CONCISE REPORT

Kinetics of Circulating B Lymphocytes in Human Myeloma

By Mario Boccadoro, Paolo Gavarotti, Giorgio Fossati, Massimo Massaia, Alessandro Pileri, and Brian G. M. Durie

The tritiated thymidine labeling index (LI%) of peripheral B lymphocytes was studied in eight myeloma patients using simultaneous immunofluorescence and autoradiography. The LI% values were low (0.3%-5.1%), but significantly increased as compared to normal controls. In addition, there was excellent correlation between the LI% values and myeloma disease activity. Lowest LI% values were observed in remission patients and the highest at the time of relapse. Simultaneous LI% evaluation of bone marrow myeloma cells in five patients gave concordant results, indicating the same kinetic behavior in both these compartments, particularly in the relapse phase. These data indicate both that circulating B lymphocytes include the neoplastic clone and that these B lymphocytes and bone marrow myeloma cells have similar kinetics.

Human myeloma is characterized by proliferation of monoclonal plasma cells that usually secrete homogeneous immunoglobulin (Ig) or M-component. Several workers have demonstrated that a high percentage of circulating B lymphocytes in human myeloma express surface Ig sharing the same idiotypic specificity as the circulating Ig. These idiotypic lymphocyte populations are considered to be neoplastic progenitors, even although direct proof of maturation toward the more mature myeloma cell population is currently lacking.

In human myeloma, extensive kinetic studies have been carried out to evaluate the prognostic significance of the tritiated thymidine labeling index (LI%) of myeloma cells. Patients with low LI% (<1%) at the time of diagnosis have a survival superior to those patients with a higher LI%. Moreover, a significant increase in this parameter usually occurs at the time of relapse. These studies of LI% of bone marrow myeloma cells have demonstrated the clinical significance of this parameter. However, if the idiotype malignant population includes B lymphocytes, some questions arise as to the level of maturation at which the most critical cell proliferation occurs and whether or not proliferation at the level of the myeloma cells is a true reflection of increases in myeloma cell burden. Myeloma cell expansion could be the result of proliferation and differentiation of a tumor cell population in a progenitor compartment.

In an effort to clarify the kinetic relationship between circulating B lymphocytes and myeloma cells, it was felt important to evaluate the LI% of both these compartments and to determine if there were fluctuations in the B lymphocyte kinetics, as have been demonstrated for the myeloma cells at different phases of the disease.

In this article, we present the results of B-lymphocyte evaluation in 8 human myeloma patients as compared to normal controls plus simultaneous bone marrow myeloma cell LI%. One patient was studied during remission and relapse.

MATERIALS AND METHODS

Peripheral blood lymphocytes were separated on a Ficoll-Hypaque discontinuous gradient and washed twice. Macrophages were removed by adherence on plastic Petri dishes for 1 hr at 37°C. The cells were resuspended at a concentration of 3 x 10⁶/ml in RPMI 1640 supplemented with 10% FCS, incubated with 5 μCi/ml of tritiated thymidine (specific activity 5 mCi/m mole) for 1 hr at 37°C and then washed twice in cold PBS containing NaN₃ 10⁻³ M. The cells were incubated with 20 μl of a fluorescent anti-Ig diluted 1:10 (goat anti-IgG + IgA + IgM, Cappel Laboratories) at 4°C for 20 min and washed twice. Slides were prepared by gently smearing and fixed with 95% ethanol and 5% acetic acid at ~20°C for 10 min and washed twice.

Bone marrow myeloma cells were separated using 3% dextran sedimentation. The supernatant was collected, the cells washed twice, and incubated for 1 hr at 37°C with 5 μCi/ml of tritiated thymidine (specific activity 5 mCi/m mole). The cells were washed again twice and smears were prepared using a Shandon cyt centrifuge. The slides were fixed in 95% ethanol and 5% acetic acid for 10 min. The fixed plasma cells were stained for 20 min with a fluorescent anti-human Ig (Cappel Laboratories) at the appropriate dilution and washed in cold PBS for at least 12 hr.

For the autoradiographic processing, prewarmed slides were dipped in K2 Floro Emulsion at 54°C and then exposed for 7 days at 4°C. The slides were developed in Kodak D19 developer. At least 500 fluorescent cells were counted in each LI% determination using a Leitz Orthoplan microscope equipped with a Plenomapack vertical illuminator with a 100-W mercury lamp.

It must be emphasized that in the present paper the lymphocyte
LI% was evaluated in over 20 patients. Since the fluorescence was substantially reduced by the heating of slides during the necessary processing and reading of the autoradiographs, only 8 of the over 20 gave meaningful results and are reported herein. Results in the excluded cases were qualitatively similar to those reported, but could not be accurately quantitated. This was not the case for combined autoradiography and immunofluorescence at the plasma cell level, because of the much larger amount of Ig in the cytoplasm. This latter combined technique has been used routinely for myeloma cell evaluations in clinical studies.\(^\text{12,13}\)

**Patients**

Patient no. 1 (Table 1) presented with multiple osteolytic lesions, no monoclonal component in the serum or urine, and no significant increase in bone marrow plasma cells. Diagnosis of nonsecreting multiple myeloma was made by pathologic examination of biopsies of the sternum and the tenth right rib. All the other patients had characteristic IgG myeloma and were classified according to the Durie and Salmon system.\(^\text{14}\) The patients received intermittent courses of Alkeran and prednisone. Remission was defined as >75% tumor reduction and stable disease as <20% change in M-component for 6 mo after stopping the chemotherapy. Patients studied at relapse had both significant increases in M-component and development of new osteolytic lesions.

**RESULTS**

In the 3 normal subjects studied, the LI% of the peripheral B lymphocytes was so low that only 1 subject had even a single labeled cell detectable after 500 cell differential counting. In contrast, in the myeloma patients, a low but significant labeling was observed (Table 1). Moreover, the patients studied during the remission phase had the lowest values (median 0.51 ± 0.20, range 0.3–0.8). In the relapse phase, LI% values were significantly higher (median 3.0 ± 1.36, range 1.6–5.1). Even with the small number of patients studied, the difference between remission and relapse was statistically significant (Wilcoxon test \(p = 0.05\)).

Table 1 also shows the LI% measured simultaneously in peripheral B lymphocytes and bone marrow myeloma cells in 4 myeloma patients in relapse and in 1 patient in remission phase. In all 5 patients there was concordance between the values observed in both these compartments: highest values were observed at the relapse and lowest during the remission. Therefore, peripheral B lymphocytes and bone marrow myeloma cells showed a similar kinetic pattern.

In one patient it was possible to demonstrate B lymphocytic kinetic fluctuations corresponding to the disease status. Patient 5, studied during the evolution of the disease, showed an increase in LI%: 0.3 in remission (5a) and 1.9 at the relapse (5b) (Table 1).

**DISCUSSION**

In the present study we have shown that peripheral B lymphocytes from myeloma patients display a low but significant increase in LI% compared to normal individuals. There was an excellent correlation between disease activity and LI%; the lowest LI% were observed during the remission and highest values during the relapse phase. In addition, one patient was shown to have fluctuations in the LI% corresponding to change in disease status.

A large number of studies evaluating the nature of the circulating B lymphocytes in multiple myeloma have been carried out.\(^\text{15,17}\) Nonetheless, the neoplastic origin of a B-lymphocytic subpopulation is still controversial. Several authors, using antiidiotypic sera, have demonstrated the presence of a higher number of circulating B lymphocytes sharing the same idiotypic specificities as the M-component.\(^\text{1}\) Other workers consider the phenomenon the result of a passive absorption involving the M-component.\(^\text{15}\) Despite this, the preponderance of evidence would seem to support the presence of a subpopulation of lymphocytes, representing the myeloma cell precursors. The most impor-

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**Table 1. LI% Evaluated by Means of Simultaneous Autoradiography and Surface Immunofluorescence (Peripheral B Lymphocytes) or Cytoplasmic Immunofluorescence (Bone Marrow Myeloma Cells)**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Staging</th>
<th>Disease Activity</th>
<th>Peripheral B Lymphocytes LI%</th>
<th>Bone Marrow Myeloma Cells LI%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IIIA*</td>
<td>Diagnosis</td>
<td>3.3</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>IA</td>
<td>Diagnosis</td>
<td>0.6</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>IA</td>
<td>Remission</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>4</td>
<td>IA</td>
<td>Remission</td>
<td>0.4</td>
<td>ND</td>
</tr>
<tr>
<td>5a</td>
<td>IIIA</td>
<td>Remission</td>
<td>0.3</td>
<td>ND</td>
</tr>
<tr>
<td>5b</td>
<td>IIIA</td>
<td>Relapse</td>
<td>1.9</td>
<td>2.4</td>
</tr>
<tr>
<td>6</td>
<td>IIIA</td>
<td>Relapse</td>
<td>1.6</td>
<td>4.3</td>
</tr>
<tr>
<td>7</td>
<td>IIIA</td>
<td>Relapse</td>
<td>5.1</td>
<td>6.2</td>
</tr>
<tr>
<td>8</td>
<td>IIIA</td>
<td>Relapse</td>
<td>3.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Normal controls</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1</td>
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<td></td>
<td>0.2</td>
<td></td>
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<tr>
<td>2</td>
<td></td>
<td></td>
<td>&lt;0.2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td>&lt;0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Nonsecretant myeloma, stage III based on multiple bone lesions.
ND, not done.
tant points supporting this conclusion are: (A) the capacity of the idiotypic lymphocytes to reexpress their surface Ig;\textsuperscript{4,6,16} (B) the non-self-renewal characteristics of the mature myeloma cells;\textsuperscript{17} (C) the aneuploid pattern of the peripheral lymphocytes in some patients;\textsuperscript{18} (D) the murine plasmacytoma model in which maturation in vivo from lymphocytoid cells was clearly demonstrated.\textsuperscript{19} Therefore, the increased proliferative activity in our patient population supports these arguments favoring the neoplastic nature of this subpopulation.

Simultaneous studies in five patients indicated that the same kinetic pattern was shown at both lymphocyte and myeloma cell levels. The four patients studied at relapse had the highest LI\% for both lymphocytes and myeloma cells. The patient studied during the remission had a low LI\% in both these compartments. In our opinion, this is an important point supporting the validity of the LI\% at the myeloma cell level as an index of tumor progression. It can be argued that myeloma tumor mass may be increased by an input of neoplastic cells from a B-lymphocytic compartment without any change in the myeloma LI\%. Apparently this is not the case in the patients studied. Thus, the LI\% performed at the myeloma cell level appears to truly reflect both the kinetics at the precursor stage and the disease activity. Although measurement of peripheral blood B-lymphocyte LI\% would be clinically simpler and give comparable results, this cannot yet be recommended for routine use because of the technical difficulties discussed.

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REFERENCES

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