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BONE MARROW T-CELL SUBSETS IN PATIENTS WITH MONOCLONAL GAMMOPATHIES: CORRELATION WITH CLINICAL STAGE AND DISEASE STATUS

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ABSTRACT

Background and Objective. The existence of an imbalance in T-cell subpopulations in patients (pts) affected by monoclonal gammopathies (MG) has been well established. This imbalance might be correlated with different control of plasma cell growth and, particularly in MM, with the severity of the disease. The aim of this study was to verify whether the alteration of the T lymphocyte subsets in bone marrow correlates with the diagnosis, clinical status and disease phase in patients with monoclonal gammopathies.

Methods. We performed a study on bone marrow (BM) T-cell subsets in 49 multiple myelomas (MM) and in 17 monoclonal gammopathies of uncertain significance (MGUS), using as controls 20 BM aspirates from normal subjects.

Results. The percentages of BM CD4 cells in MM pts at onset were slightly lower than in controls and in MGUS pts, who showed normal percentages of CD4. The percentages of CD8 cells were lower than in controls in both MM and MGUS (p=0.02 and p=0.007, respectively), and consequently the CD4/CD8 ratios were significantly higher than in normal subjects (p=0.01 and 0.008, respectively). Analysis of BM T-cell subpopulations in MM pts showed a progressive decrease in the percentage of CD4 cells from stage I to stage III (I vs III p=0.008) and an increase in CD8 cells, although not statistically significant. The same trend was observed when the different phases of MM (onset, plateau, progression) were analyzed: a lower percentage of CD4 cells and an increase of CD8 cells characterized the advanced phases. Treatment did not seem to alter significantly the distribution of T-cell subsets in MM patients.

Interpretation and Conclusions. The imbalance in T cells involving the bone marrow lymphocytic populations that exist in MG is somewhat different in MGUS and MM. In MM patients this disturbance is related to the disease stages and phases, reflecting an important role for T-cell subsets in tumor cell control.

Key words: T-cells, multiple myeloma, monoclonal gammopathies, bone marrow

Monoclonal gammopathies (MG) are immunoproliferative disorders involving a differentiated B cell and associated with an abnormal production of a monoclonal protein. Due to the role of the T-cell compartment in suppressing B cell proliferation and differentiation in normal subjects,[1] several authors have turned their attention to this phenomenon in order to define the impact in MG of T lymphocytes on the regulation of the neoplastic clone that affects the clinical presentation and progression of the disease.[2-4] The majority of the studies in the literature were performed on peripheral blood and demonstrated an imbalance in the CD4/CD8 ratio due to a marked reduction in both the percentages and absolute numbers of CD4 cells and to an increase in CD8 cells.[5-8] Some authors have also explored the relationship between this imbalance and clinical-biological parameters showing prognostic significance for the reduction of CD4 peripheral lymphocytes[9,10] and a correlation with the disease stage.[7,9,11,12] A few of the studies also reported on the distribution of T lymphocytes in the bone marrow of patients with MG,[13] but without focusing on correlations with other relevant clinical parameters. The aim of the present investigation was to verify whether the alteration of the T-lymphocyte subsets in bone marrow correlates with diagnosis, clinical status and disease phase in patients with monoclonal gammopathies.

Patients and Methods

Patients

Sixty-six patients with MG, 49 MM and 17 MGUS, were included in the present study. Diagnosis and staging were

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determined according to the criteria of Durie & Salmon.[14] The characteristics of the MGUS patients were as follows: median age 51 years (range 27-77 yrs); 9 were males and 8 females; 13 had an IgG M-component, 3 IgA, 1 biclonal. The characteristics of the MM patients were: median age 56 years (range 37-74 yrs); 36 were males and 13 females; 38 had an IgG M-component, 6 IgA, 4 BJ and 1 NS; 9 were in stage I, 5 in stage II, and 35 in stage III; 14 patients were at diagnosis, 16 were in a *plateau* phase (13 on chemotherapy and 3 off therapy), and 19 were in progression (18 treated and 1 not receiving any chemotherapy due to clinical status). All treated patients were studied at least three weeks after the last day of chemotherapy. Patients were studied at least two weeks after acute infections, antibiotic treatment, and transfusions.

We used 20 bone marrow aspirates from healthy bone marrow donors as controls.

Cytometric analyses

Fluorescein isothiocyanate (FITC)-conjugated anti-CD3, FITC anti-CD4 and phycoerythrin (PE)-conjugated anti-CD8 were used for phenotypic analysis of BM T-cell populations by direct immunofluorescence and flow cytometry with FACS (Epics-C) (Coulter). Percentages of positive cells were always referred to the total number of the lymphoid subset.

Statistical analysis

Continuous data are expressed as mean±standard deviation (SD) and ages as median and range; frequencies are reported for categorical variables. Between-group (stages and disease phases) comparisons of CD3, CD4 and CD8 percentages were performed by means of the unpaired Student's t-test. A 5% type I error (P-level <0.05) was retained and Bonferroni's correction was applied when required. The Spearman rank R statistic was computed in order to verify whether a monotonic relation was present between stage or disease phase and CD3, CD4 and CD8. The statistical package Statistica/w 4.5 (Statsoft) was used.

Results

Due to the wide range of bone marrow cellularity in normal subjects related to the extreme variability in bone marrow aspiration,[15,16] our analysis was carried out considering only the percentages of the T-cell subsets. Table 1 reports the percentages of bone marrow lymphocytic subpopulations. Patients with MGUS showed a reduction of CD3 and CD8 lymphocytes (p=0.02 and p=0.007, respectively) as compared to normal controls, while the percentage of CD4 cells remained similar to that of normal subjects. As a consequence the CD4/CD8 ratio in this group was higher than normal (p=0.02).

A similar situation was found in patients with MM in stage I (compare Tables 1 and 2); however, if MM patients were considered at onset we found a similar distribution of CD3 cells and CD8 cells and a reduction of CD4 cells, in relation to both controls and MGUS, that did not reach statistical significance. Table 2 shows a clear correlation between clinical stage and the worsening of the imbalance in the T-cell compartment, with a pronounced decrease of CD4 cells (stage I vs stage III, p=0.008) and a non significant increase of CD8 cells. Disease phases (Table 3) seem to be related only to an increase of CD8 cells (p=0.05), even though an important but not statistically significant reduction of CD4 cells is seen when these percentages are compared at onset and during progression.

If MM patients are considered all together treatment significantly affects the distribution of bone marrow T cells, but if the analysis is conducted in a more homogeneous fashion, namely taking into account only treated and untreated stage III patients, the statistical significance is lost.

We evaluated the existence of monotonic functions between CD3, CD4 and CD8 percentages and clinical stages or disease phases. As shown in Table 4, for patients with MM monotonic associations are present between the reduction of CD4 cells and clinical stage, and between the increase of CD8 cells and disease status.

Table 1. Bone marrow T-cell subsets in patients with MGUS and MM.

no. of patients	*Controls (20)*	*MGUS (17)*	*MM (14)*
% CD3	60.3±12*,°	49.8±15*	48.8±19°
% CD4	24.6±9	26.9±13	22.5±9
% CD8	32.4±9.5#,@	23.8±8.5#	23.8±12@
CD4/CD8	0.7±03^,§	1.24±0.8^	1.05±0.5§

**p=0.02; °p=0.03; #p0.007; @p=0.02; ^p=0.008; §p=0.01.*

Table 2. Distribution of T-cell subsets in MM patients according to clinical stage.

no. of patients	*Stage I (9)*	*Stage II (5)*	*Stage III (35)*
% CD3	53±7.9	49.4±22	44.1±19
% CD4	25±6*	19.4±9	17.3±8*
% CD8	22.4±5	30.4±15	29±14
CD4/CD8	1.15±0.4°	0.7±0.2	0.75±0.4°

**p=0.008; °p=0.02*

Table 3. Distribution of T-cell subsets in MM patients according to disease phase.

no. of patients	*Onset (14)*	*Plateau (16)*	*Progression (19)*
% CD3	48.8±19	42±16	47.4±19
% CD4	22.5±9	16.7±8	17.4±7.3
% CD8	23.8±12*	25.5±12	33±13*
CD4/CD8	1.05±0.5°	0.78±0.5	0.65±0.3°

**p=0.05; °p=0.008.*

Table 4. Linear correlation between the percentages of T-cell subsets and disease status in MM patients.

Pair of variables	*N. pts*	*Spearman R*	*t (N-2)*	*p- level*
Stage & CD3 %	49	–.174940	–1.21811	.229261
Stage & CD4 %	49	–.310980	–2.24319	.029637
Stage & CD8 %	49	.119852	.82763	.412063
Phase & CD3 %	49	–.039365	–.27008	.788281
Phase & CD4 %	49	–.239179	–1.68874	.097894
Phase & CD8 %	49	.305214	2.19729	.032964

Discussion

The present study shows the existence of an imbalance of bone marrow T-cell subsets in MG. A T-cell imbalance in MG has been reported mostly in studies performed on peripheral blood.[5-7,9,12,17] In particular, one of these carried out by San Miguel *et al*.[9] correlates a reduction of CD4 cells (< 0.7×10^9/L) with other adverse prognostic factors (advanced clinical stage, anemia, high β2 microglobulin levels) and with poor survival. They show, through multivariate analysis, that the number of CD4 cells adds independent prognostic information to the assessment of disease outcome in MM patients. The few studies carried out on T-cell subsets in normal bone marrow reveal an excess of CD8 cells. A similar distribution was found by Mills *et al*.[6] in the BM of MM and benign monoclonal gammopathies, while a lower ratio has been reported by others.[7,13] Functional studies also show a malfunction in the T-cell compartment in MM that reflects inadequate control over tumor cells, mostly in the advanced stages of the disease.[18,19] MM is in fact characterized by a marked activation state, which illustrates how T-cells are influenced by tumor evolution. However, in the absence of adequate costimulatory signaling this unproductive interaction predisposes T-lymphocytes to activation-induced cell death (AICD), which in the end renders them ineffective in tumor control.[19] There is probably also a timing mechanism in MM regarding the involvement of the different T-cell populations (CD4, CD8). In fact, a murine model has shown that $CD4^+$ cells are required in the early phase, whereas $CD8^+$ cells play a major role later on.[20] This is in keeping with a study conducted by Bianchi *et al*.[21] in which it was demonstrated that an *in vitro* activation wave of $CD4^+$ cells by CD3 stimulation precedes that of $CD8^+$ lymphocytes and serves to induce subsequent expansion of $CD8^+$ cells through the production of cytokines (mainly IL-2) – phenomena that underline the important role of T-cell lymphocytes in regulating MM tumor cells.

Our report shows that the imbalance of T-cell populations described by other authors in the peripheral blood is also present in the bone marrow. Nevertheless, unlike what is reported for peripheral blood, BM CD4 cells are not reduced in MGUS patients with respect to normal controls, while CD8 cells are present in lower percentages. Furthermore, this distribution of T cells in MGUS is similar to that of MM patients in stage I and, as a consequence, both MGUS and stage I MM patients have a higher CD4/CD8 ratio than controls. This is probably consistent with an abnormal T-cell tumor response and with a predominant role of $CD4^+$ lymphocytes in the early phases of the myelomatosis. It reflects *in vivo* what has been evidenced by other authors *in vitro*.[20,21]

MM patients show a marked reduction of CD4 cells and an increase of CD8 cells related to the severity of the clinical presentation. The reduction in CD4 cells becomes less pronounced when disease phases are considered. Actually no statistically significant differences are detected between patients at onset and during progression except for an increase in CD8 cells, partly due to the size of the study sample and partly probably the result of the continuous variability of the activation state present during the course of MM. Finally, a comparison between the percentages of T lymphocytes in treated and untreated patients does not reveal significant differences between the two groups when only patients in stage III are considered. This is consistent with a lack of influence of chemotherapy on the T-cell compartment disturbance in MM patients.

In conclusion, our findings support previous evidence of a T-cell imbalance in monoclonal gammopathies both in peripheral blood and in bone marrow. This imbalance is different in MM and MGUS patients; in the former it correlates with the stage and the phase of the disease, underlining the important role of T-cell subsets in controlling tumor cells.

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