Myelomonocytic Antigen Positive Multiple Myeloma

By Thomas M. Grogan, Brian G.M. Durie, Catherine M. Spier, Lynne Richter, and Elizabeth Vela

In a four year span, between 1983 and 1987, 215 bone marrow and cell culture samples from 125 myeloma patients were immunotyped and coexpression of myelomonocytic and plasma cell antigens occurred in 16 (13%). We employed both immunohistochemical and flow cytometry methods including coplots and double labelling. Three types of myeloma cases were found: (1) those with isolated myeloid antigen coexpression, usually Leu M1 or esterase (BE, CE) positive (11 cases); (2) those with multiple myeloid antigens (Leu M1, M3, M5, MY7, BE, CE) (four cases); and (3) one case beginning as 1 and ending as 2. Isolated myeloid antigen expression was generally associated with typical features of myeloma with survival close to the anticipated median (33 months), while multiple myeloid antigen expression was associated with more aggressive disease and shorter survival duration (median survival 16 months). The latter subgroup also had other poor prognostic factors including high labelling index and common acute lymphoblastic leukemia antigen (CALLA) positivity. Other features found overall were frequent abnormal karyotypes (seven of 12 abnormal) and coexpressed IgA (eight of 16); all IgA+ cases also coexpressed Leu M1. We conclude that there is an unusual and unexpected predilection for coexpression of myelomonocytic antigens in myeloma cells. The reasons are not immediately obvious. Whether the coexpression indicates that myeloma cells truly have latent multilineage potential or just aberrantly coexpress other hematopoietic antigens as a manifestation of malignancy remains to be explained. However, a cell line established from the bone marrow of one patient is a valuable scientific tool allowing detailed analysis of these questions.

AS TERMINALLY DIFFERENTIATED B cells, plasma cells are characterized by expression of cytoplasmic immunoglobulin and surface plasma cell associated antigens including PC-1,1 PCA-1,2 Leu 17,3 and OKT10.4 Plasma cell myeloma as a malignancy of plasma cells is known to have comparable plasma cell antigen and immunoglobulin expression, although the latter occurs in a monoclonal fashion.5,6 Occasionally malignant plasma cells are known to manifest aberrant B-cell antigen expression.5,7 Specifically, they may coexpress common acute lymphoblastic leukemia antigen (CALLA), Tdt, and other immature B-cell antigens in the form of novel pre-B-cell hybrid phenotypes sometimes referred to as CALLA positive myeloma.7,8 The latter phenomenon portends a poor prognosis9,10 and is thought to reflect aberrant gene expression in a pluripotential stem cell.7,9 In this study we document the occurrence of unusual hybrid myelomonocytic plasma cell phenotypes in certain myeloma cases. These aberrant phenotypes indicate that “lineage infidelities” as found in other hematopoietic malignancies11,12 may occur in myeloma. Since some of these aberrant myelomonocytic phenotypes occurred prior to therapy these phenomena appear to be part of the natural history of plasma cell neoplasia.12-20

MATERIALS AND METHODS

Patient and Tissue Selection

Between 1983 and 1987 we immunotyped 215 bone marrow and cell culture samples from 125 myeloma patients. There were 16 patients (13%) whose myeloma cells coexpressed myelomonocytic-plasma cell antigens. We used both immunohistochemical and flow cytometry methods including coplots and double labelling to identify this myeloma subpopulation. In 13 cases we studied direct marrow material; in three cases (cases nos 10, 13, and 16) studies were performed on both initial direct marrow myeloma and subsequent cell culture material. Material studied using both immunoperoxidase and immunofluorescence methods included cell suspensions, cytopsin preparations, narrow snap-frozen clot sections, and core biopsy sections.

Immunologic Marker Studies

Immunoperoxidase typing. We used snap-frozen marrow clot section and core biopsies as well as cytocentrifuge (Shandon Instruments, Sewickley, PA) slide preparations of the myeloma cell material for immunologic evaluation as previously reported.5,7 Biotin-avidin conjugates and horseradish peroxidase labeled with diaminobenzidine tetrahydrochloride (DAB) as the detection agent were used as before.7,9

Cytocentrifuge slides were fixed in acetone at 4°C for ten minutes and air-dried at room temperature. Mouse monoclonal antibody (MoAb) was applied for 30 minutes to detect human cell surface (and/or cytoplasmic) antigen. Mouse ascitic fluid (Bethesda Research Laboratories, Gaithersburg, MD) was applied to one slide in each run instead of the primary antibody as a negative control.

The first stage included MoAbs to the following B-cell antigens (cluster designations [CD] follow in brackets): B1 [CD20], B2 [CD21], B4 [CD19] (Coulter Immunology, Hialeah, FL); Leu 12 [CD19], Leu 14 [CD22] (Becton Dickinson, Mountain View, CA); immunoglobulins (kappa, lambda, mu, gamma, delta, and alpha; Becton Dickinson); plasma cell antigens: PCA-1 (Coulter); PC-1 (kindly supplied by Drs Kenneth Anderson and Lee Nadler, Dana-Farber Cancer Institute, Boston); Leu 17 (Becton Dickinson); CALLA [CD10] and HLA-DR (Ia), T-cell antigens Leu 1-9 [CD1-5,7,8], Human progenitor cell antigen (HP), transferrin receptor (TRF), myelomonocytic antigens, (LM1 [CD15], LM3, LM5) (all obtained from Becton Dickinson) and myeloid antigen, MY7 (Coulter). Anti-interleukin-2 (anti-IL-2) [CD25] receptor MoAb was obtained from Coulter. Anti-leukocyte common antigen (LC) was obtained from Dakopatts, Copenhagen.

Flow Cytometric Analysis

Flow cytometry. Flow cytometry was conducted with fluorescent antibodies analyzed by a Coulter EPICS V flow cytometer (EPICS Division, Coulter Immunology, Hialeah, FL) as previously described.
described. Data obtained by flow cytometry were analyzed by using MDADS (Multiparameter Data Acquisition and Display System) “Immuno” program; the coplot display of composite histograms was created by using the EASY 88 (Extended Analysis System with Intel 80/88 Microprocessor) analysis system.

**Indirect immunofluorescence.** With a previously described method, antigens were detected by flow cytometric analysis using indirect immunofluorescence (IF). Briefly, cultured myeloma cells were washed in RPMI medium; trypan blue dye exclusion was used to establish viability. Before staining, the cells were divided into two aliquots, and one was incubated at 37°C with blocking serum (10% rabbit serum). The myeloma cells were then incubated with the same battery of MoAbs as before for 30 minutes at 4°C. Then a second-stage goat antimouse IgG fluorescein isothiocyanate (FITC) conjugate was applied and incubated as above. After cold phosphate-buffered saline (PBS) washing the cells were kept on ice in the dark and analyzed immediately.

**Two-color direct immunofluorescence (double-labelling).** A modification of the indirect immunofluorescent technique was used for two-color direct fluorescence (DF) study. The myeloma cells were prepared the same as for the IF study. The two aliquots of cells, those that had been preincubated (incubated at 37°C and in 10% normal rabbit serum) and those that had not, were then stained. The combination of antibodies used depended on both the antigens present on the cells and the availability of directly conjugated antibodies. For the experiment illustrated in Fig 3 (case no 15) the following combinations were used: Leu 3-FITC/MY7-PE; B4-FITC/MY7-PE; B4-FITC/LM3-PE; Leu 3-FITC/LM3-PE; Leu 3-FITC/B4-PE. Controls consisted of mouse IgG-FITC/mouse IgG-PE. The cells were added to the tubes that already contained the appropriate antibody combinations as well as PBS, and were then incubated for 30 minutes at 4°C in the dark. They were then washed in cold PBS, the PBS aspirated, and fresh cold PBS added. The cells were not fixed, but were kept on ice in the dark and analyzed immediately. The subtraction was set so there was no crossover of the phycocerythrin (PE) signal into the FITC range; the control cells were run, and cursors were placed so that there was less than 5% double staining.

**Cytochemical Studies**

Marrow clot sections and cytopsin preparations of marrow aspirates were assayed for myeloid and monocytic enzyme activity using the following substrates: chloroacetate (CE) and butyrate esterase (BE), using standard cytochemical methods.

**Cell Culture Methods**

In vitro colony culture of myeloma cells was performed as previously reported. In addition, a more extended liquid culture was carried out with a new M-3 medium. After 3 months, cell cultures were well established and could be maintained using M-3 supplemented with fetal calf serum (FCS). Aliquots from each passage were frozen for later study.

**Labeling Index and Proliferative Index**

The labeling index (LI) was performed by using previously described one-hour “flash labeling” with [3H] thymidine. The proliferative index represented the percentage of cells reacting with the MoAb Ki-67 (DAKO, Dakopatts). Ki-67 identifies cells throughout the cell growth cycle and not resting cells.

**Stimulation Studies with Phorbol Ester**

Case no 10 was stimulated with phorbol ester 12-0-tetradecanoyl-phorbol-13-acetate (TPA) by using the methods and controls described by Caliganis-Cappio et al. The TPA was dissolved first in acetone and then absolute alcohol and used in the cultures at a concentration of 1.6 × 10⁻⁸ mol/L. Control and TPA-induced cells were harvested after 72 hours, assessed for viability, and then phenotyped by using biotin-avidin-horseradish peroxidase (BA-HRP) methods as detailed earlier.

**RESULTS**

Table 1 summarizes the basic clinical and laboratory features of our 16 myeloma patients coexpressing myelomonocytic and plasma cell antigens. Among the 16 patients were eight males and eight females. Their median age was 60 years (range 36 to 75 years). A monoclonal gammopathy in the form of a serum spike occurred in all 16 (with IgA expression in eight, IgG in five, IgM in one, and no heavy chain in two. Twelve patients had kappa and four lambda light chain gammapathies. Multiple lytic lesions were initially readily found in 13 of 16. Marrow plasmacytosis ranged from 14% to 99% (median = 54%). Classic plasma cell morphology with varying degrees of nuclear/cytoplasmic dysynchrony and atypia characterized the cases (see Fig 1). Three cases were morphologically distinct variants: case no 5, phagocytic myeloma with phagocytosis of normoblasts; case no 8: anaplastic myeloma; case no 15: plasmablastic myeloma. Overt leukemia was absent in all but case no 10 (which manifested as plasma cell leukemia) and case no 15 (a plasmablastic myeloma with subsequent hybrid leukemia). Staging established all but one patient as stage III.

As revealed further in Table 1, two groupings may be established based on phenotype. Group A is comprised of patients with one or two myelomonocytic antigens. Group B is comprised of patients with multiple (three or more) myelomonocytic antigens. Patient no 16 began in group A (direct marrow material) and transformed to group B (culture material) (Table 2). Group A patients have a median age of 61 and a median survival of 33 months. Group B patients have a median age of 45 and a median survival of 16 months. The mean labeling index (%) for group A is 1.8% and for group B is 17.8%, indicating the higher proliferative status of group B. Karyotypic abnormalities are found in 64% of group A and 50% of group B cases.

Phenotypic data revealing coexpression of plasma cell antigens (cytoplasmic immunoglobulin, Leu 17, PC, PCA-1) and myelomonocytic antigens (Leu M1, M3, M5, MY7, BE, and CE) are illustrated in Fig 1 (case no 14) and summarized in Table 2. Extensive flow cytometry assessment of case nos 13 and 15 using both co-plots and double labeling revealed coexpression of plasma cell, myelomonocytic, and some immature B-cell antigens in the myeloma cells (Figs 2, 3).

As revealed in Fig 3 (case no 15), faint cross-reactivity with multiple monoclonal antibodies suggested Fc receptor expression as commonly found in monocytes, not plasma cells. Incubation with 10% rabbit serum reduced these faint cross reactions reinforcing the inference that Fc receptors were present. In four cases overt phagocytic activity was found: case no 5, phagocytosis of normoblasts, case nos 13 to 15 phagocytosis of immunoglobulin was suggested. As reported separately in our companion manuscript, genotypic
Table 1. Myelomonocytic Myeloma Clinical Features

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Ig Paraprotein</th>
<th>Serum</th>
<th>Urine</th>
<th>Lytic Bone Lesions</th>
<th>Plasmaclotosis (%)</th>
<th>Myeloid Antigens on Plasma Cells</th>
<th>Prior Tx</th>
<th>LI (%)</th>
<th>Karyotype</th>
<th>Status</th>
<th>Survival†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>56 M</td>
<td>IgAα</td>
<td>+</td>
<td>+</td>
<td>31</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±</td>
<td>+</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>39 F</td>
<td>IgAα</td>
<td>+</td>
<td>+</td>
<td>49</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±, MY7±</td>
<td>+</td>
<td>3.5</td>
</tr>
<tr>
<td>3</td>
<td>60 F</td>
<td>IgAα</td>
<td>+</td>
<td>+</td>
<td>34</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>71 F</td>
<td>IgAα</td>
<td>+</td>
<td>+</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±, MY7±</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>5*</td>
<td>69 M</td>
<td>IgAα</td>
<td>+</td>
<td>+</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>70 M</td>
<td>IgAα</td>
<td>+</td>
<td>0</td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±</td>
<td>+</td>
<td>0.4</td>
</tr>
<tr>
<td>7</td>
<td>75 M</td>
<td>IgGα</td>
<td>+</td>
<td>0</td>
<td>86</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>Be± hof</td>
<td>+</td>
<td>5</td>
</tr>
<tr>
<td>8*</td>
<td>55 F</td>
<td>IgGα</td>
<td>+</td>
<td>+</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>BE diffuse</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>61 M</td>
<td>IgGα</td>
<td>+</td>
<td>0</td>
<td>37</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>Be± hof</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td>10*</td>
<td>67 M</td>
<td>IgMα</td>
<td>+</td>
<td>+</td>
<td>54</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M5±</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>11</td>
<td>60 M</td>
<td>k</td>
<td>+</td>
<td>+</td>
<td>68</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>52 M</td>
<td>IgAα</td>
<td>+</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±, M5±, MY7±, BE±</td>
<td>+</td>
<td>0.3</td>
</tr>
<tr>
<td>13</td>
<td>45 F</td>
<td>IgAα</td>
<td>+</td>
<td>+</td>
<td>62</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±, M5±, MY7±, BE±, CE±</td>
<td>+</td>
<td>4</td>
</tr>
<tr>
<td>14</td>
<td>44 F</td>
<td>IgAα</td>
<td>+</td>
<td>+</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M3±, M5±, MY7±, BE±, CE±</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>15*</td>
<td>67 F</td>
<td>k</td>
<td>+</td>
<td>0</td>
<td>99</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±, M3±, M5±, MY7±, BE±, CE±</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>16</td>
<td>36 F</td>
<td>IgGα</td>
<td>+</td>
<td>+</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>III</td>
<td>M1±, MY7±, BE±, CE±</td>
<td>0</td>
<td>55</td>
</tr>
</tbody>
</table>

*Case no. 5: phagocytosis; case no. 8: anaplastic myeloma; case no. 10: plasma cell leukemia; case no. 15: plasmablastic myeloma with subsequent hybrid leukemia.
†Total months (months from relapse).
analysis of one of the latter cases (case no 13) confirmed true genotypic monotypia as evidenced by immunoglobulin gene rearrangement. As shown in case no 15, some of our cases also expressed T-helper and immature B-cell antigens (eg, CALLA), markers previously found at a low level of monocytcs. Some cases also coexpressed HPCA, leukocyte common (LC) antigen and Ia indicating the transition to myelomonocytic phenotype was systematic, not an isolated phenotypic change.

Two cases underwent phenotypic alteration with manipulation. Case no 10 acquired Leu M5 coexpression in conjugation with TPA stimulation in culture. Case no 16 had initial isolated BE expression (16A in Table 2). Cell culture of this same specimen revealed initially an identical phenotype (not shown). Subsequent passages (16B, C in Table 2) revealed transformation to a multiple myelomonocytic (Group B) profile.

DISCUSSION

In this study we document the occurrence of unusual hybrid myelomonocytic/plasma cell phenotypes in myeloma cells. The aberrant phenotype with shared antigens from two separate lineages on one cell type, as established by co-plots and double labeling, suggest a phenotypic “platypus.” Myelomonocytic myeloma appears not as a simultaneous proliferation of two separate populations but as one hybrid population.

Myeloid or monocytic-histiocytic antigen coexpression has not previously been described in normal plasma cells so that our myelomonocytic myeloma cells do not appear derived from a normal B cell counterpart. These myeloma cells appear to be malignant cells with aberrant disorganized gene expression with novel phenotypes having no normal equivalent. Recently at least two other B-cell neoplasms (hairst cell leukemia, a malignancy of pre-plasma cells, and some cases of chronic lymphocytic leukemia) have been found to coexpress myelomonocytic antigens (eg, PCA-1, MY7, and MO1) indicating that the phenomenon we describe has a pathologic counterpart. Our previously published finding of a novel hybrid of immature B and plasma cell antigens in myeloma further corroborates our suggestion that some myelomas may eventuate from aberrant gene expression. Alternatively, hybrid myelomonocytic-plasma cell phenotypes may be found in rare normal plasma cells, which in malignancy becomes an abnormally expanded compartment.

The spontaneous occurrence of myelomonocytic phenotypes before therapy in some cases indicates myeloid coexpression may naturally occur in myeloma cells independent of therapy, in agreement with the clinical observation of Bergsagel and others. Our TPA findings in case no 10 reveal that the myelomonocytic antigens may sometimes be chemically induced on myeloma cells in culture. The latter finding is in concert with the recent induction of monocyte-macrophage–like features on cultured myeloma cells using 1,25-Dihydroxyvitamin-D3, which is known to modulate differentiation of myeloid leukemic cells. Both studies emphasize the latent potential for myelomonocytic differentiation in myeloma cells. The possibility exists that TPA and Vitamin D3 could modulate aberrant gene expression. The latent potential for myeloid/histocytic differentiation in myeloma cells is further evidenced by our case no 16. This case transformed from isolated monocytic antigen coexpression to subsequent multiple myeloid antigen expression. This reveals sequential evolutionary phenotypic change over time as a property of malignant plasma cells. The demonstration of simultaneous myeloid and monocyte capacities in myeloma cells suggests the concept of sequential lineage commitment during hematopoiesis rather than a stochastic model of development.

In some myeloma cases, the transition to monocytic-histiocytic differentiation was so complete as to include not only primary antigens LM1, M3, M5, and BE but also functional surface markers like Fc receptors and functional activities like phagocytosis. Fc receptors as commonly found on monocytes were found in abundance in several of our cases (case nos 12 to 15). These receptors frequently resulted in faint cross-reactivity with mononuclear antibodies of
### Table 2. Myelomonocytic Phenotypes

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α</td>
<td>λ</td>
<td>μ</td>
<td>γ</td>
<td>α</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>0</td>
<td>*</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>±</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

**KEY:** +, positive; 0, negative; ±, weak; R, rare; *+, + in hof; Δ = positives changed to neg after incubation; *, some cells coexpress mu; †, Ig phagocytosis; ±, early cultures identical to 16a; later cultures b, c changed.
In some cases the myelomonocytic obscurred detection of Ig expression as shown in Table 2 (case
serum reduced Fc receptor interference allowing detection of numerous lineages creating detection problems. Blocking
antigen expression at presentation was more frequently
expression while B4 was largely lost with the addition of blocking
expression while B4 was largely lost with the addition of blocking
serum suggesting cross-reactivity due to presumed Fc receptor
serum suggesting cross-reactivity due to presumed Fc receptor
expected of plasma cells, all cases tested expressed cytoplasmic immunoglobulin, Leu 17, and PC-1.5,6,7 PCA-1 was
variable expressed as previously described in myeloma. The
variable expressed as previously described in myeloma. The
phenotype.35 In some cases the myelomonocytic obscured detection of Ig expression as shown in Table 2 (case
myelomonocytic hybrids coexpressed IgA (50%) at a much
myelomonocytic hybrids coexpressed IgA (50%) at a much
myelomonicytic myelomas coexpressed B-cell antigens (eg, CALLA, B1, B4, Leu 12) not normally found on
myelomonicytic myelomas coexpressed B-cell antigens (eg, CALLA, B1, B4, Leu 12) not normally found on
some myeloid (MY7) and myelomonocytic antigens (Leu M1).

Besides the myelomonocytic antigens, several aspects of
Besides the myelomonocytic antigens, several aspects of
Besides the myelomonocytic antigens, several aspects of

Expected median survival of 30 months in stage III in these
stage III in these

Some of our cases also coexpressed T-helper antigen
Some of our cases also coexpressed T-helper antigen

Fig 2. Flow cytometry coplots in case no 13 revealing coexpressed plasma cell antigens (PC, PCA-1), some B-cell-antigens (B1) some myeloid (MY7) and myelomonocytic antigens (Leu M1). Note absent B2 (complement receptor) and negative control. Immunofluorescence histograms.

Fig 3. Flow cytometry double labelling with blocking serum incubation to evaluate cross-reactivity due to presumed Fc receptors. As shown, simultaneous expression of MY7 and Leu 3 persisted with blocking serum incubation suggesting true coexpression while B4 was largely lost with the addition of blocking serum suggesting cross-reactivity due to possible Fc receptor effect. Case no 15.

ACKNOWLEDGMENT

We acknowledge the constant support of Dr Jack M. Layton.

REFERENCES
2. Anderson KC, Park EK, Bates MP, Leonard RCF, Hardy R,


Myelomonocytic antigen positive multiple myeloma

TM Grogan, BG Durie, CM Spier, L Richter and E Vela