Evidence for Peripheral Blood B Lymphocyte but Not T Lymphocyte Involvement in Multiple Myeloma

By James Berenson, Rex Wong, Kenneth Kim, Nathaniel Brown, and Alan Lichtenstein

Because there is controversy regarding whether subsets of peripheral blood lymphocytes (PBLs) are part of the malignant clone in patients with multiple myeloma, we studied this question by immunoglobulin and T cell receptor gene analysis. Southern blot analysis with antibody probes demonstrated clonal immunoglobulin gene rearrangements in PBLs of seven of nine patients that were identical to those seen in their marrow plasma cells. Circulating plasma cells were not detected in any of these patients. In contrast, no patient demonstrated clonally rearranged T cell receptor genes. In one sequentially studied patient, PBLs obtained at diagnosis when he had stage I (Durie-Salmon) contained only germline DNA, while analysis of PBLs at relapse (stage III) revealed a clonally rearranged band. These data confirm the notion that circulating lymphocytes in patients with myeloma are part of the malignant clone and, furthermore, these malignant cells are of B cell rather than T cell lineage.

MATERIALS AND METHODS

Patients. Nine patients with multiple myeloma as defined by the Myeloma Task Force were studied. All patients underwent bone marrow examination, serum protein electrophoresis, immunoelectrophoresis, bone surveys, routine chemistries, CBCs, and 24-hour urine collection for determination of creatinine and protein. Patients were staged according to the Durie-Salmon classification.

Isolation of peripheral blood lymphocytes, granulocytes and bone marrow plasma cells. Peripheral venous blood and bone marrow samples were obtained from all nine patients after informed consent and in accordance with the Human Subjects Review Boards of the Veteran’s Administration Wadsworth and UCLA Medical Center. Peripheral blood was centrifuged to separate the cells from the serum. The cells were exposed to Histopaque (Pharmacia Fine Chemicals, Piscataway, NJ) 1077 and 1119 density gradient separations. This technique yields >98% pure granulocytes at the 1077/1119 interface, and these cells do not show antibody gene rearrangements in Southern blot analysis.

The mononuclear cells appear at the 1077/Hanks’ balanced saline solution interface, and the monocytes are removed from this fraction by adherence to plastic dishes, which leaves highly purified PBLs (>98%). Bone marrow was exposed to Ficoll-Hypaque (Pharmacia) density gradient separation to yield pure populations of plasma cells.

Southern blot analysis. Genomic DNA was prepared from PBLs, granulocytes, and bone marrow plasma cells on each patient according to the method of Perry et al. The DNA was digested with restriction endonucleases BamHI, EcoRI or HindIII (Bethesda Research Laboratories, MD). Ten micrograms of digested DNA was electrophoresed in 0.6% agarose gels at 1 to 2 V/cm for 42 hours and transferred to nylon membranes by the method of Southern. Parallel lanes of similarly digested granulocyte, PBL, and plasma cell DNA samples from each patient were electrophoresed on the same agarose gel.

Recombinant probes for the human heavy chain immunoglobulin (Ig) gene segments JH and Cμ were donated by L. Hood et al (California Institute of Technology, Pasadena, CA) and were subcloned in pBR322. The JH probe is a 3.2-kilobase pair (kb), human genomic fragment extending from JH2 to the HindIII site in the JH-Cμ intervening sequence. The Cμ probe is a 2-kb genomic clone containing the complete sequence of Cμ. This probe hybridizes with all four Cμ-subclass gene fragments, which are reported to be ≥90%
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No patient demonstrated clonal \(\beta\)-TCR gene rearrangements in the peripheral blood of two patients with active disease. It is interesting to note that the patient with the lowest tumor burden at diagnosis (patient no. 6) only demonstrated involvement in the peripheral blood after the development of clinical relapse with a marked increase in tumor burden. King and Wells\(^7\) studied two patients with active myeloma but with fairly small serum M spikes and two patients in clinical remission.

**DISCUSSION**

The aim of the present study was to investigate whether significant numbers of peripheral blood lymphocytes are part of the malignant clone in multiple myeloma. The detection of clonal Ig and TCR gene rearrangements has been used to demonstrate the clonal origin of other B and T cell-derived tumors.\(^2\) We have used these same gene probes to demonstrate that clonal antibody gene rearrangements occur in the PBLs of most patients with multiple myeloma. These results indicate that peripheral blood B lymphocytes but not T lymphocytes are part of the malignant clone in multiple myeloma.

These data confirm the work of other investigators who demonstrated peripheral blood monoclonal B cells by either abnormal surface membrane \(\kappa/\lambda\) light-chain ratios or positive surface immunofluorescence with antiidiotype reagents.\(^2\) However, these results contradict those of Pilarski et al,\(^9\) King and Wells,\(^7\) and Gobbi et al\(^8\) who failed to find monoclonal B lymphocytes in the peripheral blood of myeloma patients. Pilarski et al\(^9\) studied only three patients in remission and used antiidiotype reagents. In our study, we only studied patients with active disease. It is interesting to note that the patient with the lowest tumor burden at diagnosis (patient no. 6) only demonstrated involvement in the peripheral blood after the development of clinical relapse with a marked increase in tumor burden. King and Wells\(^7\) studied two patients with active myeloma but with fairly small serum M spikes and two patients in clinical remission.

**Table 1. Clinical Characteristics and Southern Blot Analysis**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Isotype</th>
<th>Antibody Genes in PBLs</th>
<th>Heavy Chain</th>
<th>Light Chain</th>
<th>(\beta)-TCR Genes in PBLs and Plasma Cells</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Constant Region</td>
<td>(\Delta)</td>
<td>Light Chain</td>
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<tr>
<td>1</td>
<td>(\kappa)</td>
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<td>R</td>
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<td>ND</td>
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<tr>
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<td>(\lambda)</td>
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</tbody>
</table>

Abbreviations: R, rearranged DNA identical to bone marrow plasma cells; G, germline or unrearranged DNA; ND, not done.

*Both these patients demonstrated antibody gene rearrangements in marrow plasma cells.
These investigators used a rosette assay to detect idiotypic tumor burden of this technique. This negative result may also reflect the low concentration of plasma cells bearing an endogenous idiotypic marker when using peripheral blood lymphocytes (PBLs). Failure to demonstrate PBLs bearing an endogenous idiotypic marker using pokeweed mitogen-stimulated DNA from PBls, plasma cells, or granulocytes. Southern blot analysis is exceptionally sensitive. Molecular size analysis of a cell mixture can be identified by this technique.27,28

In our study, we have not shown clonal T cell rearrangements in any of our patients. These results would suggest that clonal T cells do not exist in multiple myeloma as has been reported by other workers.6 However, these investigators used antiidiotypic antisera to demonstrate T cell involvement in a single patient with myeloma. It is well known that there is marked expansion of T cells that bear Fc receptors for the idiotypic idiom.29,30 and these “clonal” T cells were probably T cells with idiotype bound to their Fc receptors.

The common acute lymphoblastic leukemia antigen (CALLA) has been demonstrated on the surface of bone marrow cells in patients with myeloma.31 Because CALLA is normally thought to be present on B lymphoid cells during early stages of differentiation, these data suggest an oncogenic event in myeloma occurring in similar early precursor cells. The presence of pre-B lymphoid cells that express homologous idiotypic marker in two patients with IgA myeloma also supports this notion. We are presently using antibody gene analysis to determine whether earlier stages in B cell differentiation have clonal expansion of cells.

ACKNOWLEDGMENT

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