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Membrane phenotype and functional behaviour of T lymphocytes in multiple myeloma: correlation with clinical stages of the disease

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SUMMARY

The distribution of T lymphocyte subsets was assessed using monoclonal antibodies (MoAbs) in 44 untreated patients with multiple myeloma (MM) subdivided according to the clinical stage of the disease. A significant reduction ($P < 0{\cdot}001$) of T lymphocytes was observed only in stage II and III patients. The proportion and absolute number of OKT4 positive cells (helper/inducer phenotype) were significantly reduced in all stages of the disease; this quantitative abnormality was more pronounced in advanced disease. While the proportion of OKT8 positive cells (suppressor/cytotoxic phenotype) was increased above normal in all stages, the absolute number (of OKT8 positive cells) was high only in stage I patients; on the contrary in stage II–III patients the total OKT8 count was reduced compared with normal controls. A significantly reduced OKT4/OKT8 ratio was found in both groups of patients ($P < 0{\cdot}005$). Functional studies, carried out on the unfractionated T cells of patients with MM, demonstrated a consistent helper defect in the ability to induce the differentiation of normal B lymphocytes into antibody producing cells in a pokeweed mitogen driven system. However, the removal of OKT8 positive cells produces a significant increase in helper capacity, suggesting that the reduced helper function of T lymphocytes *in toto* is probably due to excessive suppressor activity. The possible

(Tanapatchaiyapong & Zolla, 1974), while limited information is presently available on the residual T cell populations (Ozer *et al.*, 1981; Hoover *et al.*, 1981).

Recently, Oken & Kay (1981) in 16 patients with MM demonstrated a significant increase of T cells with IgG receptors (Tγ) and a reduction of T cells with IgM receptors (Tμ) in patients with controlled or indolent MM. Patients with progressive disease, whilst still showing a marked decrease in Tμ cells, had a normal proportion of Tγ cells (Ozer & Kay, 1981). An increase in Tγ cells has been confirmed by others (Hoover *et al.*, 1981; Pezzuto *et al.*, 1981) together with a normal or increased suppressor activity by the Tγ cells (Perri, Oken & Kay, 1982).

To further define the immunological status of untreated patients with MM, the proportion of T lymphocyte subsets was assessed using anti-T monoclonal antibodies (MoAbs) which recently have allowed a more precise characterization of lymphoid subpopulations in normal individuals (Kung *et al.*, 1979; Reinherz *et al.*, 1979) as well as in several immunohaematological disorders (Matutes *et al.*, 1981; Mills, Worman & Cawley, 1982; Lauria *et al.*, 1982). The results were analysed according to the clinical extent of the disease based on Durie & Salmon's (1975) staging system. Furthermore, in a group of patients we studied the helper and suppressor function of enriched T lymphocytes and of purified OKT4 and OKT8 fractions in a pokeweed mitogen (PWM) stimulated system.

MATERIALS AND METHODS

Patients. Heparinized peripheral blood samples were collected from 44 patients with MM and from 20 age and sex matched normal controls. The diagnosis of MM was based on standard clinical criteria including typical skeletal lesions, presence of more than 20% of plasma cells in the bone marrow and a monoclonal (M) component in the serum and or in the urine identifiable on immunoelectrophoresis. All patients were untreated and according to Durie & Salmon's (1975) staging system: 26 were in stage I, three in stage II and 15 in stage III. In 26 of the patients (59%) the M component was IgG; in 11 IgA (25%), in two IgD (5%) while the last five (11%) showed the Bence–Jones type.

Isolation of cells. Peripheral blood lymphocytes (PBL) were separated on 'Lymphoprep' (Nyegaard) gradients and mononuclear cells were washed in RPMI (KC Biological Inc.) containing 10% fetal calf serum (FCS) (GIBCO) and depleted of adherent cells by incubation in plastic 75 mm tissue culture flasks (Falcon) at 37°C for 45 min. Lymphocytes forming rosettes with neuroaminidase treated sheep erythrocytes (RBC) (E rosetting cells) were separated from non-rosetting cells by two or more sequential centrifugations on 'Lymphoprep' gradient. In order to isolate enriched T cell subpopulations by negative selection with MoAbs, 20×10^6 unfractionated T lymphocytes were resuspended in 1 ml RPM1640 medium containing 10% FCS with 10 μg of OKT4 MoAb, and were incubated for 1 h at 4°C. Fresh rabbit complement was added at a final dilution of 1:2, and incubation was further carried out for 1 h at 37°C. The same procedure was used with 20 μg of OKT8 MoAb. Analysis of the resulting populations showed that the OKT4 treated population contained $>80\%$ of $OKT8^+$ and $<3\%$ of $OKT4^+$ cells and that the OKT8 treated population contained $>80\%$ of $OKT4^+$ cells and $<3\%$ of $OKT8^+$ cells.

Enriched B cells from normal individuals were obtained by allowing the non-rosetting cells to rosette a second time with neuraminidase treated sheep RBC followed by a further fractionation.

T lymphocyte subsets. The T lymphocyte surface phenotype was established using MoAbs reactive against all mature peripheral blood T cells (OKT3) (Ortho), T helper/inducer cells (OKT4) and T suppressor/cytotoxic cells (OKT8). The preparation and the specificity of these MoAbs have been extensively documented (Kung *et al.*, 1979). The enriched T cell fraction ($>90\%$ of T cells) was incubated with each MoAb for 30 min, and the percentage of positive cells was determined by indirect immunofluorescence microscopy (Zeiss) after labelling with fluorescein conjugated goat anti-mouse antiserum (Labtek). The percentage of positive lymphoid cells was evaluated by counting at least 200 cells.

T cell functional assay. The helper and suppressor activity of enriched T, OKT4 and OKT8 positive lymphocytes were assessed by culturing the cells in flat bottom Microtitre II tissue culture plates at 37°C in a humidified incubator with 5% CO_2 in air. All cell suspensions were cultured in TC

199 medium supplemented with 20% FCS, L-glutamine (0·3 mg/ml), gentamycin (10 μg/ml) and penicillin (200 μ/ml).

To test helper T cell function, 5×10^5 normal B enriched lymphocytes were incubated with an equal number of T and OKT4 positive cells from MM patients and from normal individuals (in control experiments) in a final volume of 0·2 ml/well and in the presence of 0·004 mg PWM (GIBCO)/well. T suppressor function was evaluated by incubating 5×10^5 normal B enriched lymphocyte with 5×10^5 normal OKT4 positive cells and with 4×10^5 OKT8 positive cells from MM patients or from normal controls in a final volume of 0·2 ml/well and in the presence of 0·004 mg PWM/well. All cultured cells were harvested after 7 days, counted and tested for viability by trypan blue dye exclusion. The cells were washed twice with phosphate-buffered saline (PBS) and counted. Air dried cytocentrifuge slides were fixed in 5% acetic acid, 95% ethanol at −20°C for 30 min, rehydrated in PBS and stained with a polyvalent goat anti-human Ig antibody conjugated with fluorescein isothyocyanate (Behringwerke A.G.). Cells containing cytoplasmic Ig (cyIg) were scored using a Zeiss fluorescence microscope and their absolute number was calculated as a function of the total number of cells recovered per well.

The Student's *t*-test was used for statistical analysis.

RESULTS

The immunological data of the 44 patients studied subdivided according to the clinical stage of the disease are reported in Table 1. In stage I patients, the mean peripheral blood lymphocyte (PBL) count and the percentage and absolute number of T lymphocytes, determined by E rosettes and by OKT3 MoAb, were practically normal, while the PBL count and the absolute number of T lymphocytes were significantly reduced in stage II–III patients compared both with stage I patients ($P < 0{\cdot}005$) and with normal controls ($P < 0{\cdot}001$). Analysis of the distribution of T cell subsets showed in both groups of patients (stage I and stage II–III) a significant reduction in the proportion of OKT4 positive cells compared with normal controls ($P < 0{\cdot}01$, respectively). The reduced absolute number of OKT4 positive cells observed in both groups was more pronounced in stage II–III patients compared with stage I patients, probably due to the marked lymphopenia in the former group. The proportion of OKT8 positive cells was increased in both groups of patients compared with controls; however, the absolute number, increased in stage I patients, was reduced in patients with more advanced disease. This reduction was statistically significant ($P < 0{\cdot}01$). Due mainly to the reduction of OKT4 positive cells, the mean OKT4/OKT8 ratio was significantly reduced ($P < 0{\cdot}005$) both in stage I and in stage II–III. No correlation was found between immunological findings and myeloma immunoglobulin subtype.

Table 1. Immunologic characteristics of 44 patients with MM according to clinical stages

	PBL $\times 10^9/1$	E Ros % (a.n.)*	OKT3% (a.n.)	OKT4% (a.n.)	OKT8% (a.n.)	$\frac{\text{OKT4}}{\text{OKT8}}$ ratio
Patients in stage I (26)	1·95±0·5	62±10 (1·20)	66±15 (1·25)	37±9 (0·70)	35±11 (0·68)	1·12
Patients in stage II & III (18)	1·26±0·4	57±9 (0·71)	62±7 (0·79)	39±5 (0·49)	30±6 (0·38)	1·25
Normal controls (15)	2·20±0·4	68±7 (1·49)	70±8 (1·50)	49±6 (1·06)	23±8 (0·50)	2·13

* a.n. = absolute number ($\times 10^9/1$).

In 14 patients (nine in stage I and five in stage III), the capacity of enriched T lymphocytes to promote the differentiation of normal B lymphocytes into antibody producing cells was evaluated in a PWM assay. In a smaller group of patients (four in stage I and two in stage II) the helper and suppressor capacities were assessed on the isolated OKT4 and OKT8 positive cells. As illustrated in Fig. 1, the proliferation of cells containing cytoplasmic immunoglobulins in cultures set up with MM T lymphocytes was significantly reduced compared with normal T lymphocytes cultured at the same time ($P < 0{\cdot}01$). However, purification experiments indicate that isolated OKT4 positive cells are consistently effective in promoting the maturation of B cells into plasma cells, while the addition of isolated MM OKT8 positive cells produced a consistent suppressor activity.

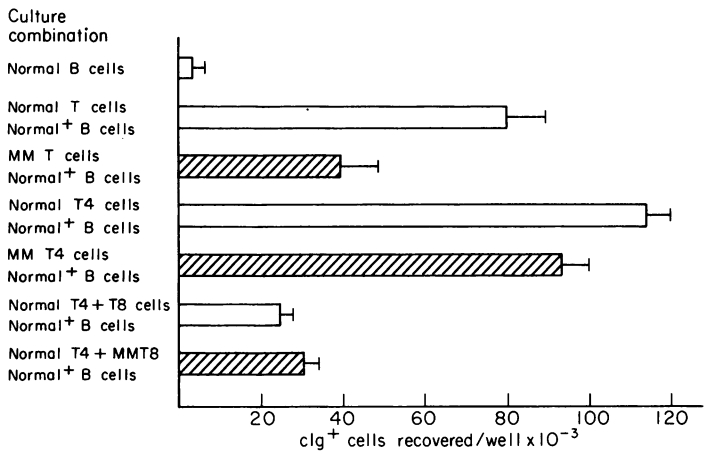

Fig. 1. Helper and suppressor capacity of MM T cells. Helper capacity of unfractionated T lymphocytes from 14 MM patients and 10 normal individuals was assessed. The two histograms are representative of seven experiments each. In six patients and four normal controls the helper and suppressor capacity were assessed on the enriched OKT4 and OKT8 positive lymphocytes. Each histogram is representative of four experiments.

DISCUSSION

In this study an abnormal distribution of circulating T cell subsets is reported in a group of untreated patients suffering from MM. Using MoAbs directed against different T cell subsets, we have observed, in the majority of patients, a significant decrease of OKT4 positive cells (helper/inducer phenotype), more evident in patients with advanced clinical stage. Conversely, an increase in the proportion of OKT8 positive cells (suppressor/cytotoxic phenotype), was found only in stage I patients, while a significant reduction in the absolute number was observed in stage II–III patients compared with stage I patients.

These findings confirm and extend results obtained using Ox IgG and Ox IgM antibodies which showed in MM a significant increase in Tγ suppressor cells mainly in patients with treatment responsive or indolent disease (Oken & Kay 1981; Pezzuto *et al.*, 1981), together with a significant reduction in Tμ helper cells in all phases of the disease. More recently, similar preliminary results have been reported using OKT MoAbs (Mellested *et al.*, 1982; Ludwig *et al.*, 1982).

Functional abnormalities have also been described in MM, firstly by Broder *et al.* (1975) who provided preliminary evidence that in some patients, mononuclear phagocytic cells were capable of suppressing the polyclonal Ig synthesis and that the removal of these cells could eliminate the suppressor activity. An increased suppressor function was subsequently reported by Paglieroni & McKenzie (1977; 1980) who demonstrated in MM a significant increase of suppressor activity by the lymphocyte population after removal of adherent cells. Finally, Perri *et al.* (1982), reported a normal helper capacity of enriched MM Tμ cells, whilst Tγ cells were more effective than normal Tγ

cells in suppressing myeloma B cells. Moreover, MM B cells appeared more sensitive to the suppressor activity of MM T cell than normal B cells, suggesting the possibility that in stage I patients the increased number of T suppressor cells may exert their activity predominantly on the MM B cell population leading to a certain control of the disease. Our findings are consistent with previous immunoregulatory cell studies in MM and provide additional data delineating the function of helper and suppressor T cell.

The reduced helper activity of the unseparated T lymphocyte population of MM observed in our study may be related: (a) to the impaired T cell subset distribution namely the reduced proportion of OKT4 positive cells, (b) to an excessive suppressor activity and (c) to an intrinsic abnormality of the T helper cell population, as observed, for instance, in B cell chronic lymphocytic leukaemia. Co-culture experiments carried out with enriched OKT4 and OKT8 lymphocyte subpopulations, isolated in a complement mediated cytotoxicity assay, suggest that OKT4 positive cells exert a helper capacity similar to that observed with the same cell fraction from normal controls. On the other hand the suppressor activity of isolated OKT8 positive cells was also comparable with that of the normal counterpart. Additional studies are underway to assess whether variations in the helper capacity can be related to the clinical stage of the disease and/or whether excessive suppressor activity can be demonstrated in a larger series of patients. Taken together, these data suggest that the depressed helper capacity of MM unseparated T lymphocytes is due to the significant reduction both in the percentage and in the absolute number of OKT4 positive cells.

Although the exact role of T cell subsets in MM remains to be fully understood and although it is still unclear whether MM cells are partly responsive to T cell interactions, our data suggest that immunoregulatory T cells may have an important role in this disease. The increased number of OKT8 positive cells in stage I patients and the important decrease in more advanced disease confirm previous suggestions that this T cell subset may play a role in controlling the proliferation of the neoplastic B cell clone. Furthermore, the marked reduction in OKT4 positive T cell population, and consequently the depressed helper capacity of MM T cells, suggest that these abnormalities may be implicated in the progressive hypogammaglobulinaemia observed in MM and thus in the severe infections which are a frequent complication in this disease.

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