Cytogenetic Abnormalities in a Plasmacytoma

By Sergio Mancinelli, John R. Durant, and William J. Hammack

TWO SOMEWHAT CONSISTENT cytogenic changes in patients with multiple myeloma have recently been reported. Houston et al.,1 using phytohemagglutinin (PHA) stimulated peripheral blood cells, observed abnormal chromosomes in fifteen of twenty-four patients. The abnormal chromosomes were as large or larger than normal A group members with centromeric positions ranging from subtelocentric to submedian. These changes were usually but not invariably supernumerary and were present in a minority of cells examined. Because this abnormality was found in both myeloma and macroglobulinemia, Houston et al.1 termed this the MG (monoclonal gammapathy) chromosome. Tassoni et al.2 studied the chromosomes in the bone marrow of fourteen patients with multiple myeloma. In five patients, they found, in a small percentage of cells, an acrocentric marker approximately the size of the long arms of the B group. This marker was also probably present in the bone marrow cells of two additional patients and was definitely present in the pleural fluid of a fifteenth patient whose effusion was proven to be due to myeloma. One patient had a few bone marrow cells with an abnormal chromosome similar in appearance to that reported by Houston et al.1 Numerous other nonspecific abnormalities have been noted, as recently reviewed by Das and Aikat.3

The significance of these markers is uncertain. The appearance of a marker in a small percentage of phytohemagglutinin sensitive circulating cells in a nonleukemic myeloma patient is surprising, especially in view of the usually normal marrow studies. It might be postulated that the abnormal cells were lymphocytic appearing circulating tumor cells or abnormal lymphoid precursors of myeloma cells. The general absence of the MG chromosome in marrow preparations could be due to dilution with more rapidly dividing normal myeloid elements, as suggested by Tassoni et al.2 to explain the low percentage of marker positive cells in their patients. Neither of these studies provided data indicating whether myeloma cells from marker positive patients usually, often, or rarely contained the abnormalities reported.

The purpose of this report is to outline the cytogenetic abnormalities obtained from a patient with diffuse myelomatosis and a solid plasmacytoma. The studies performed on the tumor disclosed the presence of both types of

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marker in many cells and one or the other of them in almost three-fourths of the cells. This represents the first such report known to us and strongly suggests that the abnormalities reported by both Houston et al. and Tassoni et al. are actually present in myeloma cells.

CASE REPORT

Mrs. G. C., A fifty-five year old white female first developed back pain in early 1965. An examination by her physician and extensive x-ray studies failed to reveal a cause, and the pain subsided. It reappeared suddenly in January 1966, requiring admission to the University Hospital. Investigation at that time revealed a slight anemia with a hematocrit of 37 and extensive lytic lesions of the sacrum, ileum, right clavicle, skull, and possibly the left femur. Marked proteinuria was noted, but the heat and acetic acid test for Bence Jones protein was negative. A serum protein electrophoresis revealed a slightly decreased albumin and definite hypogammaglobulinemia (0.58 Gm. per cent). A biopsy of the clavicular lesion was diagnostic of plasmacytic myeloma. Electrophoresis of the urine revealed a homogeneous peak in the region of the beta globulin. Immunelectrophoresis indicated that the proteinuria was due to kappa type light chains (Bence Jones proteinuria).

Shortly after admission the patient developed hypercalcemia and mild uremia. She was treated with prednisone, phenylalanine mustard, and repository testosterone (Delatestryl) after which she gradually improved and was able to be discharged. Her response to treatment was excellent with decrease in pain, and increase of the serum gamma globulin to normal, and the disappearance of Bence Jones proteinuria. The lesions noted on x-ray, however, progressed.

In May 1967 she had to be readmitted to the hospital because of severe bone pain. Bone marrow examination was normal, but hypogammaglobulinemia was again evident. There was only a trace of proteinuria. A repeat course of phenylalanine mustard and prednisone failed to improve the pain, and it became necessary to give her radiation therapy. Over the course of nineteen days she received a tissue dose of 2240 rads (Cobalt60) to a portal including the fourth and fifth lumbar vertebrae, sacrum, and medial aspects of the iliac bones. At the same time a lesion in the head of the humerus was treated with a tissue dose of 3500 rads (Cobalt60). Low back pain persisted. In September 1967 an additional 3000 rads were administered to this area, relieving the pain. Follow-up x-ray studies showed that the lesion in the humerus had undergone considerable healing.

In February 1968, the patient complained of a soft tissue mass on the upper portion of the left arm, but outside of the previously irradiated area. A biopsy of this mass revealed a plasmacytoma (Fig. 1). A bone marrow was again within normal limits. Peripheral blood studies at this time revealed a hemoglobin of 9.2 Gm. per cent, a white count of 2600, and platelets of 237,000. The differential included 11 per cent lymphocytes, some of which had plasmacytoid characteristics. The urine contained a trace of Bence Jones protein, but the serum protein electrophoresis was normal. The cytogenetic studies were performed on material obtained during this admission.

MATERIALS AND METHODS

Cytogenetic analysis was performed on three different types of cells, tumor, bone marrow, and peripheral blood. The bone marrow was prepared using the method of Tijo and Whang with slight modifications. The peripheral blood was cultured in the presence of phytohemagglutinin using Difco's TC-microtest kit. After three days colcemid was added to produce metaphase arrest, and the slides prepared according to the method of Moorhead et al. Tumor cells were obtained under sterile conditions by mincing approximately 2 cu. mm. of tumor tissue, suspending the minced tumor in 20 per cent fetal calf serum and Medium 199, and allowing this preparation to stand at room temperature for three minutes. After this time the supernatant was removed and cultured for twenty-four hours at 37 C. and then treated in the same way as peripheral blood. All slides were stained with Giemsa.

The metaphase plates thus obtained were all sketched and scored visually. Approximately
20 per cent of these were photographed and karyotyped directly in order to check on the accuracy of the visual scoring.

RESULTS

The karyograms obtained from the tumor, peripheral blood, and bone marrow are shown in Table 1. Both peripheral blood and tumor cells yielded many good metaphase plates, but the direct bone marrow preparation had only twenty-six metaphase plates suitable for analysis. Hypodiploidy was a prominent feature of many cells in the peripheral blood and tumor, but the bone marrow was essentially normal.

Analysis (Table 2) of the forty-nine metaphase plates obtained from the tumor showed the most common finding to be absence of one or more D group chromosomes. This abnormality was present in all forty-eight of the aneuploid cells. Thirty-eight of these cells contained an abnormal acrocentric marker (Fig. 2) similar to the one reported by Tassoni et al.² Twenty-three

Table 1.—Karyograms of Plasmacytoma, Peripheral Blood, and Bone Marrow in G. C. Hypodiploidy of the Tumor and Peripheral Blood are Evident.

<table>
<thead>
<tr>
<th>Cells Karyotyped of Cells</th>
<th>Total Number</th>
<th>40</th>
<th>41</th>
<th>42</th>
<th>43</th>
<th>44</th>
<th>45</th>
<th>46</th>
<th>47</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td></td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>14</td>
<td>12</td>
<td>4</td>
<td>—</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>15</td>
<td>27</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Bone</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Marrow</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
</tbody>
</table>

Fig. 1.—Section of plasmacytoma. (H & E × 480).
Table 2.—Major Cytogenetic Abnormalities Noted in Plasmacytoma and Peripheral Blood of G. C. The most frequent is a missing D group chromosome, noted in all aneuploid cells in the tumor but not the peripheral blood. The frequency of the acrocentric marker was much higher in the tumor than the peripheral blood where the MG-like marker was more common.

<table>
<thead>
<tr>
<th>Source of Material</th>
<th>Number of Cells</th>
<th>Aneuploid Cells</th>
<th>Cells with Missing D Group Chromosome</th>
<th>Cells with Acrocentric Marker</th>
<th>Cells with Missing A Group Chromosome</th>
<th>Cells with MG-Like Marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUMOR</td>
<td>49</td>
<td>48</td>
<td>48</td>
<td>38</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>BLOOD</td>
<td>54</td>
<td>27</td>
<td>8</td>
<td>5</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>MARROW</td>
<td>26</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2.—Karyotype from a hypodiploid (43) tumor cell showing a typical acrocentric marker in the top middle of the metaphase plate on the upper left. It is labeled "AM" in the karyotype where it appears in the upper right hand corner. 2 D group chromosomes are missing as well as single members of the A.C.E. & G groups. Except for the missing D, the other changes are considered to be random.

of the aneuploid cells were missing at least one member from the A group, and nineteen of these contained a marker morphologically similar to the MG chromosome (Fig. 3). Eighteen of the forty-eight abnormal cells contained all four of these abnormalities (Fig. 4). Findings in the peripheral blood were similar, although some of the abnormal cells were pseudodiploid. There were eight cells in which one or more D group chromosomes were absent. Five of these contained an abnormally large acrocentric marker. Three of these five cells also contained an MG-like chromosome and were missing a member of the A group as well. Three additional cells contained an MG-like chromosome. All cells with the MG-like chromosome were missing at least
Fig. 3.—Karyotype from a pseudodiploid peripheral blood cell. The large MG-like chromosome is seen at 12 o'clock in the metaphase plate in the upper left hand corner and is designated MG in the upper right hand corner of the karyotype. A member of pair #1 is missing. There is a missing #16. An extra chromosome most like an F group member is present. The latter two changes are considered to be random.

One member of the A group. Thus, eight of fifty-four peripheral blood cells examined contained a marker similar to those seen in tumor cells. In all marker-positive tumor and peripheral blood cells, the same relationship between the markers and missing chromosomes were present. Whenever the acrocentric marker was found, there was at least one absent D group member. Whenever the MG-like chromosome was present, at least one A group member was absent. When both markers were present, there was always a missing member from both the A and D groups. Random additional cells in the tumor and peripheral blood demonstrated missing chromosomes and various additional markers. With the exception of the absence of a D group chromosome, present in every aneuploid tumor cell examined, these random losses are assumed to be technical and of no meaning.

Discussion

There is no doubt that the cytogenetic abnormalities found in the solid plasmacytoma in this patient were actually in myeloma cells. Seventy per cent of the cells studied contained an acrocentric marker similar to the one reported in myeloma patients by Tassoni et al., and 39 per cent contained one similar to that described in the report by Houston et al. Many of the cells contained both markers. Cells similar to these were found in the peripheral
Fig. 4.—Karyotype from a hypodiploid tumor cell (42). The acrocentric marker can be seen at 7 o'clock in the metaphase plate in the upper left hand corner. The MG-like marker is at 1 o'clock in the middle of the plate. These are designated AM & MG respectively in the karyotype. There are 2 missing A group members and a missing D chromosome. In addition there is an extra chromosome ("M" in the upper left hand corner). Missing C, E, & F members are also noted. These latter changes are considered to be random.

blood, suggesting that, in this patient, there were circulating tumor cells in the absence of obvious marrow involvement.

Whether the markers reported in this patient are similar in origin to those reported by Houston et al.,1 and by Tassoni et al.,2 is open to conjecture. Radioautographic studies in this patient were unsuccessful, and none have been reported in other marker-positive patients. The most likely explanation for the origin of the "marker" appears to be translocation from some other chromosome to a normal D group member, producing a giant acrocentric. The association of a missing normal acrocentric with the acrocentric marker was noted by Tassoni et al.,2 so that it is possible that the origin of the marker in their patients was similar to that postulated for this patient. Neither our studies nor those of Tassoni et al.,2 provide information as to the origin of the translocated material. In the case of the MG marker, Houston et al.,1 believed the likeliest explanation for its origin was that of Patau6 for the similar chromosome reported by others in Waldenström's Macroglobulinemia.7,8 He suggested that pericentric inversion and mitotic nondisjunction led to the appearance of an isochromosome, the marker. He further suggested that hypodiploid cells with a missing A group chromosome would be nonviable, leaving only hyperdiploid progeny of the original abnormal cell. Our findings clearly suggest that the MG-like chromosome in our patient arose from a
### Table 3.—Abnormalities in Karyotyped Cells

<table>
<thead>
<tr>
<th>Cells Karyotyped</th>
<th>Tumor</th>
<th>Peripheral Blood</th>
<th>Marrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Chromosomes in</td>
<td>7</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>A</td>
<td>0</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>E</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>F</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>G</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Markers</td>
<td>Acro</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>MG</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Misc.</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Details of the cytogenetic abnormalities noted in karyotyped cells from the tumor, peripheral blood, and marrow. It can be noted that there are abnormalities in all the groups of chromosomes. The only consistent abnormalities were in the A and D groups (see text). N=normal number of chromosomes. –1=1 less than the normal diploid number for the group in question; +1=1 more, etc.

normal A group member. This could have resulted from pericentric inversion without mitotic nondisjunction resulting in a marker of approximately A group size but abnormal position of the centromere and five other normal A group members.

It is of interest that many of the abnormal cells in this patient contained both markers. Such an abnormality has been noted in myeloma previously. Smalley has noted a patient with myeloma and hypodiploid cells containing two markers, one acrocentric and one MG-like. In his case, both an A and D group chromosome were missing. Dubrova et al. reported on six cases. In two of these, abnormalities were found. D group chromosomes were missing in both patients, one of whom had markers similar to both the MG
and acrocentric ones. The patient with the MG-like chromosome had a normal A group similar to the patients of Houston et al. The other patient had a possible acrocentric marker but no MG and no missing A chromosome.

In summary, the karyotypic analysis of myeloma cases with the acrocentric and MG-like markers suggests an origin from the normal D group for the acrocentric marker and from an altered A group member for the MG-like marker.

The relationship of the cytogenetic abnormalities to myeloma is obscure. No association has been noted between them and the abnormal protein produced. Treatment is probably not responsible for the abnormalities since they have often been noted prior to therapy in other studies. The findings in this study suggest that abnormalities in the D group are sufficiently frequent to merit consideration as relatively early cytogenetic changes in the course of evolution of myeloma. Since some of the peripheral blood cells with the MG-like marker had perfectly normal D groups, it seems unlikely that a deletion of a D chromosome represents the initial change in myelomatous evolution. Furthermore, lack of specificity of a large acrocentric marker for myeloma should be noted. Morphologically similar markers, as noted by Tassoni et al. have been reported in ovarian and testicular tumors, as well as in Burkitt's lymphoma. A further understanding of the cytogenetics of myeloma awaits the successful application of radioautography to a number of the marker positive patients.

**SUMMARY**

Cytogenetic studies from a plasmacytoma, bone marrow, and peripheral blood of a patient with myelomatosis have been described. Studies on the plasmacytoma revealed a marker chromosome in 72 per cent of the cells. Ninety-eight per cent of these contained an abnormal acrocentric marker similar to that reported by Tassoni et al. and approximately 50 per cent of the marker-positive cells contained an MG-like chromosome. The origin of the acrocentric marker appeared to involve a translocation from an unknown chromosome to a normal D group member, and that of the MG-like marker to involve pericentric inversion of a normal A group member.

The high percentage of cells from the tumor which contained the marker chromosomes makes it likely that these markers, described in low percentage in marrow or peripheral blood by others, actually were obtained from myeloma cells.

**SUMMARIO IN INTERLINGUA**

Es describite studios cytogenetic a base de specimens ab un plasmacytoma, le medulla ossee, e le sanguine peripheric de un paciente con myelomatosis. Studios del plasmacytoma revelava un chromosoma marcatori in 72 pro cento del cellulas. In 98 pro cento de istos, un anormal marcator acrocentric esseva trovate, simile a illo reportate per Tassoni et al., e aproximativamente 50 pro cento del cellulas marcatorio-positive contineva un chromosoma MG-simile. Le origine del marcator acrocentric pareva comportar un translocation ab un noncognoscite chromosoma ad un membro de gruppo D normal, durante que le origine del marcator MG-simile pareva comportar le inversion pericentric de un membro de gruppo A normal.
CYTOGENETIC ABNORMALITIES

Le alte procentage de cellulas plasmacytomal continente le chromosomas marcatori rende probabile que iste marcatores—describes per altere autores como occurrente in basse procentages in le medulla e le sanguine peripheric—esseva de facto obtenite ab cellulas myelomatose.

REFERENCES

Cytogenetic Abnormalities in a Plasmacytoma

SERGIO MANCINELLI, JOHN R. DURANT and WILLIAM J. HAMMACK