patients who have received an autologous bone marrow transplant (BMT) and in patients following completion of intensive chemotherapy for acute myeloid leukaemia (AML). These findings suggest that these CD3+ CD5- cells are a normal component of the regenerating lymphoid system after BMT or chemotherapy.

weight glycoprotein present on the surface of most human thymocytes and peripheral T cells and on a subpopulation of B cells.¹⁻⁴ A small proportion of CD3+ T cells in normal subjects do not express CD5.⁵ These cells are CD2+, CD8+, CD4- and CD16- and express low levels of CD7.⁵ They seem to be functional in that they have been shown to express normal cytolytic activity.⁵ An increase in these CD3+ CD5- cells has been reported after allogeneic bone marrow transplantation, and a correlation has been found between the circulating CD3+ CD5- cells in the first 90 days after transplantation and the eventual development of all types of graft versus host disease (GVHD).⁶ In this study we measured CD3+ CD5- cells after autologous bone marrow transplantation and after drug treatment.

Methods

Samples of whole blood were collected in

Department of
Haematology, Western
Infirmary, Dumbarton
Road, Glasgow
G11 6NT

P J McKay
A McLaren
L M Andrews
N P Lucie

Correspondence to:
P J McKay

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The CD5 molecule is a 67 000 molecular

Table 1 Consistency of CD3+ CD5- population in normal subjects (five samples a person)

	Percentage of CD3+ CD5- cells		Absolute counts of CD3+ CD5- cells	
	Mean (SD)	CV	Mean (SD)	CV
1	5.4 (1.67)	30.9	0.13 (0.08)	61.5
2	8.0 (3.03)	37.9	0.14 (0.08)	57.1
3	7.66 (2.16)	28.2	0.19 (0.04)	21.1
4	7.5 (3.88)	51.7	0.13 (0.08)	61.5

EDTA and analysis was performed by optical flow cytometry using a Coulter CS Plus. Preconjugated monoclonal antibodies, phycoerythrin conjugated anti-CD3-RT1 (Coulter), and anti-CD5 fluorescein isothiocyanate (Dako) were used. Initial experiments were conducted using normal control samples to determine the optimal antibody concentration. The appropriate amount of antibody was added to 100 µl of whole blood and incubated at room temperature for 30 minutes. Erythrocyte lysis and leucocyte membrane fixation were performed using Immunoprep (Coulter). The total lymphocyte count of peripheral blood was estimated using a Technicon H1.

Sixty one normal healthy adults (28 men and 33 women, age range 20–61 years) were studied to establish our normal range for the CD3+ CD5+ and CD3+ CD5- cells. Four (two men and two women) were also studied serially over a nine month period.

Six patients who had received an autologous transplantation and six patients who had received drug treatment for acute myeloid leukaemia (AML) were studied.

Results

From the 61 healthy subjects studied, we established a normal mean (SD) of range 1.32 (0.88) × 10⁹/l for the CD3+ CD5+ population and 0.196 (0.24) × 10⁹/l for the CD3+ CD5- population. The CD3+ CD5- population represented 9.5 (10.48)% of total lymphocytes (mean ± 2SD). Table 1 shows the coefficient of variation in the four healthy

subjects studied over a nine month period.

Table 2 outlines the results obtained from the six patients who underwent an autologous bone marrow transplantation for either acute leukaemia or Hodgkin's disease. Recovery of CD3+ CD5+ cells is defined as the day when the count reaches 0.5 × 10⁹/l, or the nadir doubles, if this was above 0.5 × 10⁹/l. No CD3+ CD5- peak was demonstrable in cases 2 and 5, but in case 2, no sample was obtained between days 43 and 104 and case 5 required further treatment within one month of transplantation because of recurrent disease. In three of the six patients a definite peak was demonstrable which lasted from three to 13 days. In case 4 the CD3+ CD5- count remained increased 88 days after the initial peak. This patient was receiving granulocyte-macrophage colony stimulating factor (GM-CSF). Figure 1 shows the behaviour of the CD3+ CD5- and CD3+ CD5+ populations after autologous transplantation in case 1.

In the first 12 days after treatment the CD3+ CD5- population was consistently low. Thirteen measurements in six patients showed a mean of 2 (1.47)% and a mean absolute value of 0.02 (0.03) × 10⁹/l. In contrast, the CD3+ CD5+ population was much less affected (fig 2). Table 3 shows the treatment regimens of the six patients, all of whom had acute myeloid leukaemia. No further chemotherapy was given in the study period. Cases 1 and 2 showed a CD3+ CD5- peak 30 and 35 days, respectively, after recovery of the CD3+ CD5+ cells. Case 4 showed an early peak at day 13 associated with a transient fall in CD3+ CD5+ cells. Case 6 differed from the others as the CD3+ CD5- cells at the day of the peak were distinguished from the rest of the lymphocytes in that they were present in a distinct population of cells which were larger than the normal lymphocytes and were strongly CD3 positive. The blood picture at this time showed a clinically important proportion of larger atypical lymphoid cells lacking granularity. Similar cells were seen in

Table 2 Preparatory regimens and recovery of T cell populations after autologous bone marrow transplant in six patients

Case No	Age (years)	Diagnosis	Pre-transplant conditioning	Day of recovery of CD3+ CD5+ cells	Day of peak of CD3+ CD5- cells	Absolute count CD3+ CD5- peak (× 10 ⁹ /l)	Percentage of CD3+ CD5- cells at peak
1	39	AML**	Cyclophosphamide 60 mg/kg/day × 2 TBI 200 cgy × 7	31	53	1.03	43
2	44	T cell ALL***	As for patient 1 + T-cell depletion of marrow (CD7, CD8)	17	—	—	—
3	29	HD****	BCNU 300 mg/m ² × 1 Etoposide 200 mg/m ² /day × 3 Cytosine arabinoside 200 mg/m ² bd × 3 Melphalan 100 mg/m ² × 1	44	44	1.51	55
4*	33	HD	Cyclophosphamide 6 g/m ² × 1 Etoposide 1 g/m ² × 1	15	15	0.53	42
5	16	HD	Cyclophosphamide 6 g/m ² × 1 Etoposide 1.3 g/m ² × 1	38	—	—	—
6	33	HD	BCNU 300 mg/m ² × 1 Etoposide 200 mg/m ² /day × 3 Cytosine arabinoside 200 mg/m ² bd × 3 Melphalan 140 mg/m ² × 1	13	13	0.51	34

*received GM-CSF after marrow re-infusion.

**AML = Acute myeloid leukaemia.

***T-cell ALL = T-cell acute lymphoblastic leukaemia.

****HD = Hodgkin's Disease.

Table 3 Treatment regimens and recovery of T cell populations following intensive chemotherapy for acute myeloid leukaemia in six patients

Case No	Age (years)	Diagnosis	Treatment prior to monitoring CD3+ CD5- cells	Day of recovery of CD3+ CD5+ cells	Day of peak of CD3+ CD5- cells	Absolute count CD3+ CD5- peak (× 10 ⁹ /l)	CD3+ CD5- % at peak
1	61	AML	Etoposide 100 mg/m ² × 5 Cytosine 200 mg/m ² bd × 5	9	39	1.14	51
2	40	AML	Amsacrine 100 mg/m ² × 5 Cytosine 200 mg/m ² × 5 Etoposide 100 mg/m ² × 5	6	41	0.71	39
3	36	AML	Mitozantrone 10 mg/m ² × 5 Cytosine 1 g/m ² bd × 3	45	—	—	—
4	41	AML	Daunorubicin 50 mg/m ² × 3 Cytosine 100 mg/m bd × 8 Thioguanine 100 mg/m ² bd × 8	—*	13	0.81	43
5	52	AML	Mitozantrone 10 mg/m ² × 5 Cytosine 1 g/m ² bd × 3	38	—	—	—
6	76	AML	Daunorubicin 50 mg/m ² × 2 Cytosine 100 mg/m ² bd × 7 Thioguanine 100 mg/m ² bd × 7	16	18	0.55	20

*There was no significant reduction in the CD3+ CD5+ population in this patient.

Figure 1 Case 1, autologous transplant recipient, showing recovery of CD3+ CD5+ and CD3+ CD5- populations after treatment.

two other patients (case 6 after transplantation and case 4 after chemotherapy) at the time of the CD3+ CD5- peak. In cases 3 and 5, although no peak was shown the CD3+ CD5- cells did show a gradual return to within normal limits. Interestingly, in these two patients the recovery of the CD3+ CD5+ cells was prolonged at 45 and 38 days, respectively. Both had received high doses of cytosine arabinoside.

Discussion

We have shown that an increase in CD3+ CD5- lymphocytes occurs in a proportion of patients receiving autologous bone marrow transplantation and after intensive chemotherapy. As far as we are aware, the rise in CD3+ CD5- cells after chemotherapy has not been documented previously. Four of the six autologous transplant recipients showed a

rise in CD3+ CD5- cell count. Of the other two, a rise may have been missed in one and the reinstitution of chemotherapy may have affected the other. Four of the six patients recovering from chemotherapy also showed a distinct peak in cell count. In two, the peak occurred well after CD3+ CD5+ recovery, and in one the peak was at the same time. The two patients who showed no CD3+ CD5- peak, also had delayed recovery of their CD3+ CD5+ cells and this may have been related to high doses of cytosine.

It would seem unlikely that the CD3+ CD5- population is simply a precursor of the CD3+ CD5+ population because an increase in CD3+ CD5+ cells occurred before or at the same time as the CD3+ CD5- population in most patients. In contrast to the transplant recipients, where both populations of cells were noticeably reduced, the patients receiving chemotherapy showed a pronounced reduction in the CD3+ CD5- population immediately after treatment but a variable reduction in the CD3+ CD5+ population, suggesting that CD3+ CD5- cells were more sensitive to the effects of chemotherapy.

An association has been found between the CD3+ CD5- cells and the later or simultaneous appearance of clinical GVHD, but it was thought unlikely that these cells were involved in the production of GVHD.⁶ Our findings suggest that this distinct chemosensitive subpopulation of T lymphocytes, which may show a transient rise to above normal limits following autologous transplantation or chemotherapy, is a normal component of the regenerating lymphoid system.

Figure 2 Case 1 after chemotherapy showing recovery of CD3+ CD5+ and CD3+ CD5- populations after treatment.

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