Delay and Not Deficiency in Cap Formation of Peripheral Blood B Cells in Patients with Multiple Myeloma

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A major problem in the study of peripheral blood (PB) B cells from patients with multiple myeloma (MM) is the distinction between the cells really able to synthesize membrane (m) immunoglobulins (Ig) and those able only to absorb serum Ig passively, since the lymphocytes of such patients are bathed in very high concentrations of monoclonal Ig. In order to reappraise PB B cells (including putative pre-B cells) in MM, we have used three different criteria: (a) the capacity of PB B cells to cap mlg when triggered by an anti-Ig; (b) the presence of B-cell differentiation antigens (CD19, CD20, CD21, and CD37) as specific B-cell markers; and (c) the expression of cytoplasmic μ heavy chain as a marker of pre-B cells. We have found that, in active myeloma (N=13), the percentages and absolute numbers of PB B cells able to cap mlg (4.25%; 45.43 cells/mm³) were significantly lower than those in healthy donors (8.4%; 151.2 cells/mm³) and those in stable MM (7.67%; 134.39 cells/mm³). In addition, the capping formation in patients with stable or active MM was significantly delayed compared to that in healthy donors. For all the normal individuals and patients investigated, there has been found an excellent correlation between the percentages and absolute numbers of PB B cells able to cap their mlg and those of PB mononuclear cells bearing the four B cell-specific differentiation antigens: CD19, CD20, CD21, and CD37. Finally, virtually no pre-B cells bearing cytoplasmic μ chains have been identified in the peripheral blood from healthy donors and patients with MM.

KEY WORDS: B cells; capping; multiple myeloma.

INTRODUCTION

Multiple myeloma (MM) is a B-cell neoplasia characterized by the clonal expansion, mainly in bone marrow, of malignant plasma cells producing a monoclonal immunoglobulin (Ig) which is defined by its idiotypic determinants (Id) (1). As only a minor proportion of malignant plasma cells is proliferating, the search for the precursor (clonogenic) cell that may feed the plasma-cell compartment is an important goal for understanding this disease. One of the critical problems in this search is that investigations of B-cell numbers expressing membrane immunoglobulins (mlg) have, until recently, yielded conflicting results since increased or reduced numbers of mlg-bearing B cells have been reported (2–8). In these reports, published from 1970 to 1980, B cells were identified by using anti-Ig antibodies labeled with fluorescein. This might lead to the identification not only of B cells but also of the cells able to attach the circulating monoclonal protein (9), as the lymphocytes of patients with MM are bathed in very high concentrations of monoclonal Ig. Recently, the ability of B cells to cap their mlg when triggered by anti-Ig was used in order to distinguish the cells able to synthesize mlg from those able to absorb serum Ig passively (10). This leads to the following conclusion: in patients with MM, there are virtually no circulating B cells but an expansion of μ-chain-positive (cμ+) circulating pre-B cells (10,11).

In this study, we have not been able to reproduce these results. Three different criteria were used: B cells were identified by their capacity to cap mlg and by the presence of specific differentiation antigens recognized by monoclonal antibodies (CD19, CD20, CD21, and CD37); pre-B cells were recognized by the presence of cytoplasmic μ chains (cμ).
Because the process of capping is time dependent (12), the cells have been cultured at 37°C for different durations. The mean percentage and absolute number of B cells able to cap their mIg were 7.67% and 134.39 cells/mm³ in the PBMC from MM patients with stable disease, not significantly different from the mean values found in the healthy donor (HD) group (8.40% and 151.2 cells/mm³). In the group of MM patients with active disease, these values were 4.25% and 45.43 cells/mm³ and were significantly lower than those in the group of patients with stable disease and in the HD group. Moreover, no cy+ pre-B cells have been found in the PBMC from patients with MM and HD.

MATERIALS AND METHODS

Patients

The studies were performed in 19 patients with MM. MM was defined according to the Southwest Oncology Group (USA) diagnostic criteria (13). Thirteen patients had an active disease at the time of the study, and the six others were studied at the time of complete remission and the plateau phase. There were 13 IgG MM, 3 IgA, 1 IgD, and 2 Bence-Jones MM. Eleven patients had the k light-chain subtype, and eight the k light chain. The sex ratio (M/F) was 1:4 and the mean age was 60±10 years (range, 39-79 years). The clinical evaluation and management of our patients with MM were previously published in detail (14).

Antibodies

Monoclonal antibodies (mAb) against human IgM, IgD, and IgG were purchased from Immuno-tech (Marseilles Luminy, France). CD19,SB4 antibody was a generous gift from Dr. Poncelet (Sanofi, Montpellier, France). The CD21, BL13 and CD37,BL14 were derived by one of us [Dr. Brochier (15)]. The CD20,B1 was purchased from Beckton Dickinson (Mountain View, CA).

Cell Preparations

Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation of heparinized blood over Ficoll–Hypaque gradients. The cells recovered at the interface were washed twice in PBS–BSA (phosphate-buffered saline, 0.15M, pH 7.2–bovine serum albumin, 1%) before processing.

Identification of Membrane Antigens

For this study, we used an indirect immunofluorescence assay. Briefly, PBMC (5 × 10⁶) were resuspended in 50 μl of test murine anti-human antibodies diluted in PBS–BSA at an appropriate concentration or in mouse immunoglobulin (Immuno-tech, Marseilles, France) as a control sample. The cells were incubated at 4°C for 60 min, spun down, washed twice in cold PBS–BSA–NaN₃ (sodium azide, 0.02%), and resuspended in 50 μl F(ab')₂ fragments of sheep anti-mouse Ig labeled with fluorescein isothiocyanate (FITC) (NEN, France) for 45 min at 4°C. The cells were then washed three times with cold PBS–BSA–NaN₃. A part of them was fixed in 1% paraformaldehyde for flow cytometry analysis (Dr. F. Favier, Laboratory of Flow Cytometry, Service Commun INSERM, Montpellier, France). With the other part, slides were prepared (10,000 cells per slide) and dried in air.

Determination of the Antigen Density

Antigen densities were determined using a cytofluorometric method previously described (16). In each experiment, 10 tubes were dedicated to the biological standards, generating a calibration curve which converts the fluorescence intensity into the number of cell-bound mAb molecules. These standards consisted of five subclones of the T-cell line CEM, which, after indirect staining with CD5 mAb (ST1) or with mouse immunoglobulin as a control, consistently bound various amounts of mouse IgG (i.e., from 500 to 95,000 molecules per cell). All mAbs were used at saturating doses since, under such conditions, the binding of mAbS is essentially monovalent (P. Poncelet, personal communication) and the calculated number of cell-bound mAb molecules can be assimilated to the density of antigenic sites expressed on the cell surface.

Capping Assay

The cells were first labeled at 4°C with murine anti-human Ig mAbS and FITC-F(ab)₂ fragments of sheep anti-mouse Ig as described above. Then they were incubated in PBS and 1% fetal calf serum at 37°C for 10, 30, 60, and 120 min to allow capping.