Decreased Ecto-5'-Nucleotidase Activity of Peripheral Blood Lymphocytes in Human Monoclonal Gammopathies: Correlation With Tumor Cell Kinetics

By Massimo Massaia, David D.F. Ma, Mario Boccadoro, Francesco Golzio, Paolo Gavarotti, Umberto Dianzani, and Alessandro Pileri

Ecto-5'-nucleotidase (5'NT) activity of peripheral blood (PB) lymphocytes was determined in 31 patients with serum monoclonal gammopathies (MG). Twenty-one patients had a diagnosis of multiple myeloma (MM), and ten patients had monoclonal gammopathy of undetermined significance (MGUS). The proliferative activity of the bone marrow plasma cells (LI%) was investigated in 28 of these MG patients by means of tritiated thymidine uptake evaluated by simultaneous autoradiography and cytoplasmic immunofluorescence. 5'NT activity was significantly lower in MG patients as compared with normal controls. MM patients had lower 5'NT activity than MGUS patients, but the difference was not significant. By contrast, MM had significantly higher LI% than MGUS patients. There was a significant linear regression of 5'NT on LI% which was statistically significant: the higher the LI%, the lower the 5'NT. Because the LI% is an accurate prognostic and monitoring factor in MG, this correlation indicates that 5'NT may be of assistance in predicting the clinical progress of MG patients. In seven MGs, the PB T and B lymphocytes were studied separately. The T cell subpopulation was 5'NT deficient compared to the normal controls, shown as a significant linear regression of T cell 5'NT on the LI%. This suggests that in MG there may be an alteration of non-neoplastic T lymphocytes correlated with tumor growth. The OKT8+ lymphocytes were mainly responsible for the 5'NT deficiency of unseparated T lymphocytes.

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**MATERIALS AND METHODS**

**Patients**

Thirty-one MG patients were studied (age, mean ± SE: 63 ± 2). MM was diagnosed in 21 according to the SWOG criteria. All MM patients were observed at diagnosis and staged according to Durie and Salmon. Six were IgG, 5 were IgA, 16 carried a light chain, and 5 carried a light chain. Six patients had Bence-Jones proteinuria. Ten patients had MGUS, defined as previously reported: eight were IgG and two were IgA. All MGUS patients had an initial follow-up time of at least two years. Normal controls were healthy subjects screened for platelet aggregation and healthy volunteers working in our lab. The control group was matched for age and sex and not significantly different from the MG patients.

**Isolation of PB Lymphocytes**

PB lymphocytes were isolated from heparinized venous blood by centrifugation on Ficoll density gradient. Monocytes were removed by the plastic adherence method. In seven MG patients and eight normal controls, E-rosetting and nonrosetting lymphocytes were separated by means of 2-aminoethylisothiouronium bromide hydro-

**HUMAN MONOCLONAL gammopathies (MG)** are a heterogeneous group of B cell disorders characterized by monoclonal proliferation of B lymphocytes fully differentiating to immunoglobulin-producing cells. The clinical picture ranges from aggressive multiple myeloma (MM) to smoldering myeloma (SM) or indolent myeloma. Several MG are totally asymptomatic and are discovered by chance. They are classified as monoclonal gammopathies of undetermined significance (MGUS). Precise diagnosis is mandatory because active MM patients must be treated whereas MGUS and SM patients need only careful observation. Several prognostic factors have been proposed to distinguish these conditions: serum β2-microglobulin, plasma cell acid phosphatase, and (i-glucuronidase, the presence of J chains in the serum fl2-microglobulin, plasma cell acid phosphatase, and (i-glucuronidase, the presence of J chains in the

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Table 1. PB Lymphocyte 5'NT Activity and BM Plasma Cell LI% in MG Patients by Clinical Diagnosis and Stage

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>5'NT Activity (U/10⁶ Cells)</th>
<th>LI% (CVIF+ Cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MM patients</td>
<td>5.9 ± 0.9</td>
<td>1.6 ± 0.4</td>
</tr>
<tr>
<td>Stage I</td>
<td>10.5 ± 2</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Stage II</td>
<td>4.7 ± 1.1</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>Stage III</td>
<td>5.5 ± 0.7</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

bromide (AET)-treated SRBC. In three MG patients and two normal controls, OKT4+ and OKT8+ lymphocytes were studied separately. The LI% was not determined in these patients. In brief, E-rosetting lymphocytes were incubated with monoclonal antibody (four cases OKT8, one case OKT4) and fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG as the second layer.23 A Becton Dickinson 420 fluorescence-activated cell sorter (FACS) was used to separate reacting from nonreacting lymphocytes. Cells were washed twice in Tris-buffered normal saline before 5'NT assay. The viability of all suspensions was greater than 95% when checked with the trypan blue exclusion test.

5'NT Assay

The 5'NT assay was performed by a modification24 of the method of Edwards.25 In brief, activity was assessed on intact fresh cells by conversion of tritiated 5'AMP to tritiated adenosine, inosine, and hypoxanthine. The final concentration of cold 5'AMP in the reaction mixture was 0.1 mmol/L. Substrate and products were separated by descending chromatography on DE 81 paper in 5 mmol/L ammonium formate. 5'NT activity was expressed in units/10⁶ cells (1 unit = number of nanomoles of substrate converted per hour).

LI% of BM Plasma Cells

The LI% was calculated in 28 MG patients by combining cytoplasmic immunofluorescence (CyIF) with autoradiography, as previously reported.12 In brief, BM mononuclear cells were separated by dextran sedimentation. After incubation with tritiated thymidine, they were cytocentrifuged (Shandon 2 cytocentrifuge) on glass slides, fixed and stained with FITC-conjugated swine antihuman immunoglobulins (Dako Immunoglobulins, Denmark), and processed for autoradiography with K2 Ilford emulsion and Kodak D19 developer.

Surface Markers

Characterization of unseparated and separated cells was done by indirect immunofluorescence (IF) with monoclonal antibodies OKT4, OKT8, and OKT3 (Ortho Diagnostic System, Raritan, NJ), and FITC-conjugated rabbit anti-mouse IgG (Dako Immunoglobulins, Denmark) as the second layer.23 Surface and CyIF were evaluated in a Leitz Orthoplan microscope with a Ploomopack vertical illuminator and a 100 W mercury lamp. Sorted OKT4+ and OKT8+ subsets were reanalyzed by FACS and were always >95% pure.

Cells were counted with an automatic counter (Sysmex CC 120). Student's t distribution and the F test were used for statistical analysis of the difference between means and 5'NT regression on LI%, respectively.

RESULTS

Mean 5'NT activity of PB lymphocytes from 28 MG patients was statistically lower (P < .001) than in 18 normal donors (Fig 1). It was higher in MGUS than in MM, but the difference was not significant (P > .05) (Table I); by contrast, MGUS patients displayed a significantly lower LI% (P < .001) than MM patients (Table I).

A significant linear regression (P < .01) of 5'NT on LI% was found in 28 MG patients (Fig 2). 5'NT activity was significantly higher (P < .01) in 15 subjects with LI% < 1.0 (nine MGUS, six MM) than in 13 subjects (12 MM, one MGUS) with LI% > 1.0 (Fig 3).

E-rosetting lymphocytes isolated in seven MG
patients contained 85% ± 6% OKT3+ cells. Of these, 26% ± 5% were OKT8+ lymphocytes and 55% ± 5% were OKT4+ lymphocytes. PB T lymphocytes were isolated in eight normal controls (85% ± 6% OKT3+, 64% ± 4% OKT4+, 20% ± 2% OKT8+ cells).

The difference between the proportions of OKT4+ subsets was significant (P < .01).

Mean 5'NT activity of PB T lymphocytes from seven MG patients was significantly lower than in the controls (P < .001) (Table 2). In these patients, there was a statistically significant regression of T cell 5'NT deficiency on the LI% (y = -2x + 11.01, F = 16.31, P < .01). This correlation was better than that with the unfractionated PB lymphocytes (y = -1.3x + 8.57, F = 7.27, P < .05).

5'NT activity of regulating T cell subsets in three MG patients and two normal controls is shown in Table 3. 5'NT activity of OKT8+ subset was found to be greatly reduced by comparison with the controls in two MG patients with low T cell 5'NT activity. In contrast, one MG patient with normal T cell 5'NT activity showed an enzyme pattern coordinated with that of the controls.

Mean 5'NT activity of B lymphocytes was not significantly different from normal controls and no significant relationship was found with the BM LI% (data not shown).

DISCUSSION

Mean PB lymphocyte 5'NT activity was significantly lower in 28 MG patients than in normal controls. Values ranged from normal to very low, but this was not surprising in view of the clinical heterogeneity of MG. Mean 5'NT activity was lower in MM than in MGUS, but this difference was not significant.

The proliferative activity of the BM plasma cell was evaluated. MM and MGUS patients had very different LI% values. The LI% can be used to distinguish MM and MGUS patients, and predict their clinical progress. A statistically significant regression of 5'NT activity on LI% was observed in each patient: the higher the number of proliferating plasma cells in the BM, the greater the enzyme deficiency in the PB.

A cutoff value of 1.0 was used to maximize diagnostic separation and survival. MG patients with a LI% of <1.0 are expected to have a slow progression. In this study, they presented a significantly higher 5'NT activity than those with a LI% of >1.0, suggesting that 5'NT could also serve as a pretreatment prognostic factor.

The 5'NT assay was carried out on separated T and B lymphocytes in seven MG patients to identify the subpopulation responsible for the deficiency. We previously reported 5'NT activity of normal PB T and B lymphocytes. The MG T lymphocytes were found to have a significantly lower 5'NT activity than in normal controls. The regression of T cell 5'NT activity on LI% was significant. These data point to a T cell enzyme alteration closely related to tumor cell growth in the
BM. The T cell subpopulation is altered in suppressor function and subset distribution in MG, but the correlation with the clinical disease status is controversial.

Low T cell 5'NT activity may be related to an unbalanced OKT4-OKT8 ratio. In normal controls, the OKT8+ lymphocytes have a significantly higher 5'NT activity than OKT4+ lymphocytes. The proportion of OKT8+ cells in our seven MG patients was increased, while the OKT4+ cells were statistically fewer. Similar results have been reported in MG. It would seem that this T cell deficiency is not due to an unbalanced OKT4-OKT8 ratio, but to an intrinsic defect of the lymphocyte, mainly in the OKT8+ subset. Similar results have been observed in hypogammaglobulinemia. To test this hypothesis, a 5'NT assay was performed on FACS-purified OKT4+ and OKT8+ subsets in three MG patients and two normal controls. 5'NT deficiency of T cells in two MG patients was proved to be caused by the low 5'NT activity of OKT8+ cells. One MG patient with normal T cell 5'NT activity displayed an enzyme pattern in T cell subsets well correlated with that of the controls. In conclusion, the numerical proportion of OKT8+ lymphocytes is increased in MG patients, but their 5'NT activity is sometimes very low. This deficiency is more evident when the BM plasma cell LI% is high and is mainly responsible for the 5'NT deficiency of unseparated T lymphocytes.

The functional role of a 5'NT deficient T cell subpopulation is not known. Thompson et al have correlated this deficiency in vitro with an increased suppressor activity toward a PWM-driven differentiation. A deficiency of 5'NT has been clearly demonstrated in infectious mononucleosis, in which an active suppressor T cell subpopulation is expanded (Ia+, OKT8+). We suggest that a peripheral T cell subpopulation with an altered suppressor activity correlated to the tumor cell kinetics is present in MG. An increased suppressor function of OKT8+ cells has been recently reported in MG. Low 5'NT activity of OKT8+ lymphocytes may serve as an indicator of increased suppressor activity. A functional assay is thus warranted in patients whose PB OKT8+ lymphocytes are 5'NT deficient.

NOTE ADDED IN PROOF

Subsequent to the submission of this work, Silber and Conklin have demonstrated a marked deficiency in 5'NT activity in the OKT8+ subpopulation from patients with B cell chronic lymphocytic leukemia.

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