Abnormal Clonogenic Potential of T Cells From Multiple Myeloma Patients

By Linda M. Pilarski, Michael J. Mant, Bernard A. Ruether, George Carayanniotis, David Otto, and John F. Krowka

Peripheral blood lymphocytes (PBLs) from multiple myeloma patients are defective in both proportion and absolute numbers of OKT4+ cells and have a normal proportion but reduced absolute number of OKT8+ cells. To assess the functional capabilities of the T cells in myeloma patients, we cloned the T cells in PBLs using limiting dilution conditions in which 100% of OKT4+ and OKT8+ T cells in normal PBLs are able to form a clone. In contrast, the OKT8+ cells from PBLs of five of seven multiple myeloma patients were severely compromised in their clonogenic potential; only 7% to 25% of OKT8+ T cells appeared to give rise to a clone. Clonogenic potential of the OKT4+ cells in patients was more nearly normal. Analysis of two multiple myeloma patients with abnormally low numbers of T cells in PBLs revealed the existence of abnormalities in the progenitors of T cell clones. In both patients, two to three times as many T cell clones were observed as would have been expected based on the number of PBLs cultured, suggesting that OKT4-8+ cells in PBLs are capable of giving rise to OKT4+ and, at lower frequency, to OKT8+ clonal progeny in vitro.

We conclude that purely quantitative assessment of T cell subsets should be interpreted with caution, since proportionately normal numbers of OKT8+ cells in patient PBLs are seriously compromised in their ability to give rise to clonal progeny in vitro, and since there appears to be a OKT4-8+ population of T cells in PBLs that are committed to become OKT4+ or OKT8+ T cells, but are unable to do so in vivo.

PBLS from randomly chosen Red Cross donors or from patients were purified by centrifugation over Ficoll-paque (Phamacia, Dorval, Quebec), and the composition of the resulting cell population was determined by a differential count. Aliquots of these preparations were characterized for number of T cells by indirect immunofluorescence. A second aliquot was cultured at limiting dilution to grow clonal progeny.

PBLS were cloned by a modification of the method of Moretta et al.13 Various concentrations (0.1 to 3.0 cells per microculture well) of unfractionated PBLs were mixed with 50% conditioned medium containing growth factors, 1% PHA vol/vol (GIBCO Canada, Burlington, Ontario), and 104 irradiated (5,000 rad) normal PBL feeders from Ficoll-paque purified buffy coat preparations were cultured in flat-bottomed 0.2-mL microculture wells (Linbro, Flow Laboratories, Mississauga, Ontario). Culture medium was RPMI 1640 (GIBCO Canada) plus 10% fetal calf serum (FCS) (Flow Laboratories). Each microculture was fed weekly with 102 irradiated normal PBLs resuspended in 100 μL of conditioned medium or diluted Jurkat fraction III, plus 1% phytohemagglutinin (PHA).

Conditioned medium was the filtered supernatant from irradiated (1,500 rad) normal PBLs grown at 106 cells/mL in RPMI + 10% FCS + 1% PHA (vol/vol) for three days. For clones from some normal subjects and for patients 138 and 144, a semipurified supernatant was obtained from the T lymphoma line Jurkat after stimulation with concanavalin A and phorbol myristic acid as a source of growth factors. This preparation was concentrated by ammonium sulfate precipitation, followed by fractionation on a Sephadex G-100 column to obtain interleukin 2(IL-2)-enriched fraction III. It contained 400 U/mL of IL-2 activity as assessed by its ability to support the growth of an IL-2-dependent cell line, and was used at a final concentration of 8 units of IL-2 per microculture well. The efficiency of cloning was 1.0, and the distribution of OKT4+ and OKT8+ clones was the same from normal PBLs cultured with PHA-conditioned medium or from the Jurkat Fraction III.

Cultures were monitored weekly by microscopic screening; clones were first visible at about ten days and continued to grow for up to six more weeks. When a clone reached sufficient numbers to cover most of the surface of the microwell and at least seven days after the last feeding, cells were removed, divided into two to four aliquots and typed for surface phenotype by indirect immunofluorescence. Clonal phenotype was constant for at least eight weeks. Clones assessed by two time points. In most cases, the clones were typed at three to four weeks after culture initiation. Approximately 50 clones representing both OKT4 and OKT8 phenotypes, from both normal and patient donors, were typed again at six to eight weeks after culture initiation.

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and the results obtained were consistent with data obtained for each
close to three at four weeks.

**Immunofluorescence (IF).** For this study, the assay method was
an indirect IF assay. Every set of assays included a normal PBL
sample as a control to ensure that T cells could be detectable with the
reagents used on a particular day. Purified PBLs (5 x 10^9 to 10^10)
or one half to one fourth of a clone were aliquoted into v-bottomed
microtiter wells, pelleted, and resuspended in 50 μL of the test
murine anti-human antibody diluted in normal saline to an appro-
siate concentration, and incubated for 60 minutes at 4 °C. Plates were
centrifuged and washed twice in saline, and the cell pellet was
resuspended in 50 μL of a one-tenth dilution of F(ab)2 fragments of
sheep anti-mouse immunoglobulin (Ig) labeled with fluorescein iso-
thiocyanate (FITC) or tetramethyl rhodamine isothiocyanate
(RITC) (Tago, Burlingame, Calif), incubated for 60 minutes at 4
°C, and washed twice. Cells were kept in 0.02% sodium azide
throughout the procedure and were fixed in 1% formalin after the
final wash. An aliquot of each sample was always incubated with
saline rather than antibody prior to the addition of the fluorescent
anti-mouse antibodies as a negative control. In addition, each PBL
sample was also tested for IF with anti-human Ig as well as other
antibodies. The number of positive cells was different for different
antibodies, indicating that IF with OKT antibodies was specific; this
was confirmed in many cases using double IF techniques. IF was
examined using a Zeiss microscope with fluorescence epi-illumina-
tion, and selective filters. Cell samples were counted for total cells in
the field and for total number of cells with ring fluorescence by
means of a hemocytometer. At least 200 cells were screened for PBL
samples and 30 to 100 cells were counted for each clonal aliquot.
Double IF was performed as previously described and always
included negative control aliquots of PBLs for both fluorochromes.

**Antibodies.** BA-1 is a monoclonal antibody specific for a marker
expressed on sIg+ B cells and some granulocytes (Hybridtech, San
Diego). BA-2 was also from Hybridtech. Anti-K and anti-h and
anti-lgM were monoclonal reagents from BRL (Gaithersberg, Md).
OKT3, OKT8, and OKT10 were from Ortho (Raritan, NJ). 7H.3 is
a monoclonal Ab specific for monomorphic determinants on
HLA.DR, and 41H.16 recognizes an antigen expressed only on
sIg+ B cells; both were provided by Dr B.M. Longenecker, Univer-
sity of Alberta.

## RESULTS

**T cell subset distribution in PBLs.** PBLs from multiple
myeloma patients, patients with MGUS, and normal donors
were assessed for expression of OKT3, 4, and 8 (Table 1). As
has been reported by others, we find that most myeloma
patients in all treatment categories have a reduced propor-
tion and absolute number of OKT4+ and normal proportion
but, unlike the other reported patients, have a reduced
absolute number of OKT8+ T cells. Of MGUS samples, 42%
(11 of 26) had a number of OKT4+ cells within the normal
range (mean ± 2 SD), whereas only 25% (20 of 81) of
myeloma samples had a normal number of OKT4+ T cells.
Untreated patients had a somewhat higher average number
of OKT4+ cells than did patients on chemotherapy. Of
untreated patients, 39% (7 of 18) were within the normal
range of OKT4+ cells, whereas 23% (13 of 57) of samples
from patients on chemotherapy were normal.

**Cloning of normal T cells.** T lymphocytes from normal
individuals were cultured at limiting dilution with feeder
cells, PHA, and T cell growth factors. Under these condi-
tions, 100% of the T cells were able to form a colony
consisting of from 1 to 3 x 10^5 progeny lymphocytes. The
progeny are T cells as defined by their absence of any B cell
markers (sIg-, BA-1+, 41H.16+) and their expression of
OKT3, OKT4 and OKT8 antigens (Table 2). Progeny T cells
were HLA.DR+ and strongly positive for BA-2, an antigen
thought to occur mainly on hemopoietic and pre-B progenitor
cells; our data indicate that it is expressed on actively
growing cultured T cells but not on freshly isolated periph-
lar T cells (Table 2). The progeny of normal T cells cultured
at limiting dilution is homogeneous and therefore clonal for

### Table 1. Proportion and Absolute Number of OKT4+ and OKT8+ T Lymphocytes in MM, MGUS, and Normal Donors

<table>
<thead>
<tr>
<th>Subjects</th>
<th>OKT3+ (% Total)</th>
<th>OKT4+ (% Total)</th>
<th>OKT8+ (% Total)</th>
<th>OKT4+-OKT8+ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>42 ± 5</td>
<td>33 ± 5</td>
<td>21 ± 3</td>
<td>1.9 ± 1.2</td>
</tr>
<tr>
<td>On chemotherapy</td>
<td>41 ± 4</td>
<td>24 ± 3</td>
<td>19 ± 2</td>
<td>1.5 ± 1.1</td>
</tr>
<tr>
<td>Off chemotherapy</td>
<td>24 ± 11</td>
<td>20 ± 9</td>
<td>23 ± 10</td>
<td>0.7 ± 0.4</td>
</tr>
<tr>
<td>MGUS</td>
<td>45 ± 5</td>
<td>36 ± 5</td>
<td>18 ± 2</td>
<td>2.4 ± 1.7</td>
</tr>
<tr>
<td>Normal</td>
<td>69 ± 3</td>
<td>56 ± 2</td>
<td>18 ± 1</td>
<td>3.1 ± 1.2</td>
</tr>
</tbody>
</table>

MM, multiple myeloma; MGUS, monoclonal gammopathy of undetermined significance; PBLs, peripheral blood lymphocytes.

*Values represent the mean ± SE. All assays involved indirect immunofluorescence using the indicated antibody followed by F(ab)2, fragments of
fluorescein isothiocyanate (FITC)-labeled sheep anti-mouse Ig. The above values were obtained from a total of 53 individual patients, 15 untreated, 32 on
treatment, and 6 off treatment (81 samples total); 24 individual MGUS patients (26 samples tested); and 22 individual normal donors (25 samples
tested).

†The average ratio ± SD of OKT4+ to OKT8+ cells was calculated from the ratios in individual samples.

‡The reduced mean number of OKT3 relative to OKT4 plus OKT8 T cells seen in patients off therapy held true in four of six individual patients.

Preliminary work suggests that this reflects a transiently reduced density of OKT3 on the PBL T cells of these patients relative to that in most normal T
cells. This pattern was observed in 27% of the total patient PBL samples analyzed and in 12% of normal PBL samples.
The total number of PBLs cultured was 0.16 to 0.28. On the cells cultured: 0.53, 0.34/0.18; 0.67, 0.95/0.24; 0.90, 1.2/0.25; 0.91, 0.96/0.17; 0.60, 0.67/0.07. These were also seen in clonal populations from normal donors (Table 3). Although fresh PBLs from multiple myeloma patients had a proportion of OKT8+ cells within the normal range and a reduced proportion of OKT4+ cells, upon culture, most cells capable of giving rise to a colony yielded OKT4+ progeny T cells. Unlike T cells from normal donors in which we observed 100% efficiency of cloning for OKT4+ and OKT8+ cells, the cloning efficiency in some patients was <1.0 for OKT4+; in five of seven patients, it was severely reduced for OKT8+ cells (0.07 to 0.25). However, in calculating this number we must make the assumption that the PBLs typed as OKT4+ or OKT8+ are the same cells that give rise to clonal progeny in culture. Although this assumption is probably correct for normal PBLs, in which 60% to 70% of the cells are T cells, with a cloning efficiency of 0.7 to 0.8, it may be incorrect in the case of patient PBLs in which proportion of T cells is <50% and the efficiency of cloning for total T cells (OKT4+ + OKT8+) is <1.0. Clonal progenitors and OKT4-bearing or OKT8-bearing cells may be non-overlapping populations in PBLs.

The validity of the above caveat was verified by observations on the clonogenic potential of PBLs from two patients, both with an abnormally low proportion of OKT4+ T cells. PBLs from patient 138 gave rise to a set of clones with the expected distribution of OKT8+ phenotype (Table 4); however, the efficiency of cloning for OKT4+ cells, including the

<table>
<thead>
<tr>
<th>Patient</th>
<th>Class of Paraprotein</th>
<th>Chemotherapy Treatment Status</th>
<th>Phenotype of Freshly Isolated Peripheral T Cells (%)</th>
<th>Clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>053</td>
<td>IgGK</td>
<td>Off</td>
<td>OKT3 64</td>
<td>16/26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT4 48</td>
<td>11/26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT8 36</td>
<td>1/26</td>
</tr>
<tr>
<td>067</td>
<td>IgG4</td>
<td>On</td>
<td>OKT3 71</td>
<td>21/30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT4 24</td>
<td>7/30</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT8 21</td>
<td>1/27</td>
</tr>
<tr>
<td>090</td>
<td>IgAK</td>
<td>On</td>
<td>OKT3 34</td>
<td>22/27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT4 23</td>
<td>3/27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT8 12</td>
<td>1/27</td>
</tr>
<tr>
<td>091</td>
<td>Light chain</td>
<td>On</td>
<td>OKT3 76</td>
<td>19/22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT4 29</td>
<td>2/22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT8 15</td>
<td>1/22</td>
</tr>
<tr>
<td>050</td>
<td>IgGK</td>
<td>On</td>
<td>OKT3 40</td>
<td>44/52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT4 23</td>
<td>7/52</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OKT8 37</td>
<td>1/52</td>
</tr>
</tbody>
</table>

Cloning efficiency (OKT4/OKT8) based on number of OKT4 or OKT8 T cells in peripheral blood lymphocytes (PBLs) corrected for the differential count on the cells cultured: 0.34/0.18; 0.67; 0.96/0.24; 0.90; 1.2/0.25; 0.91; 0.96/0.17; 0.60, 0.67/0.07. The efficiency of cloning as calculated from the total number of PBLs cultured was 0.16 to 0.28.

The observation that the number of OKT3+ cells considerably exceeded the number of OKT4+ plus OKT8+ cells held true in 11% of the patient samples tested. Additional experiments are required to define this subset of OKT3+ 4-8 cells further. This pattern was not seen in samples of normal PBLs.
ABNORMAL T CELLS IN MYELOMA

Table 4. Abnormally High Cloning Efficiency in Some Myeloma Patients

<table>
<thead>
<tr>
<th>Patient No./Clones</th>
<th>Percentage in PBLs</th>
<th>Expected No.</th>
<th>Observed No.</th>
<th>Cloning Efficiency*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 138</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total T cells</td>
<td>34</td>
<td>5.4</td>
<td>12</td>
<td>2.2</td>
</tr>
<tr>
<td>OKT4+</td>
<td>21</td>
<td>3.4</td>
<td>8</td>
<td>2.3</td>
</tr>
<tr>
<td>OKT8+</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>1.0</td>
</tr>
<tr>
<td>OKT48+ BA-2</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>Patient 144</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total T cells</td>
<td>22</td>
<td>3.7</td>
<td>11</td>
<td>3.0</td>
</tr>
<tr>
<td>OKT4+</td>
<td>10</td>
<td>1.7</td>
<td>5</td>
<td>2.9</td>
</tr>
<tr>
<td>OKT8+</td>
<td>12</td>
<td>2.0</td>
<td>4</td>
<td>2.0</td>
</tr>
<tr>
<td>OKT48+ BA-2</td>
<td>—</td>
<td>—</td>
<td>2</td>
<td>—</td>
</tr>
</tbody>
</table>

Patient 138 was newly diagnosed as multiple myeloma but had been treated for hypercalcemia with high-dose prednisone with mithramycin. Patient 144 was in remission and off treatment.

*Calculated based on the frequency of the phenotypes in peripheral blood lymphocytes (PBLs) and particularly subject to the caveat expressed in the Results section; for patient 138, 73% of PBLs gave rise to a clone even though only 34% of the cells expressed OKT4 or OKT8; for patient 144, 76% of PBLs gave rise to a clone, while only 32% of PBLs expressed T cell markers.

OKT48+ BA-2 doubles was abnormally high (208%) suggesting a contribution by OKT4+ progenitors. A similar pattern of cloning was observed from PBLs of patient 144, who had been off therapy for over a year and was in remission. For patient 144, the apparent efficiency of cloning was abnormally high for both OKT4+ and OKT8+ clones (2 to 2.9) (Table 4). Unlike the other patients tested, for both individuals, the proportion of lymphocytes in PBLs able to produce a clone (73% to 76%) was higher than the proportion expressing an OKT4 or 8 marker (22% to 34%).

BA-2 expression on clonal progeny and on patient PBLs. In a series of clones from patients and normal subjects, the cells in each culture well were divided into four aliquots and assayed for OKT4, OKT8, BA-2, and HLA.DR. All clones were HLA.DR+, but only a subset were BA-2+, suggesting that BA-2 may be a valuable marker to analyze further T cell subset distribution in patients. OKT48+ BA-2+, OKT48+ BA-2−, OKT8+ BA-2− and OKT48− BA-2+ subsets were observed (Table 5). These BA-2+ T cell subsets were not found in uncultured PBLs.

Table 5. Heterogeneity Between Clones in the Expression of BA-2 Surface Phenotype of T Cell Clones

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>OKT4+ BA-2+</th>
<th>OKT4+ BA-2−</th>
<th>OKT8+ BA-2+</th>
<th>OKT8+ BA-2−</th>
</tr>
</thead>
<tbody>
<tr>
<td>053</td>
<td>7/16</td>
<td>4/16</td>
<td>5/16</td>
<td>—</td>
</tr>
<tr>
<td>067</td>
<td>5/12</td>
<td>2/12</td>
<td>2/12</td>
<td>—</td>
</tr>
<tr>
<td>090</td>
<td>3/13</td>
<td>9/13</td>
<td>1/3</td>
<td>—</td>
</tr>
<tr>
<td>091</td>
<td>3/9</td>
<td>8/9</td>
<td>—</td>
<td>1/9</td>
</tr>
<tr>
<td>Normal</td>
<td>7/27</td>
<td>14/27</td>
<td>2/27</td>
<td>4/27</td>
</tr>
</tbody>
</table>

The contents of each microtiter well were divided into four aliquots and assayed in the immunofluorescence assay for expression of OKT4, OKT8, BA-2, and HLA.DR. Most clones were HLA.DR+ positive (4/50). Only wells from microtiter plates with <30% positive wells were used to minimize chances of more than one precursor T cell per well.

The possibility existed that in patient PBLs, cells with a density of T cell markers below the threshold of detection in from most myeloma patients, MGUS, or normal donors (Table 6).

DISCUSSION

The distribution of T lymphocyte subsets in PBLs from multiple myeloma patients and from many donors with MGUS is abnormal, with reduced proportion and absolute numbers of OKT4+ and normal proportion but reduced numbers of OKT8+ cells (Table 1). This observation has been interpreted as suggesting enhanced suppressor T cell function coupled with reduced helper function, although some functional studies indicate a reverse pattern. We were unable to detect any correlation between stage of disease and quantitative T cell abnormalities (data not shown). Like some, and unlike others, we could not delineate any features of the T cell population to distinguish between patients with multiple myeloma and those with MGUS. In order to analyze and describe the T lymphocytes in PBLs of myeloma patients more accurately, we have utilized technology that permits cloning of 100% of the T cells in an aliquot of PBLs. Clonal analysis of the T cells from normal individuals occurred with an efficiency of 1.0 for both OKT4+ and OKT8+ T cells, which in five to six weeks produced homogeneous clones of OKT4+ or OKT8+ progeny and a small proportion of homogeneous OKT48+ clones as predicted by Kutlaca et al. Unlike their precursors, the progeny T cells had strong surface expression of both HLA.DR and BA-2 antigens. Progeny cells did not express B cell markers such as surface IgM, K, or λ, 41H.16, or BA-1. In contrast, T lymphocytes from most multiple myeloma patients, cloned under identical conditions to those used for normal PBL, had a reduced cloning efficiency and an unexpected distribution of T cell subsets within the clonal progeny. In four of seven patients, 70% to 86% of the T cell clones were OKT48+ even though the PBL T cells originally cultured were only 38% to 66% OKT4+. This appears to be due to a very low efficiency of cloning within the OKT8+ subset. However, for patient 053, the distribution of OKT4+ or OKT8+ clones was exactly that predicted from the distribution of OKT4+ and OKT8+ cells in the PBLs cultured, probably because the clonogenic potential of both subsets was abnormally low.

Table 6. Expression of BA-2 on Freshly Isolated PBLs: Double Immunofluorescence Studies

<table>
<thead>
<tr>
<th>No. of Samples</th>
<th>No. of Individuals Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT4+</td>
<td>OKT8+</td>
</tr>
<tr>
<td>BA-2+</td>
<td>BA-2−</td>
</tr>
<tr>
<td>BA-2+</td>
<td>BA-2−</td>
</tr>
</tbody>
</table>

PBLs, peripheral blood lymphocytes; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma.

*The two individuals with BA-2+ T cells expressed this phenotype in a transient manner. In samples taken from the same individuals one month later, no BA-2+ T cells were detected.
the IF assay could give rise to T cell clones. Because, unlike those in normal subjects, the number of T cells in patient PBLs was usually <50%, one cannot with certainty say that cells bearing OKT4 or OKT8 are the same subset as those with clonogenic potential. Analysis of two patients, both with very low numbers of T cells in PBLs, suggests that abnormalities do exist in the clonal progenitor populations from patients. In both patients 138 and 144, two to three times as many OKT4* clones were observed than were expected. For patient 144, twice as many OKT8* clones were observed than were expected. In addition, the number of PBLs able to give rise to a clone was twofold to threefold greater than the number expressing OKT4 or OKT8 markers. This indicates that in some patients with abnormally low numbers of T cells there exists a population of OKT4* - OKT8* progenitors that give rise to OKT4*, OKT8*, or OKT4* OKT8* clones in vitro.

These observations raise serious questions concerning the assumption that T cells typed as OKT4* or OKT8* actually give rise to any of the clonal progeny analyzed from the multiple myeloma patients. In most patients, unlike in normal subjects, only 16% to 28% of total PBLs generate a clone. Thus, it is possible that is most such patients, neither OKT4* nor OKT8* cells grow. The existence in two patients of substantial numbers of OKT3* OKT4* OKT8* lymphocytes (Table 4) lends support to this interpretation. Double IF and cell-sorting experiments will be required to define this unexpected population further. The precursors of clones from patients may be OKT4* OKT8* cells, for the most part predisposed to the OKT4 pathway, that are unable to differentiate in vivo but are fully able to do so in vitro. The reasons for their existence in PBLs from patient donors but not normal donors could be complex and multifaceted. A precedent for this hypothesis derives from the work of Ozer et al. who suggested a defect in T suppressor precursors in multiple myeloma based on analyses of immune function. Our observations may also provide an explanation for the apparent paradox that patients with reduced numbers of OKT4* cells in PBLs have apparently normal helper function in vitro. Cloning experiments using T cells sorted into OKT4*, OKT8* or OKT4* OKT8* subpopulations are planned to test this hypothesis.

Both the OKT4* and OKT8* populations of clones are heterogeneous in their expression of BA-2. Clones were either BA-2* or BA-2++. Similar heterogeneity, although transient, has been observed in PBL T cells of two patients, indicating that BA-2 is a valuable marker for detection of qualitative changes in patient T cells and particularly in their clonal progeny.

The observations reported here indicate that purely quantitative descriptions of the T cell populations in PBLs have limited power to distinguish T cell abnormalities and may in fact be misleading. Although multiple myeloma patients have a normal proportion of OKT8* T cells, these cells are qualitatively defective in their ability to generate clonal progeny in vitro under conditions in which normal OKT8* T cells very efficiently give rise to clones. Furthermore, T cell clones arise from PBLs with undetectable levels of T cell markers. Experiments are in progress to determine whether OKT4* patient T cells are similarly defective and if OKT4*, and possibly OKT8*, T cell differentiation is reversibly arrested in vivo at an OKT4* OKT8* but otherwise undefined progenitor stage. Although other interpretations are possible, we favor the hypothesis that differentiation of T cell progenitors is controlled by an immunoregulatory differentiation arrest analogous to that described by us for both pre-B cells and B cells in PBLs of multiple myeloma patients.

ACKNOWLEDGMENT

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