Increase and inhibition of pre-B cell proliferation in culture, by T cells

P. PAOLUCCI,* LORNA LAYWARD, A. R. HAYWARD,† N. T. RAPSON &
J. F. SOOTHILL *Departments of Immunology and Haematology, Institute of Child Health and
The Hospital for Sick Children, London*

SUMMARY

T cells may either increase or decrease *in vitro* proliferation of marrow pre-B cells from patients with acute lymphatic leukaemia after withdrawal of successful treatment. There is less proliferation when T cells are removed by E rosetting, and repletion of T cells restores proliferation. When additional T cells from the patients were added to the patients' marrows, proliferation was increased more effectively than with T cells from healthy subjects; there was no evidence of an allogeneic effect. In contrast, normal T cells stimulated with concanavalin A suppress proliferation. There was no evidence of differentiation into B cells.

INTRODUCTION

Pre-B cells are increased in number in the marrow of children who have recovered from acute lymphoblastic leukaemia after withdrawal of treatment (off-treatment ALL), and they divide in culture more readily than do the pre-B cells of normal marrow (Paolucci, Hayward & Rapson, 1979; Paolucci *et al.*, 1981). This suggests that a normal control mechanism was suppressed and T cells are an obvious possibility. We have therefore studied the effect of T cells on the proliferation of pre-B cells *in vitro*.

METHODS

Twenty-three children (median age 8·5 years; range 4–14) who had been successfully treated for ALL were studied 1–16 months after withdrawal of treatment (see Paolucci *et al.*, 1979, for details). Cell culture and staining methods are described in detail by Paolucci *et al.* (1981). Aliquots of bone marrow (aspirated from the iliac crest for clinical purposes) were studied; red cells were lysed with ammonium chloride and nucleated cells were washed. To count pre-B and B cells, cell suspensions were stained for surface IgM with fluorescein-conjugated anti-μ. They were then washed, cytocentrifuged, fixed in ethanol–acetic acid and stained for cytoplasmic IgM with rhodamine-conjugated anti-μ. T cells were detected by rosette formation following incubation for 30 min at 4°C with neuraminidase-treated sheep red blood cells (E_N).

* Present address: Istituto di Clinica Pediatrica, Universita di Bologna, Via Massarenti 11, 40138 Bologna, Italy.
† Present address: Department of Pediatrics, University of Colorado Medical Center, Denver, USA.
Correspondence: Professor J. F. Soothill, Department of Immunology, Institute of Child Health, 30 Guilford Street, London, WC1N 1EH.

T cells were depleted from bone marrow by rosetting with E_N followed by Ficoll–Triosil centrifugation at 4°C; interface cells were harvested and washed. T cells were isolated from heparinized blood from patients and healthy adults by the method of Hayward *et al.* (1978). Briefly, mononuclear leucocytes were separated by Ficoll–Triosil centrifugation and washed, and the adherent cells were removed by incubation on plastic. E_N were added and the rosetted T cells were separated at 4°C on Ficoll–Triosil; this procedure was repeated and the E_N were lysed with ammonium chloride. T cells were washed and either added directly to marrow cultures or incubated for 24 hr at 37°C with or without 10 μg/ml concanavalin A (Con A) (Sigma), then washed four times before being added to the cultures.

Nucleated bone marrow cells (2×10^5 in 0·2 ml in each of three wells) were cultured in flat-bottomed microtitre plates (Falcon) in RPMI 1640 with glutamine, gentamicin and 10% fetal calf serum in humidified 5% CO_2 (Paolucci *et al.*, 1981). In co-culture experiments, $1·5 \times 10^5$ blood T cells in 0·15 ml was added to each of three wells containing marrow cells. After 24 hr triplicate wells were harvested and pooled, cells were washed and viable cells were counted. The proportion of T cells bearing receptors for IgG (T_G cells) in isolated blood T cells was determined by rosette count with IgG-coated ox red blood cells after 10 min of incubation at 4°C according to Moretta *et al.* (1976). Statistical analysis was by paired *t*-test.

RESULTS

The proportion of both pre-B and T cells in marrows of ALL patients on remission maintenance treatment is low (Paolucci *et al.*, 1979) but after stopping the treatment, the proportion of pre-B cells rises to abnormally high values by 2 months and remains high throughout the period of the study; T cells remain below normal levels for about 6 months (Fig. 1).

To determine whether the lack of T cells contributed to this rise in pre-B cells, we studied their effect on pre-B cell culture. The number of pre-B cells in 24-hr cultures of patients' marrows after removal of T cells by E rosetting were compared with similar cultures of patients' marrows similarly processed on Ficoll–Triosil but without addition of sheep red cells. Depletion of T cells reduced the *in vitro* division of pre-B cells (Fig. 2). In three experiments, proliferation was restored when autologous or normal heterologous T cells were added back to the T-depleted bone marrow cultures. The cultures contained half the standard cell concentration (hence the lower numbers of pre-B cells without added T cells) and equal numbers of blood T cells were added. Pre-B cell numbers rose consistently during 24-hr culture (Table 1).
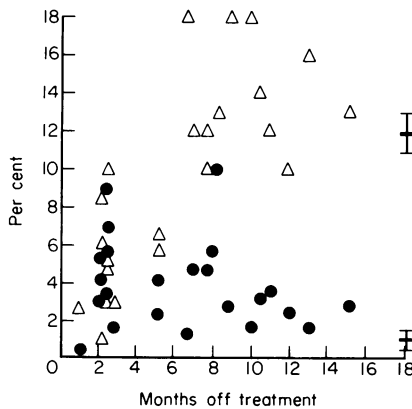
Fig. 1. Percentage of pre-B cells (●) and T cells (△) in the nucleated cells of bone marrow of patients with ALL in the months following the stopping of successful maintenance treatment. Bars indicate mean ± 1 s.e. for normal marrow from 12 children with various diseases (Paolucci *et al.*, 1981); the lower bar is for pre-B cells, the upper bar for T cells.

The addition of isolated adult blood T cells to cultures of patients' bone marrows increased the number of pre-B cells recovered after 24 hr (Fig. 3). This was not analysed because it could be an effect of increased cell number. A T cell effect was confirmed since patients' T cells increased the pre-B cell number significantly ($P < 0.05$) more than did T cells from healthy adults; such a difference was not seen in our restoration experiments (Table 1). Patients' T cells were similarly effective whether they were from the same or a different donor as the pre-B cells, i.e. there was no allogeneic effect.

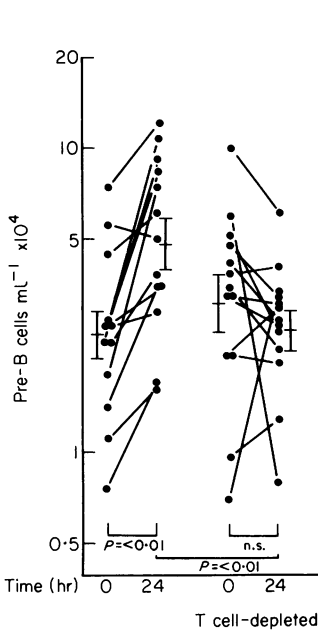These results suggested that T cells are required for the *in vitro* division of pre-B cells. To
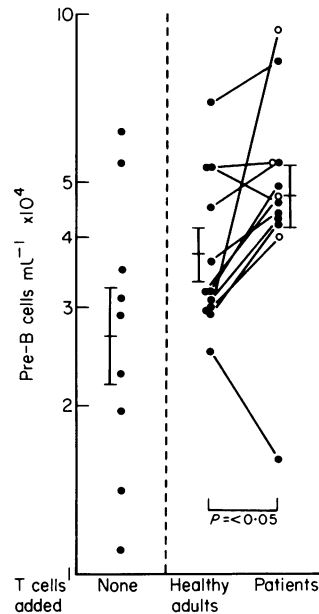

Fig. 2.   **Fig. 3.**

Fig. 2. Concentration of pre-B cells (i.e. CIgM$^+$, SIgM$^-$ cells; since the numbers of marrow cells and the dilutions were constant, concentration is directly related to number in each pool of triplicate cultures) before and after 24-hr culture of 10^5 bone marrow cells from off-treatment ALL patients, with or without T cell depletion by E$_N$ rosetting.

Fig. 3. Concentration of pre-B cells in cultures of 1.5×10^5 bone marrow cells from off-treatment ALL patients, with or without addition of 0.5×10^5 T cells from the blood of healthy adults or from off-treatment ALL patients. Autologous co-cultures (o) and heterologous co-cultures (●).

Table 1. Pre-B cell numbers at 0 and 24 hr in cultures of T cell-depleted off-treatment ALL bone marrow with or without addition of blood T cells – autologous or from healthy donors

T cells added	Patient 1		Patient 2		Patient 3	
	0 hr	24 hr	0 hr	24 hr	0 hr	24 hr
None	1·6	1·6	0·4	0·75	2·1	1·7
Autologous	1·8	4·2	0·37	2·2	1·7	4·7
Healthy donor	1·4	5·3	0·37	3·15	1·7	5·4

determine whether T cells can also suppress pre-B cell division, we added control or Con A-activated heterologous blood T cells to bone marrow cultures and compared the number of pre-B cells recovered after 24 hr. Preliminary studies with marrow to T cell ratios of 6:1, 3:1 and 1:1 showed the greatest difference in counts between stimulated and unstimulated T cells at the 1:1 ratio; this was therefore selected for further studies. There were significantly more pre-B cells in the cultures to which unstimulated T cells had been added ($P < 0.005$; Fig. 4). The high viability ($> 80\%$) of the recovered cells makes it unlikely that this difference was due to non-specific cytotoxicity by the Con A-activated T cells alone. There was a trend for cultures with unstimulated T cells to have more pre-B cells than did the cultures without added T cells, as was found in Fig. 3. As reported previously (Paolucci *et al.*, 1981) using a different cell concentration, there was no rise—indeed, a significant fall in B cell numbers in the marrow cultures—but cell concentrations were suboptimal for B cell culture.

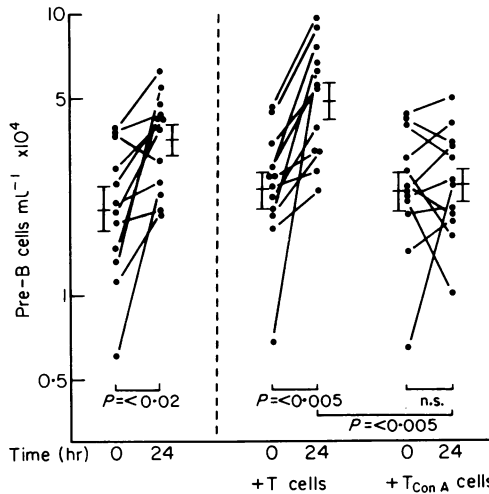Most of the T cells which suppress B cells are probably T_G cells (Moretta *et al.*, 1977). The

Fig. 4. Pre-B cell numbers before and after 24-hr culture *in vitro*, with or without addition of normal blood T cells, unstimulated or Con A-stimulated. The probabilities of differences by paired *t*-tests are shown.
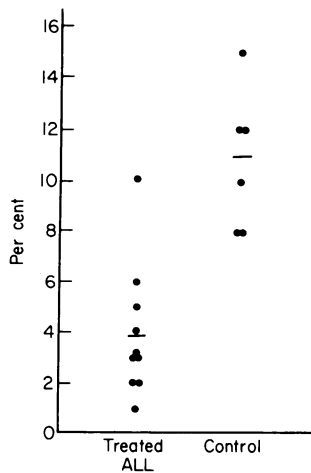

Fig. 5. Percentage of T_G cells in blood of off-treatment ALL patients and healthy adults.

marrow samples were too small for us to measure T_G cells in them, but the proportion of T_G cells in the blood of our off-treatment ALL patients was lower than in the healthy adult controls ($P < 0.001$) (Fig. 5); the proportion of normal T_G cells had reached adult values by the ages at which the patients were investigated (Trompeter, Layward & Hayward, 1978). However, this is not strong evidence that T_G cells normally perform this function, since this may be an independent effect of the disease or its treatment.

DISCUSSION

We have shown that T cells may both increase and reduce the proliferation in culture of pre-B cells from marrow of off-treatment ALL children. As with B cells (Hayward *et al.*, 1978), concanavalin A-treated T cells strongly suppressed pre-B cell proliferation in culture, but T depletion also prevented proliferation and T cells from healthy subjects and the patients restored it. Developmental (Gathings, Lawton & Cooper, 1977; Hayward *et al.*, 1977) and B cell suppression studies (Burrows *et al.*, 1978) are consistent with the view that pre-B cells are the precursors of B cells and their lack of surface immunoglobulin suggests that they are antigen-unresponsive. The T cell contribution to pre-B cell proliferation, which our results suggest, is therefore likely to be different from their help for B cell differentiation into plasma cells. It is more likely to resemble the non-specific help which T cells provide for erythrocyte development (Nathan *et al.*, 1978). Though our findings suggest that T cells promote pre-B cell division *in vitro*, they do not establish its significance *in vivo*. The development of B cells in T-deficient nude mice and patients with thymic hypoplasia suggests that it is not essential, though it is possible that subtle defects of B cells, such as diminished clonal diversity, might go unnoticed.

The T cells from the blood of off-treatment ALL patients were better than those from adult controls at promoting pre-B cell proliferation. These findings suggest that a disturbance of T cell control may account for the increased number of pre-B cells in the patients' marrows and for the ease with which we have been able to culture the cells *in vitro*. We have commented previously (Paolucci *et al.*, 1981) that the pre-B cell division *in vitro* is probably not solely the result of drug withdrawal, since we saw it in marrow from untreated children with cancer. The four possibilities (not necessarily mutually exclusive) that the increase in pre-B cells after ALL treatment might be due to the disease, or a primary abnormality predisposing to the disease, or to an oncogenic virus or to drug withdrawal, could all work through a disturbance of T cell function. It is conceivable that changes in the T cell-dependent regulation of pre-B cells may contribute to the functional immaturity of B cells from patients recovering from post-transplant immunosuppression (Ringden *et al.*, 1980). Pre-B cell suppression by T cells may explain the association of hypogammaglobulinaemia with thymoma (Good, 1954).

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