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T lymphocyte regeneration after transplantation of T cell depleted allogeneic bone marrow

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SUMMARY

The regeneration of T cell subsets was studied with double immunofluorescence marker methods in 37 patients who received HLA matched T lymphocyte depleted bone marrow transplants (BMT) as part of the treatment for their haematological disease. A cocktail of anti-pan-T (CD6: MBG6) and anti-suppressor/cytotoxic-T cell (CD8: RFT8) monoclonal antibodies was used with rabbit serum as a source of cytolytic complement to achieve selective T cell lysis. The $T8^+$ cells reached low normal values around 60 days post-transplant and remained within the normal range throughout the study (> 150 days). This observation is in contrast to our previously published results in patients who, after receiving BMT without efficient T cell depletion, had increased numbers of circulation $T8^+$ cells from 60 days post-transplant. In the present study Leu-7^+, $RFT8^-$ cells reached normal values rapidly but the reconstitution of $T4^+$ lymphocytes was slow: low normal levels were reached only around day 150 following BMT. The degree of graft-versus-host disease (GVHD) seemed to be related to the number of residual T cells infused: two of the three patients who received the highest numbers of T cells developed Grade II and III; otherwise GVHD was minimal. Among the clinical parameters studied cytomegalovirus (CMV) immune status moderately influenced reconstitution: at 55–90 days post-transplant $T8^+$ cells were present at the upper normal levels in seven out of 15 patients receiving BMT from CMV seronegative donors, but in none of the 16 individuals receiving BMT from seropositive donors. CMV related complications were relatively uncommon. Thus the most significant factor in preventing '$T8^+$ cell overshoot' and T cell imbalance during regeneration appears to be the depletion of T (including $T8^+$) lymphocytes from marrow. The differences of $T8^+$ cell reconstitution in this and previous studies may reflect a different regeneration pattern from T cell precursors as opposed to inoculated mature T cells.

Keywords T lymphocyte depleted bone marrow transplants graft-versus-host disease T lymphocyte regeneration

INTRODUCTION

Following bone marrow transplantation (BMT), given as rescue from chemoradiotherapy in leukaemia treatment, the donor bone marrow (BM) is the source of both haematopoietic and

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lymphoid precursors. Animal experiments have demonstrated that BM derived pre-T precursors are educated in the recipients' thymus (Zinkernagel *et al.*, 1978; Wagner *et al.*, 1981), and mature T lymphocytes can be depleted from the bone marrow in order to prevent graft-versus-host disease (GVHD) (Rodt *et al.*, 1979; van Bekkum *et al.*, 1972). In man removal of T lymphocytes from donor BM has also reduced the incidence of GVHD (Prentice *et al.*, 1984; 1985). In this study we have explored the pattern of T cell reconstitution in recipients of T cell depleted bone marrow grafts.

In previous studies, following BMT without effective T lymphocyte depletion of the donor BM, an imbalance of $T4^+$ and $T8^+$ lymphocyte subsets with a $T8^+$ lymphocytosis has been observed (Favrot *et al.*, 1983; Schroff *et al.*, 1982; Friedrich *et al.*, 1982; DeBruin *et al.*, 1981; Atkinson *et al.*, 1982). Many $T8^+$ lymphocytes co-expressed Class II (HLA-DR) and HNK-1 (Leu-7) antigens (Favrot *et al.*, 1983). This T cell regeneration is different from fetal development in which these cell types are virtually absent (Abo, Cooper & Balch, 1982). The specific aims of the present study were, therefore, to investigate in recipients of T-lymphocyte depleted bone marrow grafts the reconstitution of the various T-lymphocyte subsets identified by sensitive double marker methods. In addition, we have studied the clinical parameters, such as the numbers of residual identifiable T cells infused, development of GVHD, and the cytomegalovirus (CMV) immune status of donors and recipients pre- and post-transplant (Gratama *et al.*, 1984; Verdonck & De Gast, 1984), factors which might influence the developing T cell immunity.

MATERIALS AND METHODS

Patients. From a group of 37 patients receiving BM from HLA-matched sibling donors follow up samples were available in 31 individuals. The other six patients were not studied because of early death (2), graft rejection (1) or insufficient data (2). These donor-recipient pairs were fully matched for HLA-, B antigens (and HLA-C, -DR antigens, where tested), and were unreactive in mixed lymphocyte cultures. Seven patients had acute myeloid leukaemia (AML): six in first complete remission (1CR) and one in relapse. Eighteen had acute lymphoblastic leukaemia (ALL): seven in 1CR, seven in second CR (2CR) and four in relapse. Eight patients had chronic granulocytic leukaemia (CGL): five in the chronic phase (CP), two in accelerated phase (AP) and one in blast transformation (CGL-BC). Finally, one patient had Fanconi's anaemia, one thalassaemia major and one severe aplastic anaemia. All patients with leukaemia in 1CR or CP received conditioning of high dose cyclophosphamide (60 mg/kg $\times 2$) and a single dose, total body irradiation (TBI) delivered with a fast dose rate (7·5 Gy at 26 cGy/min in air; mid plane received dose approximately 15 cGy/min). This conditioning has permitted sustained engraftment in all but one of these patients, as discussed by Patterson *et al.* (1985). In the later part of the investigation patients in 2CR or relapse were transplanted following an intensified conditioning protocol: Ara-C (3 g/m^2 12 hourly six times), cyclophosphamide (45 mg/kg $\times 2$) with a single dose of TBI as above (Prentice *et al.*, 1985). Patients with non-leukaemic diseases received Busulphan (4 mg/kg) on four consecutive days and cyclophosphamide (50 mg/kg) on 4 days with or without anti-lymphocyte globulin (ALG). After marrow harvest (mean $= 3{\cdot}4 \times 10^8$/kg; range 1·8–9·0), the BM mononuclear cells were separated using an IBM 2991 cell processor (Gilmore *et al.*, 1982). A mean of $3{\cdot}9 \times 10^7$/kg cells (range 1·4–9·9) were infused into the patients following incubation with a cocktail of pan-T antibodies and cytolytic complement (see below). Recovery of the WBC to $> 10^9$/l WB*g*C was achieved by 23 days (mean; range 12–37); $> 5 \times 10^8$/l neutrophils were observed by 27 days (mean; range 13–72 days). Engraftment was sustained in all 31 patients who are the subject of this study.

T lymphocyte subsets were studied at monthly intervals from 3 weeks to 6 months after BMT. The clinical assessment of GVHD was based on skin, liver and gut involvement (Thomas *et al.*, 1975). CMV infections were assessed by weekly culture of urine, blood and saliva, and, in cases of pneumonitis, by culture from bronchoalveolar lavage, in combination with a monoclonal antibody (MoAb) based test for early CMV antigens (Griffith *et al.*, 1984). Patients who were seropositive (anti-HSV $> 1:8$) for herpes simplex virus received prophylactic acyclovir (Hann *et al.*, 1982). Patients who, along with donors, were seronegative for CMV received blood from CMV seronegative donors.

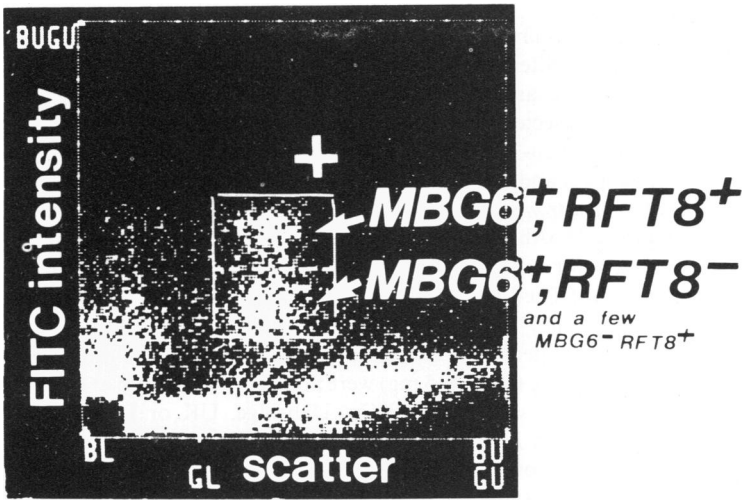

Fig. 1. Flow cytometric analysis of peripheral T cells with the cocktail of MBG6 (CD6) and RFT8 (CD8) Mc Abs on the EPICS V analyser (Coulter). Most T cells of suppressor/cytotoxic type are labelled by both Abs (MBG6$^+$, T8$^+$) and show stronger staining than those of helper type (MBG6$^+$, T8$^-$). A few T cells are MBG6$^-$, T8$^+$; see text.

Monoclonal antibodies with lytic activity. Two murine antibodies (Abs), MBG6 and RFT8, were selected on the basis of reactivity with peripheral T lymphocytes and negativity with all other BM cell types. Both were analysed in suspensions, tissue sections and in functional assays, such as phytohaemagglutinin (PHA) cultures and clonogenic assays for BM precursor cells. MBG6 (CD6), is a pan-peripheral T cell Ab of IgM class, which recognizes an antigen of 120 kD (CD6). This Ab reacts with $>93\%$ of T lymphocytes and some cases of B-CLL but is negative on normal B cells and on identifiable BM precursor cells including CFU-GEMM, CFU-GM, BFU-e and CFU-e (Bastin *et al.*, 1981). The RFT8 (CD8), also of IgM class, recognizes a 32 kD antigen on T cells of suppressor/cytotoxic type, and is unreactive with other cells (Batory *et al.*, 1984). The two Abs, used in combination, label the majority of T8$^+$ cells very strongly and the T4$^+$ cells moderately strongly (Fig. 1).

Fluorescence analysis. This was mostly performed on Ficoll-Hypaque separated cells using a Zeiss microscope equipped with epifluorescence attachment. Two staining combinations were used. The cells labelled with anti-T Abs of IgM class were visualized using fluoresceinated goat anti-mouse Ig (G-anti-M-Ig-FITC). Dead cells were studied in the same samples by double labelling with ethidium bromide (van Rood *et al.*, 1976; Granger *et al.*, 1982). MoAb of IgM and IgG classes were used in combination with G-anti-M-IgM-FITC and rhodamine labelled G-anti-M-IgG-TRITC second layers (Biotechnological Associates, Birmingham, AL, USA). In the BM samples the CD6+CD8 cocktail of IgM Abs was used together with CD3 (UCHT1) Ab of IgG class, the latter being an independent T cell marker. Other reagent combinations were used on Ficoll-Hypaque separated blood mononuclear cells. The proportions of T4$^+$ (CD4) and T8$^+$ (CD8) cells were determined by double staining using Leu-3 (CD4)-FITC and RFT8 (CD8)-TRITC. Similar tests were performed using Leu-7 (HNK-1)-FITC labelled together with RFT8-TRITC. The proportions of T3(CD3) positive cells were also determined, and corresponded to the total of T4$^+$ plus T8$^+$ cells and to 65–75% of Leu-7$^+$ cells within the lymphoid population. The percentage of SmIg$^+$ B cells was also studied. With the help of these values and the total lymphocyte count, the absolute numbers of various lymphocyte types in the blood were calculated. The two sets of control values were normal volunteers ('c') and patients in remission after having been treated for leukaemia ('p').

Fluorescence analysis on EPICS-V. Dual colour fluorescence studies using fluorochromes phycoerythrin (PE; orange red colour) and fluorescein isothiocyanate (FITC; green) were

performed on a Coulter EPICS V cell sorter with the following settings: laser 300 mW at 488 nm, photomultiplier tube 700 V, 515 nm blocking filter, 560 nm dichroic mirror, 560 nm SP filter for the FITC chanel and a 570 nm LP filter for the PE chanel. Electronic subtraction was used to eliminate fluorescence spillover between chanels. The lymphoid cells were gated on forward angle light scatter and studied at 488 nm with selected filters for FITC and phycoerythrin (Lanier *et al.*, 1983). The double combinations were Leu-3 (CD4)-FITC with Leu-2 (CD8)-phycoerythrin (Leu-2-PE; Becton-Dickinson-Labimpex) and Leu-7 (HNK-1)-FITC with Leu-2-PE.

Analysis of T cell removal from the BM. The C′ batches were standardized for optimal efficacy (on T cells in the presence of MoAb) and lack of 'unwanted' toxicity (on non-T lymphocytes and BM clonogenic myeloid progenitor cells in the presence and absence of MoAb; Granger *et al.*, 1983). The large scale preparation of T cell-depleted BM was carried out on an IBM 2991 cell washer using Ficoll-Hypaque (Gilmore *et al.*, 1982). BM cells were washed to remove anti-complementary activity. The cells were resuspended at 10^8 concentration in buffered salt solution (HBSS with Ca^{2+} and Mg^{2+}). MoAb (MBG6, 5 mg + RFT8, 5 mg) were added for 15–20 min at 20°C. Then, without washing, rabbit serum (Northeast Biomedical Co. Uxbridge, UK or Research Sera Ltd, East Grinstead, UK) was admixed in 1:4 final dilution as a source of cytolytic C′. The mixture was incubated for 45 min at 37°C, followed by a second round of incubation with C′. It has recently been found that when the 'T cell contamination' of the BM sample was high (>20–30%), both the amount of Abs and that of incubating medium (HBSS with 25% rabbit serum) had to be increased to maintain >98% lysis of T cells.

CMV diagnosis. Sera, obtained from patients and their donors prior to BMT, were tested by radioimmunoassay for IgG Abs against CMV (Schroff, Gale & Fahey, 1982; Griffith *et al.*, 1984). For the analysis of CMV infection following BMT methods were used in parallel. For detecting early antigen fluorescent foci (DEAFF) a pool of seven MoAb directed against different CMV specific proteins was used (Griffith *et al.*, 1984). The cytopathic CMV effects in cultures of human embryo lung fibroblasts were also studied. These two tests are fully specific and give positive results within 27 h and 17–18 days, respectively.

RESULTS

Residual T cells in the BM and GVHD

Twenty six of the 31 patients received $< 1 \times 10^6$/kg T cells (Table 1); 19 had no GVHD. Mild chronic GVHD developed with no pre-dating acute phase in patient 21 and resolved with steroids. Six patients had acute Grade I GVHD with mild skin involvement only (patients 20 & 22–26). In patient 22 the GVHD has resolved with no further treatment, in three patients (nos 20, 23 & 24) steroids were given in low dose, and two further patients progressed to mild chronic GVHD which resolved with steroids in patient 25 and with steroids plus azathioprine in patient 26.

T cell depletion was less complete in Patients 27–31 who received $> 1{\cdot}3 \times 10^6$/kg T cells; all of these patients subsequently developed GVHD. This was mild in patients 27, 28 and 29 and resolved with steroids. Patients 30 and 31 received the highest 'dose' of T cells ($1{\cdot}95$–$2{\cdot}0 \times 10^6$/kg). Patient 30 had Grade II GVHD and died from septic shock when neutropenic due to 'autologous' anti-netrophil antibodies whilst also receiving steroids for GVHD. Patient 31 developed Grade III GVHD which progressed to chronic GVHD but resolved after 9 months treatment with steroids plus azathioprin.

Reconstitution of $T4^+$ and $T8^+$ cells

The $T8^+$ cells recovered more rapidly than the $T4^+$ population (Fig. 2a). The control range of $T8^+$ cells in healthy individuals is $3{\cdot}9 \pm 1{\cdot}2$ (s.e.m.) $\times 10^5$/ml. Following BMT $3{\cdot}0 \pm 2{\cdot}3 \times 10^5$/ml and $3{\cdot}2 \pm 2{\cdot}4 \times 10^5$/ml $T8^+$ cells were observed at 46–60 days and 61–90 days, respectively. $T8^+$ cell numbers remained around these near normal levels throughout the study ($3{\cdot}4 \pm 1{\cdot}6 \times 10^5$/ml at days 120–160).

The control range of $T4^+$ cells in healthy subjects is $6{\cdot}5 \pm 1{\cdot}5 \times 10^5$/ml. The $T4^+$ population in BMT patients reached the level $2{\cdot}4 \pm 2{\cdot}2 \times 10^5$/ml around 45–60 days, and remained subnormal

Table 1. The relationship between the number of infused T cells and GvHD

No. of patient	UPN no.	Age (years)	Percent of residual T cells in BM	Absolute no. of T cells infused $\times 10^6$/kg	Clinical course acute:chronic GVHD grade
Patients receiving $< 1 \times 10^6$/kg T cells:					
1–19	*	7–45	0·3	0–1·0	O–O
20	102	16	0·2	<0·1	I–O
21†	94	30	0·5	0·1	O–C
22	91	36	<1	<0·2	I–O
23†	99	18	2·3	0·22	I–O
24	104	39	2·1	0·27	I–O
25‡	73	10	<1	<0·3	I–C
26†	109	42	1·5	0·18	I–C
Patients receiving $> 1{\cdot}3 \times 10^6$/kg T cells:					
27	124	16	2·9	1·35	I–O
28	125	12	3·8	1·5	I–O
29†	121	21	8	1·9	I–O
30	120	19	11	1·9	II–O
31	110	31	18	2·0	III–C
Patients with no T cell removal ('control' group): Nine out of 14 patients developed GVHD Grade II–IV					

* Unique patient numbers (UPNs) are: 1=78; 2=79; 3=80; 4=82; 5=83; 6=85; 7=87; 8=89; 9=92; 10=92; 11=103; 12=105; 13=106; 14=111; 15=112; 16=117; 17=119; 18=122 & 19=126.

† These patients showed a moderately accelerated $T8^+$ cell reconstitution. Two patients (nos 21 & 23) were recipients with latent CMV infection receiving BM from CMV negative donor.

‡ This patient also had CMV infection (isolated from urine).

until 160 days post-transplant ($4{\cdot}5 \pm 2{\cdot}5 \times 10^5$/ml). This value was no longer different from the slightly lower than normal values seen in patients following chemotherapy ($4{\cdot}8 \pm 1{\cdot}7 \times 10^5$/ml; Fig. 2a).

The phenotypic features of $T8^+$ cells were further analysed in a selected group of patients using double labelling with Leu-7. Five patients were studied at days 40–60, and another five at days 150–180 post-transplant (Fig. 3). In all patients 20–66% of $T8^+$ cells were also Leu-7^+, and additional cells (10–30% of lymphoid cells) were Leu-7^+ but $T8^-$ (Fig. 3).

Finally, the expression HLA-DR (Class II) antigens was studied in the $T8^+$ subset: 10–30% of these cells were HLA-DR^+ (data not shown). These as well as the Leu-7^+ cells were absent in cord blood (A in Fig. 3).

T cell regeneration and other clinical features

We have searched for clinical events which may influence the levels of circulating $T8^+$ cells during the 55–100 days post-transplant period. Seven of 31 patients (patients 2, 3, 8, 21, 23, 26 & 29) showed a $T8^+$ cell reconstitution to the higher range of normal levels as defined by an absolute count of $6–9 \times 10^5$/ml $T8^+$ cells and a low (0·12–0·40) T4/T8 ratio. None of our patients had higher than 9×10^5/ml $T8^+$ cells. (The rest of the patients had >0·45 T4/T8 ratio).

Six of these patients received low T cell numbers ($< 0{\cdot}5 \times 10^6$/kg), and only one (patient 29) received high numbers ($1{\cdot}9 \times 10^6$/kg). This was the only case where the $T8^+$ recovery was unusually swift (day 55) with a rapid return to low normal levels in subsequent samples. Three of the seven patients (patients 2, 3 & 8) had no GVHD, but the other four had mild GVHD (Table 1).

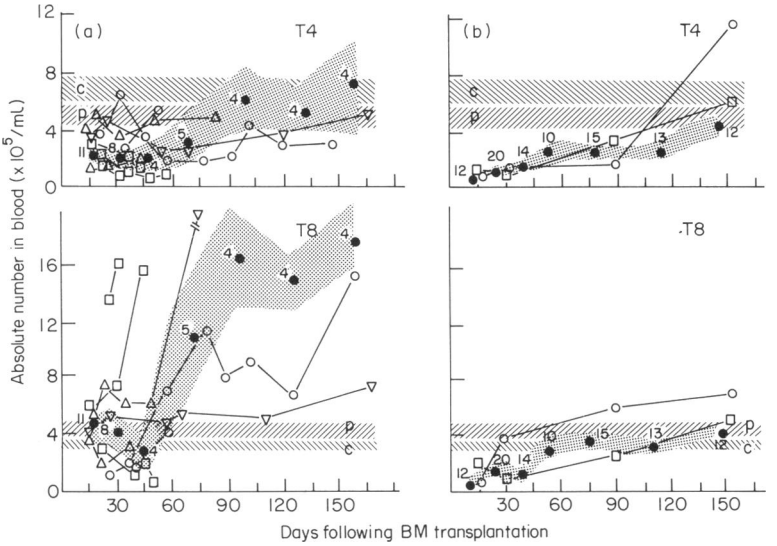

Fig. 2. Regeneration of T4+ and T8+ subsets after the transplantation of fully matched allogeneic BM. (a) Patients undergoing BMT without T cell depletion (historical control from Favrot *et al.*, 1983; with permission of the publishers); (b) patients following the transplantation of T cell depleted BM. The mean values (●) and the range of standard error (▥) are shown. Numbers refer to the observations in each group. Individuals with serious complications are separately shown as follows: patients with severe (Grade II–IV) GVHD (▽,△); patients with severe CMV infection (○); patients with both severe GVHD and CMV infection (□). In 'b' patients 21 (○) and 31 (□) are separately depicted. The controls are: normal blood ('c') and patients waiting for BMT following therapy ('p').

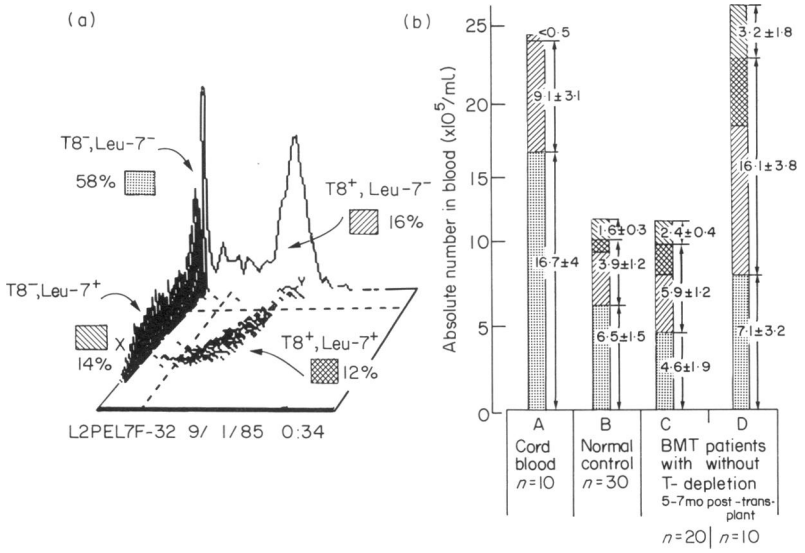

Fig. 3. Analysis of the absolute numbers of T lymphoid subpopulations in the blood following BMT. The proportions of T8+ and Leu-7+ cells were determined by double staining (a). Similar double labelling was also performed with T8 and Leu-3 (T4-like; CD4) as described in Materials and Methods. Absolute numbers were calculated on the basis of lymphocyte blood cell count (b). While T4+ (▥) and T8+ cells (▨) represent separate populations, some cells are double labelled with T8 and Leu-7 (▩). Lymphocytes from fetal cord blood (A) and normal blood (B) served as controls for the lymphocytes seen in the blood of patients 5–7 months following the transplantation of bone marrow with or without depletion of T cells (C & D, respectively).

Table 2. CMV status of recipient/donor and T8+ cell regeneration*

Group	no.	CMV serology pre-transp. recipient	CMV serology pre-transp. donor	T8+ regeneration to upper normal levels	T8+ regeneration normal	Per cent of rapid T8+ regeneration	
1	10	−	−	4†	6§	40	47
2	5	+	−	3‡	2§	60	
3	6	−	+	0	6§	0	0
4	10	+	+	0	10¶	0	

* T8+ regeneration at 55–90 days post-transplant to 6–9 × 10^5 T8+ cell/ml blood with <0·4 T4+/T8+ ratio 2–3 months after BMT.

† Patients 2, 3, 26 and 29.

‡ Patients 8, 21 and 23.

§ No signs of CMV infections were detected in these groups.

¶ Five of these patients excreted CMV in urine (patients 16–18, 24 & 31) and one had late CMV pneumonitis (patient 31)

A correlation was observed between the T8+ reconstitution and CMV immune status. All seven patients showing the recovery of T8+ cells to the upper normal range received BM from CMV-seronegative donors (Groups 1 & 2, Table 2). Three of these patients had Abs to CMV before transplantation (patients 8, 21 & 23 in Group 2), and these patients had CMV reactivation following BMT. This was manifest by the isolation of CMV from urine (patients 21 and 23), by CMV pneumonitis (patient 21) and increasing CMV Ab of IgM class (patient 8). Four patients with T8+ reconstitution to upper normal levels were in the CMV seronegative donor/recipient category (Group 1, Table 2). Patient 2 had a primary CMV infection in the early post-transplant period (day 20). Patient 29 was discussed above. There was no apparent correlation between identifiable viral infection and the rapid T8+ cell regeneration in Patients 3 and 26.

In 16 patients receiving BM from CMV-seropositive donors (groups 3 & 4 in Table 2) the T8+ cells recovered to normal levels, but not to as high values as the upper limits of normality.

Finally, the relationship of CMV morbidity and GVHD can be summarized as follows. Nine patients developed evidence of CMV infection (patients 2, 8, 16, 17, 18, 21, 23, 25 & 31). Two of these also had mild acute GVHD (patients 23 & 25) and one had severe GVHD (Grade III, patient 31). Three individuals (patients 21, 25 & 31) developed chronic GVHD. (Table 1). Apart from another patient (no. 26), these were the only cases who developed chronic GVHD. In two patients late CMV pneumonitis developed (patients 21 & 31). Following rapid diagnosis with DEAFF hyperimmune CMV immunoglobulin was given (Biotest, Birmingham, UK); both patients have recovered.

DISCUSSION

Our study confirms previous observations that the following BMT lymphocytes of the T8+ subset regenerate to normal levels more rapidly than those of the T4+ subset (Favrot *et al.*, 1983; Schroff *et al.*, 1982; Friedrich *et al.*, 1982; De Bruin *et al.*, 1981; Atkinson *et al.*, 1982). In our T cell depleted BMT patients, however, T8+ cells remained within the range seen in the normal peripheral blood. This is in contast with the findings of previous studies where depletion of T lymphocytes was not performed, and the absolute numbers of circulating T8+ cells increased from two- to four-fold above normal levels from the second months onwards post-transplant. The findings in our historical control group given HLA-matched BMT without complete removal of T lymphocytes are shown in Fig. 2a. In other studies the 'T8-overshoot' was also seen irrespective of whether the

patients received autografts (Singer, Tansey & Burnett, 1983), syngeneic transplants from their identical twins (Witherspoon *et al.*, 1982) or allogeneic grafts from their siblings (Friedrich *et al.*, 1982; De Bruin *et al.*, 1981). This phenomenon of 'T8-overshoot' was observed in patients receiving methotrexate (Favrot *et al.*, 1983; Schroff *et al.*, 1982; Friedrich *et al.*, 1982; De Bruin *et al.*, 1981; Atkinson *et al.*, 1982), Cyclosporin-A (Favrot *et al.*, 1983) or no prophylaxis for GVHD (Singer *et al.*, 1983; Witherspoon *et al.*, 1982), leading to severely depressed values of T4/T8 ratio.

$T8^+$ cells in the normal adult blood are heterogeneous and include Leu-7^- and Leu-7^+ cells (Abo *et al.*, 1982; Pizzolo *et al.*, 1984; Lanier *et al.*, 1983). High levels of $T8^+$, Leu-7^+ cells are seen in some immunosuppressed patients with CMV infections (Maher *et al.*, 1985) and/or GVHD following BMT without T cell depletion (Favrot *et al.*, 1983). Many of these cells also coexpress Class II ($HLA\text{-}DR^+$) antigens. Such cells are virtually absent in the fetus and cord blood. Thus this population is likely to be part of a response to extrinsic antigens including viruses. We have now found that after T cell depletion from the BM the absolute numbers and relative proportions of $T8^+$ and Leu-7^+ subsets in the peripheral blood are close to the values seen in the normal adult blood and different from those seen in cord blood.

In adult, but not in fetal, blood Leu-7^+, $T8^-$ lymphocytes can also be detected and partly contribute to natural killer (NK) activity. These cells are not removed from the bone marrow inoculum by our anti-T cell Ab-cocktail. The early appearance of this population in our patients is in agreement with the results of functional assays which indicate a rapid recovery of NK cells (Rooney *et al.*, 1985). These subsets regenerate from donor cells. In the blood of three patients who received sex-mismatched BM, PHA stimulated dividing cells (including $T4^+$ & $T8^+$ cells) were of donor type. Furthermore, double labelling experiments performed using Leu-7-TRITC and quinacxine, a marker for Y chromosome revealed that Leu-7^+ cells were also of donor origin (H. Walker, H. Goodall, M. Brenner, D. Campana & G. Janossy, unpublished results).

The two striking clinical observations in our patients have been the reduction of GVHD (Table 1) and the relatively low incidence of severe CMV related complications. It is, however, unlikely that the reduction in GVHD itself is responsible for the near normal T cell recovery. Only in four patients with mild GVHD could a slight increase to the upper normal range of $T8^+$ cells be observed, and three patients with no GVHD have also shown this pattern (Table 2). On the other hand, the CMV immune status may have influenced, although only moderately, the T cell reconstitution. In some patients who received bone marrow from CMV seronegative donors the $T8^+$ cells regenerated to upper normal levels, irrespective of the CMV status of the recipient. In the other group who received bone marrow from CMV immune individuals, $T8^+$ cells remained invariably below 6×10^5/ml for a period of 3 months and T4/T8 ratios were above the 0·45 value. There is some evidence to suggest that short term humoral immunity to CMV can be transferred by allogeneic BM (Wahren *et al.*, 1984; Wimperis *et al.*, 1986), so that the sparing of B lymphocytes and plasma cells in the BM by our T cell specific cocktail might be advantageous. It is known that stimulated B cells and plasma cell precursors migrate to the BM, an important site of immunoglobulin synthesis in the body (Benner, Hijmans & Haaijman, 1981). An alternative explanation for these findings is that the transfer of a putative Leu-7^+, $T8^-$ NK-cell affiliated population may regulate T cell reconstitution, and that these cells may be in a different functional state depending upon the donors' CMV immune status. The relationship of immune reconstitution and CMV immunity clearly requires further analysis.

We therefore conclude that the differences of $T8^+$ cell reconstitution between this and the previous studies are most probably due to the fact that in the present study T ($T8^+$) lymphocytes were efficiently removed from the bone marrow inoculum. It is relevant that the antibody cocktail used in our series is particularly efficient in lysing $T8^+$ cells. Thus the results may be a reflection of a different regeneration pattern from T cell precursors as opposed to inoculated mature T cells. Our findings also delineate a tentative relationship between T cell reconstitution, GVHD and the activation of T lymphoid cells during viral, most prominently CMV, infections. Previously in patients who received BMT without T cell depletion high $T8^+$ cell levels led to a marked and lasting imbalance of T4/T8 lymphocytes that is unlikely to be beneficial and may contribute to a vigorous response against minor histocompatibility antigens. GVHD, in turn, is known to disrupt immune regulation (Witherspoon *et al.*, 1981) and is further treated with immunosuppressive drugs.

Diminished control over viral infections is then an obvious possibility. Severe CMV infection, once established, may further activate suppressive $T8^+$ cells and abolish helper effects with a potentially disastrous outcome (Verdonck *et al.*, 1984). The observations summarized above indicate that this vicious circle may be aborted by the selective depletion of mature T cells from BM, and by avoiding unnecessary immunosuppressive therapy such as GVHD prophylaxis during immune reconstitution. Early diagnosis of CMV infections followed by rapid administration of hyperimmune anti-CMV globulin may also play a beneficial and complementary role in the management of patients following BMT.

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