Of the patients who had severe reactions to suxamethonium only 1 had received the drug previously. The possibility of sensitivity being acquired from other quaternary ammonium compounds has been suggested,[14] and the high frequency of positive prick-tests to 'Cetrimonium' (ICI), a quaternary ammonium compound, in patients who reacted to suxamethonium in a French series[10] is interesting.

The incidence of severe anaphylactoid reactions during anaesthesia is 1 in 10 000 cases. Consequently, screening a large population for the antibody is unlikely to be rewarding or a cost-effective preventive manoeuvre. Further studies are also needed to determine the value of this assay in the diagnosis of anaphylactoid reactions during anaesthesia. However, administration of suxamethonium to subjects who have a positive reaction with the assay should be avoided. The occurrence of minor reactions in 2 patients (17 and 19) who had negative skin-tests, but significant uptakes of radioactive anti-IgE, suggests that the assay may add useful information to intradermal testing, that the mechanism of these reactions involves IgE, that subsequent exposure to relaxants incriminated by intradermal testing is contraindicated, and that the cross-sensitivity between relaxants is likely to be a greater problem than previously believed.[6]

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IS CYTOMEGALOVIRUS INFECTION A MAJOR CAUSE OF T CELL ALTERATIONS AFTER (AUTOLOGOUS) BONE-MARROW TRANSPLANTATION?

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Summary   Peripheral-blood T cell subsets and functions were studied in 14 patients with malignancies treated with high-dose chemotherapy and radiotherapy followed by autologous bone-marrow transplantation (BMT). 8 CMV-positive patients (6 with latent infections and 2 with a primary infection) showed an inversion of the OKT4/OKT8 ratio caused by an increase in OKT8+ T cells and a decrease in OKT4+ T cells. This was accompanied by a pronounced increase in the percentage of HLA-DR+ T cells, and functionally by increased suppressor T cell activity and decreased helper T cell activity and T cell proliferation. These alterations in T cell subsets and functions were not observed in 6 patients who were kept CMV-negative by a deliberate transfusion policy. In the 6 patients helper T cell activity and T cell proliferation capacity recovered well after day 30. Differences between the two groups were most striking 60 days after BMT. This study suggests that CMV infection, whether primary or secondary, is a major cause of T cell alterations after (autologous) BMT.

Introduction

PROFOUND combined immunological deficiency is found for at least 6 months after allogeneic bone-marrow transplantation (BMT). The resulting increased incidence of opportunistic infections[1-4] seems mainly to result from alterations of T cell functions and defective B cell maturation.[5-7] Although opportunistic infections are less frequent in syngeneic[8] and autologous[9,10] BMT, similar immunological alterations are found and thus seem to be independent of the type of graft or the presence of acute graft-versus-host disease.[7,11,12] The inversion of the OKT4/OKT8 ratio has been explained as being the result of a more rapid recovery of the OKT8 subset.[7,13,14] We have studied T cell alterations in CMV-positive patients and CMV-negative patients after autologous BMT.

Patients and Methods

Patients

8 patients with a non-Hodgkin lymphoma (NHL) of high-grade malignancy and 3 patients with acute leukaemia (AL) were treated with cyclophosphamide (120 mg/kg) and total-body irradiation (800 rad). 3 patients with a testicular carcinoma were treated with cyclophosphamide (6000 mg/m²) and etoposide (1200 mg/m²). Chemotherapy or radiotherapy was followed by autologous BMT. Details of the patients are summarised in table I.

A primary CMV infection, manifesting as interstitial pneumonia or haemorrhagic gastroenteritis, developed in patients 1 and 2. In each case the source of infection was thrombocyte concentrates obtained from a relation who was CMV seropositive. Cultures from both patients were CMV positive. Serological studies of 6 patients with a latent CMV infection demonstrated reactivation or reinfection (a fourfold or greater titre rise) in only patients 3 and 5 and cultures became CMV positive in patient 3. Prompted by the experience in the first 2 patients with a primary CMV infection, we adopted a deliberate transfusion policy in the 6 CMV-negative patients. Filtered leucocyte-free erythrocytes and thrombocyte concentrates from CMV seronegative donors were used. No granulocyte concentrates were used. These patients remained negative for CMV by serology and culture during follow-up. A fever (without other symptoms or signs) developed 2 months after BMT in patient 13. Antibody titres to Epstein-Barr virus (EBV) became positive, indicating a primary EBV infection.

TABLE I—PATIENT DATA

Patient no	Diagnosis	Sex	Age (years)	Status at BMT	Response	Duration of CR (months)	Cause of death	CMV status
1	NHL	M	53	Drug resistant	CR	2	CMV-IP	Primary
2	Testis ca	M	29	Drug resistant	CR	8	Relapse	Primary
3	ALL	M	23	1st CR	CR	14+	..	Latent
4	Testis ca	M	25	Drug resistant	NR	—	Progression	Latent
5	NHL	M	39	Primary, drug resistant	CR	4	Relapse	Latent
6	NHL	M	15	Primary, drug resistant	PR	—	Progression	Latent
7	AML	F	35	2nd CR	CR	3	Relapse	Latent
8	NHL	M	38	PR	CR	4+	..	Latent
9	NHL	M	20	1st CR	CR	18+	..	Negative
10	NHL	F	35	PR	CR	15+	..	Negative
11	NHL	M	33	Primary, drug resistant	CR	5	Relapse	Negative
12	Testis ca	M	24	PR after 1st relapse	CR	6+	..	Negative
13	NHL	M	23	1st CR	CR	5+	..	Negative
14	AML	M	28	1st CR	CR	2+	..	Negative

NHL = non-Hodgkin lymphoma; AML = acute myeloid leukaemia; ALL = acute lymphoblastic leukaemia; testis ca = testicular carcinoma.
CR = complete remission; PR = partial remission; NR = no response; IP = interstitial pneumonia.

Identification of T cell subsets.—Lymphocyte subsets were studied before and at regular intervals after BMT in the mononuclear fraction of peripheral blood. T lymphocytes and T cell subsets were determined by binding of the monoclonal antibodies OKT3 (pan T cell), OKT4 (comprising the helper/inducer T cell subset), and OKT8 (comprising the suppressor/cytotoxic T cell subset) (Ortho Pharmaceutical Laboratories, Raritan, USA). HLA-DR antigen was determined on T cells by the monoclonal antibody anti-human HLA-DR (clone L 243, Becton-Dickinson, Rutherford, USA).

Immunological function tests.—In-vitro cultures were performed as described elsewhere.[15]

Mitogen-induced proliferation.—The following mitogens were used in cultures containing 4×10^4 cells: phytohaemagglutinin (PHA, 100 μg/ml, HA 15, Wellcome), concanavalin-A (con-A, 75 μg/ml, A grade, Calbiochem), and pokeweed mitogen (PWM, 50 μg/ml, Gibco Biocult). The counts per minute (cpm) in cultures stimulated with mitogens were compared with those from 2 simultaneous controls. The results are expressed as percentage of control values.

Helper and suppressor T cell activity.—T cell effector functions were determined as described previously[7] with a slight modification. In brief, T and non-T cells from patients and controls were separated and mixtures of 2×10^4 non-T cells and 2×10^4 T cells (irradiated) were stimulated with PWM.[15] B cell differentiation was studied after 6 days of culture by counting the number of cytoplasmic Ig-positive cells which were immunofluorescent. To assess helper T cell activity 2×10^4 normal non-T cells were mixed with 2×10^4 patient T cells (irradiated) and to assess suppressor T cell activity, 1×10^4 patient T cells (non-irradiated) were added to mixtures of normal non-T and normal irradiated T cells. Helper T cell and suppressor T cell activities were expressed as the percentage of a normal control by means of the following formulas:

$$\% \text{ T cell helper activity} = \frac{Bn + TpI}{Bn + TnI} \times 100$$

$$\% \text{ T cell suppressor activity} = \left[1 - \frac{Bn + TnI + Tp}{Bn + TnI} \right] \times 100$$

B = non-T cell fraction; T = T cell fraction; I = irradiated; p = patient; n = normal control.

Normal T cells exhibited a suppressor activity between 0% and 50%.

CMV infections were monitored by measurement of antibody titres in an enzyme-linked immunosorbent assay (ELISA)[16] and by viral cultures (from urine, saliva, and buffy coat).

Statistical tests.—Differences between the CMV-positive and CMV-negative groups were evaluated by Student's *t* test. p values less than 0·05 were regarded as significant.
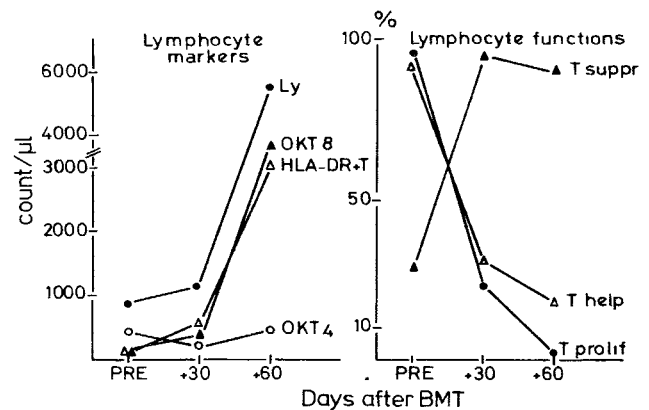

Fig 1—Primary CMV infection (2 patients).

T cell alterations were accompanied or even preceded by high suppressor T cell activity and very low helper T and proliferative T cell activity.

TABLE II—LYMPHOCYTE COUNT, OKT4/OKT8 RATIO AND PERCENTAGE HLA-DR+ T CELLS IN RELATION TO CMV INFECTION (MEAN±SEM)

| —	CMV status	Pre BMT	+30 days	+60 days	+100 days
Lymphocyte count (normal: 1500–4000μl)	Negative	1735* (±111)	1402 (±359)	752 (±108)	916 (±189)
	Positive	1249 (±157)	1745 (±247)	1591 (±249)	1279 (±254)
OKT4/OKT8 ratio (normal: 1·5–2·4)	Negative	1·7 (±0·2)	1·5 (±0·3)	1·3 (±0·1)	1·2 (±0·3)
	Positive	0·9 (±0·2)	0·6 (±0·2)	0·3 (±0·1)	0·3 (±0·2)
	†p value	<0·01	<0·01	<0·01	<0·01
% HLA-DR T cells (normal: <5%)	Negative	8 (±4)	20 (±6)	17 (±4)	11 (±3)
	Positive	30 (±8)	63 (±5)	48 (±7)	52 (±14)
	†p value	<0·05	<0·01	<0·01	NS

*Expressed as mean (±SEM).
†Student's *t* test.
NS = not significant.

Results

Lymphocyte Subsets after BMT

60 days after BMT the 2 patients with a primary CMV infection (fig 1) showed a pronounced increase in lymphocytes, which were nearly all activated T cells (HLA-DR+) with the suppressor/cytotoxic phenotype (OKT8+). On day 60 the OKT4/OKT8 ratio in these 2 patients was very
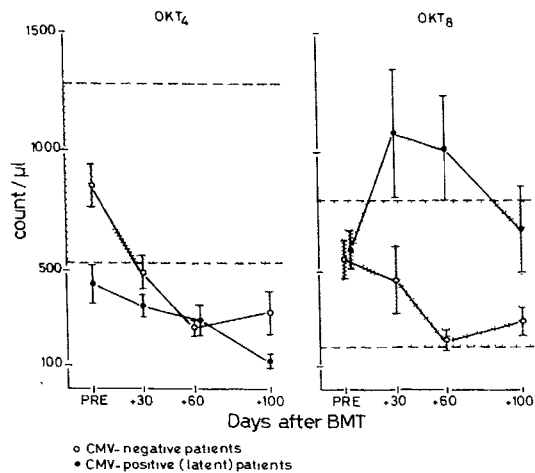
Fig 2—Counts of T cells of helper/inducer phenotype (OKT4 +) and of suppressor/cytotoxic phenotype (OKT8 +) in relation to CMV infection after BMT.

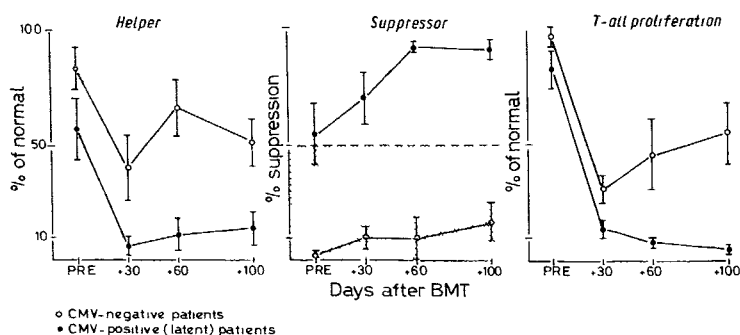Stippled bands indicate normal ranges.

low (0·02 and 0·3). The 6 CMV-positive patients with a latent infection showed a nearly normal lymphocyte count after BMT (table II). This was mainly the result of an increase in the number of OKT8+ T cells, and a concomitant decrease in OKT4+ T cells (fig 2). This resulted in an inverted OKT4/OKT8 ratio. Most T cells were HLA-DR+ too (table II).

In contrast, the 6 CMV-negative patients were lymphopenic (table II). OKT4+ and OKT8+ T cell numbers were decreased (fig 2), and the OKT4/OKT8 ratio was not inverted (table II). In 1 CMV-negative patient the OKT4/OKT8 ratio was inverted at day 60 and day 100 (0·2), probably as a result of a primary EBV infection. HLA-DR+ T cells numbers were only slightly increased in the CMV-negative patients (table II).

The CMV-positive patients with a latent infection had already an inverted OKT4/OKT8 ratio and an increased number of HLA-DR+ T cells before BMT. Difference in OKT4/OKT8 ratio and percentage HLA-DR+ T cells between the CMV-positive and CMV-negative group were significant, both before and after BMT.

Lymphocyte Function after BMT

In 2 patients with a primary CMV infection there was a pronounced increase in suppressor T cell activity and very low helper and proliferative T cell capacity after BMT (fig 1). The 6 CMV-positive patients showed similar T cell functions—ie, pronounced increase in suppressor T cell activity and very low helper T cell and T cell proliferative capacity (fig 3). Increased suppressor T cell activity was already present in 4/6 patients before BMT. In contrast, the 6

Fig 3—Lymphocyte function in relation to CMV infection.

Stippled bands indicate normal range.

CMV-negative patients showed a normal suppressor T cell activity before and after BMT (fig 3), except the patient with the primary EBV infection (50% suppression at day 60 and 89% at day 100). Differences between the two groups are significant before and after BMT if the patient with EBV is excluded. Helper T cell activity and T cell proliferation were low at day 30, but gradually recovered after that period, in contrast to the results in CMV-positive patients (fig 3). Differences between the groups were significant at day 30 and day 60 and for day 100 for T cell proliferation capacity.

Discussion

A preponderance of suppressor/cytotoxic T cells (OKT8+), an inversion of the OKT4/OKT8 ratio,[11,13,17,18] and the increase of HLA-DR+ T cells[18,19] have been reported after allogeneic BMT. The inversion was also found after autologous[11,20] and syngeneic[11] BMT. The preponderance of the OKT8+ subset is generally ascribed to a faster repopulation and maturation.[7,13,14] Most T cells in normal bone-marrow are OKT8+,[21] and the preponderance of OKT8+ T cells may also be the result of a rapid increase in numbers of already mature T cells.

In normal individuals herpes virus infections, especially CMV and EBV are accompanied by lymphocytosis and a marked increase in OKT8+ and HLA-DR+ T cells.[22-24] In this study a lymphocytosis with a high number of OKT8+ and HLA-DR+ T cells was found in 2 patients with a primary CMV infection after autologous BMT. The CMV-positive (latent) patients also showed a pronounced inversion of the OKT4/OKT8 ratio, which was the result of a low number of OKT4+ cells and a normal to increased number of OKT8+ T cells. The percentage of HLA-DR+ T cells was increased too. The CMV-negative patients displayed no inversion of the OKT4/OKT8 ratio. Both the number of OKT4+ T cells and the number of OKT8+ T cells were decreased. Only 1 CMV-negative patient showed an inversion of the OKT4/OKT8 ratio after BMT, probably as a result of a primary EBV infection.

The functional tests accorded with the alterations in T cell subsets. Patients with a CMV infection (both latent and primary) displayed a low T cell proliferation and helper T cell function and a markedly increased (non-specific) suppressor T cell function. These findings accord with other studies in BMT patients[3,5-7] and resemble findings in normal individuals with a CMV infection.[25] The CMV-negative patients in contrast, demonstrated a rather good recovery of helper T cells and T cell proliferative capacity after day 30 and a normal suppressor T cell function. Differences between the CMV-positive (latent infection) and the CMV-negative patients were significant in this small study.

Since both patient groups had received similar treatment with high-dose chemotherapy and/or radiotherapy before autologous BMT and since both groups differed solely in the presence or absence of CMV antibodies before BMT, it is unlikely that the observed T cell changes were the cause of the (secondary) CMV infection. That they were indeed the result of the CMV infection is supported by the data in the 2 patients with a primary infection, who showed a pronounced increase of OKT8+ and HLA-DR+ T cells only at day 60 and not at day 30 after BMT. In the CMV-positive patients the immunodeficiency caused by the high-dose chemotherapy and/or radiotherapy probably induced reactivation of the CMV infection, and alterations in T cell subsets resulted. None of the 6 CMV-positive patients showed clinical symptoms of CMV infection after BMT and reactivation demonstrated by a rise in titre and/or excretion of CMV was

found in only 2 patients. So T cell alterations may be the first sign of reactivation of CMV.

We conclude that a preponderance of OKT8 + T cells after BMT is not merely the result of a faster repopulation by OKT8 + T cells. In autologous BMT, CMV infections (and to a lesser degree other viral infections such as EBV infection) seem to be an important cause of immunological changes and of the preponderance of OKT8 + T cells with accompanying high suppressor T cell activity. CMV infection is probably important in allogeneic BMT too, although graft-versus-host disease also results in immunological changes. Since morbidity and mortality associated with primary CMV infections are high, a deliberate transfusion policy should be adopted in CMV-negative patients. The high suppressor T cell activity with an impairment of helper T cell and T cell proliferative capacity in patients with a latent CMV infection, probably increases the frequency of opportunistic infections. Effective measures to prevent a primary CMV infection or the reactivation of a latent CMV infection (possibly by immune plasma) may help to reduce the frequency of these complications.

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TYPING SCHEME FOR CLOSTRIDIUM DIFFICILE: ITS APPLICATION IN CLINICAL AND EPIDEMIOLOGICAL STUDIES

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Summary   Epidemiological studies of Clostridium difficile diarrhoeal disease have been hindered by the lack of a typing scheme for this organism. A typing method based on the incorporation of sulphur-35-labelled methionine into cellular proteins and their separation by sodium dodecylsulphate/polyacrylamide gel electrophoresis showed clear pattern differences between strains, and nine distinct groups within the C difficile species were established. 98% of 250 clinical strains derived from four hospitals were typable. Group X was the commonest group and was associated with outbreaks of pseudomembranous colitis and antibiotic-associated colitis in two hospitals. Groups A–D were isolated predominantly from mothers and newborn infants. In outbreaks of antibiotic-associated colitis in oncology and orthopaedic wards the same strains, group X and group E, respectively, were isolated from patients and their environment, providing strong evidence of cross-infection between patients and of hospital acquisition of C difficile.

Introduction

Clostridium difficile is well known to be the primary cause of pseudomembranous colitis and antibiotic-associated colitis.[1-5] There are several reports of clusters of such cases in hospitals.[6-11] However, the important questions of whether cross-infection occurs and whether patients acquire the organism from the hospital environment are still not answered, because a suitable system for typing this organism is lacking.

A bacteriophage typing scheme is being developed by Sell et al,[12] and Wüst et al[13] have applied various existing methods,

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