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Imbalance of T cell subpopulations in patients with chronic lymphocytic leukaemia of the B cell type

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SUMMARY

T cell enriched mononuclear cells from the peripheral blood of 20 patients with histologically and immunologically defined chronic lymphocytic leukaemia of the B cell type (B-CLL) and 20 healthy individuals of various ages were investigated with T cell-specific monoclonal antibodies (OKT 4 and OKT 8) with regard to their subpopulation distribution. In B-CLL, a significant increase of lymphocytes reacting with OKT 8 could be demonstrated. Whereas there was a ratio of OKT 4 to OKT 8 of 1·72 in the control group, an OKT 4 to OKT 8 ratio of 0·67 was found in the B-CLL as a whole. With increasing clinical stage in accordance with the Rai scheme (Rai *et al.*, 1975), a further displacement of this ratio in favour of OKT 8 positive cells was found. These results clearly show that, in peripheral blood of patients with B-CLL, an abnormal distribution pattern of circulating T cell subpopulations is present and that this also has prognostic relevance.

INTRODUCTION

Chronic lymphocytic leukaemia of the B cell type (B-CLL) is defined as a proliferation and accumulation of immunoincompetent B lymphocytes (Dameshek, 1967) which are arrested in their maturation and differentiation at an early stage (Salmon & Seligman, 1974). This results in immune defects which are manifested clinically as intensified susceptibility to infection and almost regularly occurring hypogammaglobulinaemia (Miller & Karnofsky, 1961).

Although it is homogeneous in its clinical features, B-CLL is a heterogenous disease with regard to its prognosis and course. A range from rapidly progressive progress forms to progress forms which remain stationary for a long time are observed. The cause of this is still not yet clear; suitable prognostic parameters are lacking. A recent investigation (Foa *et al.*, 1979) showed a correlation between the quantitative extent of circulating T lymphocytes and the progress form. On the other hand, it is also known that T lymphocytes play an important role in the pathogenesis of immune defect states (Moretta *et al.*, 1977; Waldman *et al.*, 1978; Bach & Bach, 1981). In the meantime, an imbalance of the circulating T lymphocytes has been demonstrated in patients with B-CLL using 'functional markers' (Tγ, Tμ) (Kay *et al.*, 1979; Lauria, Foa & Catovsky, 1980). Both groups describe a proliferation of Tγ lymphocytes, i.e. lymphocytes to which a suppressor function is partly attributed.

The object of our present study was to investigate T cell enriched lymphocyte suspensions with T cell-specific monoclonal antibodies (OKT 4 and OKT 8) in patients with B-CLL in different stages of clinical spreading.

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MATERIALS AND METHODS

Patients. Heparinized venous blood from 20 healthy individuals of various ages and 20 patients with histologically verified and immunologically defined B-CLL was investigated. The immunological phenotype SIg+, Ia+, M-R+, C3d, C3b−, E-R− proves the B cell origin. None of the patients had received a cytostatic or corticoid therapy within the last 3 months before the investigation. The absolute lymphocyte counts of the patients with B-CLL ranged from 29 to 402×10^9/l. A stage Rai II was found in seven patients, a stage Rai III in five patients and a stage Rai IV in eight patients.

Cell isolation. Peripheral blood lymphocytes were isolated from venous blood by centrifugation over conventional Ficoll-Hypaque gradient (Böyum, 1968). We removed monocytes from the suspension obtained in this way on the basis of their adherence to plastic at 37°C in 5% CO_2. The non-adherent cells were contaminated by less than 2% non-specific esterase positive cells.

T cell enriched lymphocyte suspensions were obtained by rosette formation with neuraminidase-treated sheep erythrocytes and subsequent Ficoll-Hypaque separation as described (Wybran, Chantler & Fudenberg, 1973). T cells were prepared by a sequence of two such procedures. A T cell purity of 92–97% in the control group and of 89–96% in the B-CLL group was attained in this way. In all cases, more than 97% cells were found viable as tested by trypan blue dye exclusion.

Cell identification. The methods for determining membrane-bound immunoglobulins (SIg), cells rosetting with mouse and sheep erythrocytes (M-R, E-R), the Ia-like antigen as well as the lymphocytes with receptors binding complement component C3d and C3b (C3d-R, C3b-R) have been described in detail elsewhere (Billing *et al.*, 1976; Jondal, Holm & Wigzell, 1972; Pincus, Bianco & Nussenzweig, 1972; Rabellino *et al.*, 1971; Stathopoulos & Elliot, 1974).

The T cell subpopulations were determined immediately after T cell enrichment using OKT 4 and OKT 8 as described (Kung *et al.*, 1979; Reinherz *et al.*, 1980). In brief, the enriched T lymphocytes (0·1 ml, 5×10^6/ml) were incubated for 30 min at 4°C with 0·1 ml buffer and OKT 4 or OKT 8 (5 μg/ml in each case). After incubation, the cells were washed three times in cold buffer and resuspended in 0·1 ml buffer. The cells were then incubated for 30 min with the corresponding dilution of an FITC-conjugated goat anti-mouse immunoglobulin. After another three washing procedures, the cells were resuspended once more in cold buffer, and the membrane immunofluorescence was evaluated. OKT 4 positive cells reflected the helper cell fraction and OKT 8 positive cells the suppressor cell fraction (Thomas *et al.*, 1980). The Student's *t*-test was used for statistical analysis of the results.

RESULTS

Determination of the immunological phenotype of the unseparated lymphocyte suspension in the control group (Fig. 1) revealed that $12{\cdot}35 \pm 4{\cdot}45\%$ of the cells bore membrane-bound immunoglobulins, $18{\cdot}45 \pm 4{\cdot}95\%$ of the lymphocytes were Ia-positive, $12{\cdot}8 \pm 4{\cdot}12\%$ of the cells form rosettes with mouse erythrocytes, $14{\cdot}25 \pm 4{\cdot}4\%$ possessed receptors for binding C3d, $14{\cdot}7 \pm 3{\cdot}5\%$ possessed receptors for binding C3b and $61{\cdot}75 \pm 6{\cdot}8\%$ spontaneously formed rosettes with sheep erythrocytes and thus proved to be T cells. In the B-CLL group (Fig. 2), $64{\cdot}6 \pm 12{\cdot}1\%$ of the lymphocytes displayed membrane-bound immunoglobulin, $68{\cdot}7 \pm 9{\cdot}9\%$ were Ia positive, $53{\cdot}9 \pm 9{\cdot}2\%$ form rosettes with mouse erythrocytes, $12{\cdot}8 \pm 6{\cdot}9\%$ bore C3b receptors, $41{\cdot}75 \pm 13{\cdot}5\%$ bore C3d receptors. The proportion of T cells determined as the percentage of cells forming rosettes with sheep erythrocytes was $10{\cdot}1 \pm 4{\cdot}2\%$. Thus, as described at the beginning, the immunological phenotype SIg+, Ia+, M-R+, C3d+, C3b-R−, E-R− results.

Table 1 shows the relative proportion of OKT 4 positive and OKT 8 positive in the T cell enriched suspension for the normal group and the B-CLL group. Here a significantly ($P < 0{\cdot}001$) higher proportion of OKT 8 positive cells is evident in the B-CLL group ($47{\cdot}0 \pm 6{\cdot}0\%$) than in the control group ($29{\cdot}0 \pm 5{\cdot}2\%$). The percentage proportion of OKT 4 positive cells was $31{\cdot}5 \pm 6{\cdot}6\%$ s.d. in the B-CLL and thus significantly ($P < 0{\cdot}001$) below the percentage proportion of OKT 4 positive

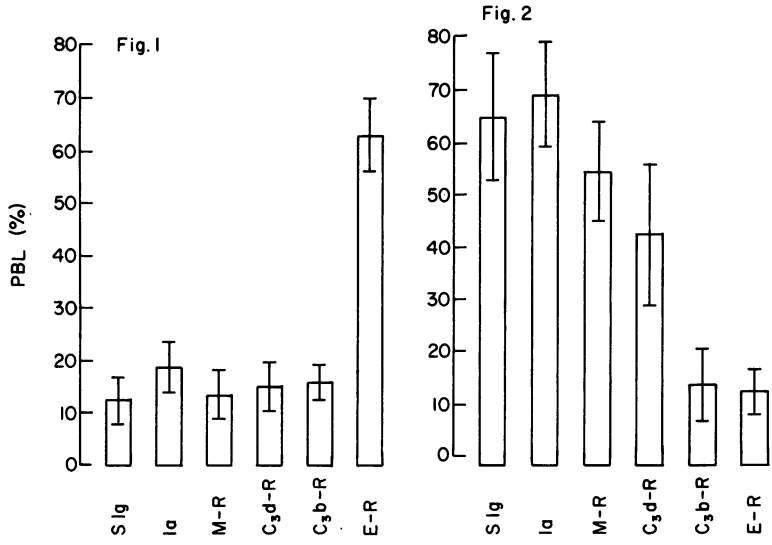

Fig. 1. Surface markers in healthy individuals ($n = 20$).

Fig. 2. Surface markers in B-CLL ($n = 20$).

Abbreviations used in these figures: PBL = peripheral blood lymphocytes; SIg = surface bound immunoglobulin; Ia = Ia-like antigen; M-R = mouse red blood cell receptor; C_3d-R and C_3b-R = receptor for complement components C_3d and C_3b; E-R = sheep red blood cell receptor.

cells in the control group (50·0 ± 6·1% s.d.). Absolute figures for the lymphocytes of the normal and B-CLL group reacting with OKT 4 and OKT 8 are likewise presented in Table 1. In all cases, the absolute number of OKT 8 positive cells was above the value of the normal control. The absolute number of OKT 4 positive cells was in the normal range in two patients and was likewise increased in the remaining 18 patients. The investigation of the dependence of the ratio of the absolute and relative number of OKT 4 positive and OKT 8 positive cells on the clinical stage according to Rai was of particular interest. The results are listed in Table 2. A positive reaction with OKT 8 was found in an average of 42·2 ± 6·96% in the peripheral lymphocytes of the patients in stage Rai II. An average of 48·8 ± 2·7% was found in stage Rai III and an average of 50·0 ± 4·14% in stage Rai IV. The absolute figures also rose as the stage increased (in Rai II an average of 2·725, in Rai III 7·280 and in Rai IV 14·567). Peripheral lymphocytes reacting with OKT 4 were identified in an average of 35·3 ± 8·9% in Rai II, in 30·8 ± 5·2% in Rai III and in 28·6 ± 2·9% in Rai IV. The mean values of the absolute numbers were 1·802 in stage Rai II, 3·682 in Rai III and 6·904 in Rai IV. Thus a ratio of OKT 4 to OKT 8 of 1·72 results in the normal group, as can be seen from Fig. 3. Seen as a whole, the

Table 1. Numbers of OKT 4 and OKT 8 positive cells in healthy individuals ($n = 20$) and in patients with B-CLL

		OKT 4		OKT 8	
		Relative (%)	Absolute ($\times 10^9$/l)	Relative (%)	Absolute ($\times 10^9$/l)
Normals	range	36–57	196–1,192	22–43	94–784
	mean	50·0	464	29·0	220
	s.d.	±6·1	n.d.	±5·2	n.d.
B-CLL	range	24–47	886–12,999	30–60	796–27,577
	mean	31·5	4,343	47·0	8,601
	s.d.	±6·6	n.d.	±6·0	n.d.

Table 2. Correlation between Rai stage and numbers of OKT 4 and OKT 8 positive cells in patients with B-CLL

		OKT 4		OKT 8	
		Absolute ($\times 10^9$/l)	Relative (%)	Absolute ($\times 10^9$/l)	Relative (%)
Stage II	range	880–2,631	25–47	796–5,040	30–49
	mean	1,802	35·3	2,725	42·2
	s.d.	n.d.	±8·9	n.d.	±6·9
Stage III	range	1,980–8,272	27–39	2,940–17,296	45–52
	mean	3,682	30·8	7,280	48·8
	s.d.	n.d.	±5·2	n.d.	±2·7
Stage IV	range	2,550–12,999	24–33	5,299–27,577	47–60
	mean	6,904	28·6	14,567	50·0
	s.d.	n.d.	±2·9	n.d.	±4·1

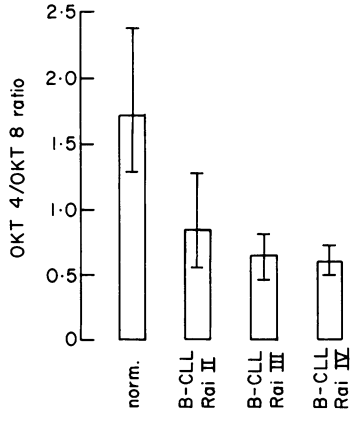

Fig. 3. The ratio of OKT4/OKT8 positive cells in peripheral blood of healthy individuals ($n = 20$) and of patients with B-CLL ($n = 20$) in different stages (Rai II $n = 7$, Rai III $n = 5$, Rai IV $n = 8$). Our results are expressed as the mean values ± s.d.

ratio in the B-CLL group was 0·67. With increasing Rai stage, an increasing displacement of this ratio in favour of OKT 8 positive cells was also found. The OKT 4 to OKT 8 ratio was 0·84 in Rai II, 0·63 in Rai III and 0·57 in Rai IV.

DISCUSSION

In chronic lymphocytic leukaemia of the B cell type, an absolute proliferation of T lymphocytes was already described in earlier papers (Catovsky *et al.*, 1974). This T cell proliferation was already characterized as being prognostically relevant (Foa *et al.*, 1979).

It is shown in the present study that this T cell proliferation is due particularly to an absolute and relative increase of OKT 8 positive cells, i.e. cells with suppressor function. Compared to the normal group, on the other hand, the relative proportion of OKT 4 positive cells, i.e. cells with helper cell function, was significantly below the value of the healthy control group, although its absolute proportion was either normal or increased. Characteristically, the patients in stages Rai III and Rai IV showed the highest absolute values of OKT 8 positive cells. We thus suspect that the progression of the disease is associated with an increase of cells with suppressor function. Comparable findings

have already been reported in earlier papers (Kay *et al.*, 1979; Lauria *et al.*, 1980) using the functional markers Tγ and Tμ. What is the significance of this phenomenon?

As is known, the mitogen reactivity of T cells in chronic lymphocytic leukaemia of the B cell type is normal (Wybran *et al.*, 1973; Rühl *et al.*, 1978). On the other hand, the B cells involved in B-CLL are not able to mature and differentiate into cells secreting immunoglobulin (Salmon & Seligman, 1974; Stein, 1978). Hypothetically, the increase of suppressor T lymphocytes is assumed to be the cause of this. However, *in vitro* experiments using a reverse haemolytic plaque assay recently showed that, in co-cultures of a PWM-driven system, the T lymphocytes of the patients with B-CLL were unable to suppress the transformability of normal lymphocytes in immunoglobulin secreting cells (Sieber *et al.*, 1981).

In a series of diseases, immune defect states or impaired B cell reactivity are doubtless the consequence of an altered suppressor activity (Moretta *et al.*, 1977; Waldman *et al.*, 1978; Bach & Bach, 1981). Although a suppressor function is attributed to OKT 8 positive cells, their increase in patients with B-CLL does not on its own appear to be adequate proof that the lowering of the serum immunoglobulin levels is also causally related to the increase of OKT 8 positive cells. Our own recent observation of the normalization of a previously raised absolute proportion of OKT 8 positive cells in a patient with B-CLL after intermittent chlorambucil–corticoid treatment without any effect on the demonstrable hypogammaglobulinaemia (unpublished observations) underscores this doubt. Thus we must summarize in conclusion that the imbalance of the T lymphocyte subpopulations in B-CLL appears to be of prognostic significance, but that the role of the T–B and T–T interaction in the pathogenesis of the disturbed B cell differentiation in B-CLL has not been sufficiently clarified. Our further studies will focus on this area.

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