Chromosomal Aberrations Common to Three Types of Monoclonal Gammopathies

By ELSIE W. HOUSTON, STEPHAN E. RITZMANN AND WILLIAM C. LEVIN

BOTTURA ET AL.1 and German et al.,2 in 1961, described a unique chromosomal abnormality in a patient with primary macroglobulinemia (Waldenström). Similar findings have subsequently been reported in 11 patients.3-9 Two types of abnormal chromosomes have been recognized: a metacentric larger than any other in the cell2 and a submetacentric or subterminal1 in the AB size range or larger. In patients with myeloma, only scanty evidence of chromosomal abnormalities has been published. Bottura,10 Castoldi et al.,11 and Lewis et al.12,13 have described inconsistent aberrations in five of nine patients studied. In none of these patients were the myeloma proteins characterized immunochemically.

This report describes a study of 24 patients with monoclonal gammopathies (MG),14,15 7 with \( \gamma \)M-MG, 3 with \( \gamma \)A-MG and 14 with \( \gamma \)G-MG, with complete characterization of the abnormal proteins. The evidence obtained suggests that certain chromosomal abnormalities, some of which have been previously described in association with primary macroglobulinemia (Waldenström) and some not hitherto reported, are common to all three types of monoclonal gammopathies.

MATERIALS AND METHODS

The 24 patients with monoclonal gammopathy included 7 patients with \( \gamma \)M-, 3 patients with \( \gamma \)A-, and 14 patients with \( \gamma \)G-globulin abnormalities based upon immunoelectrophoretic and ultracentrifugal analyses (Table 1). Type K- and L-light chain specificity was identified in 20 and 4 cases respectively (Table 1).

Most patients with \( \gamma \)G- and \( \gamma \)A-MG exhibited clinical symptomatology, bone marrow
findings and bone lesions typical for multiple myeloma, but in one, these were absent. This patient (C. S.) with γG-MG, without clinical or marrow evidence of myeloma, also had typical chronic myelocytic leukemia. Similarly, only six of seven patients with γM-MG manifested characteristic clinical features and marrow cytology of primary macroglobulinemia (Waldenström). The other (J. R.) had a protracted clinical course, and the concentration of the abnormal gamma globulin remained relatively low. The disease in such patients was classified as "idiopathic" monoclonal gammopathy. Information regarding treatment with radiation, chemotherapy, and mercaptanes administered prior to chromosomal analyses is presented in Table 1.

Chromosomal analyses were performed on peripheral blood cultured by the method of Moorhead et al. and/or direct marrow preparations by the method of Tjio and Whang. In the majority of cells, only microscopic analysis was performed. Only those cells which had obvious abnormalities or in which microscopic analysis was uncertain were selected for photographing and karyotyping.

In the three types of monoclonal gammopathies, the occurrence of certain types of chromosomal abnormalities and the involvement of certain karyotopic foci were evaluated statistically.*

Terminology

In 1964, Levan et al. suggested a new system of terminology, in the hope of increasing accuracy and uniformity in chromosomal descriptive morphology. The symbols M and T were used to designate chromosomes, the centromeres of which were located at the exact median and terminal points, respectively. The area between these points was divided into four regions of equal size, and all chromosomes not classified as M or T were designated as m, sm, st or t, according to the location of their centromeres within these regions. With the lengths of the long (1) and short (s) arms adjusted proportionately, so that 1 + s = 10, when d equals the difference between 1 and s (d = 1 − s), and r equals 1 divided by s (r = s−1), the d and r ranges of the various regions are as follows:

<table>
<thead>
<tr>
<th>Term</th>
<th>Location</th>
<th>d Values</th>
<th>r Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>Median Point</td>
<td>0.0</td>
<td>1.0</td>
</tr>
<tr>
<td>m</td>
<td>Median Region</td>
<td>0.0−2.5</td>
<td>1.0−1.7</td>
</tr>
<tr>
<td>sm</td>
<td>Submedian Region</td>
<td>2.5−5.0</td>
<td>1.7−3.0</td>
</tr>
<tr>
<td>st</td>
<td>Subterminal Region</td>
<td>5.0−7.5</td>
<td>3.0−7.0</td>
</tr>
<tr>
<td>t</td>
<td>Terminal Region</td>
<td>7.5−10.0</td>
<td>7.0−∞</td>
</tr>
<tr>
<td>T</td>
<td>Terminal Point</td>
<td>10.0</td>
<td>∞</td>
</tr>
</tbody>
</table>

In this manuscript, the above symbols will be used parenthetically together with the terminology used by the authors quoted in the review of the literature, in order to reconcile the differences in terminology which presently exist.

The abnormal chromosomes in the AB size range, or larger, previously described by others in association with primary macroglobulinemia (Waldenström), were called "W" chromosomes by Benirschke et al. Neither their size nor centromere position, which ranges from metacentric (M) to submetacentric (m, sm), are sufficiently uniform to assign to them a morphologically descriptive term which would designate them as members of a specific abnormal group. Since we have identified them in three types of monoclonal gammopathies, we shall hereafter refer to them as MG chromosomes in the interest of brevity.

*We are grateful to Dr. J. E. Overall, Director, Research Computation Center, University of Texas Medical Branch, Galveston, Texas, for the statistical analyses.
Table 1.—Synopsis of Patients Examined, Including Age, Sex, Protein Values, Chromosome Cultures, and Treatment Data

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age</th>
<th>&quot;Abnormal&quot; Globulins (Immunologic Types)</th>
<th>Ultracentrifugation</th>
<th>Chromosomes Cultures</th>
<th>Treatment Radio- or Chemotherapy, Corticosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S&lt;sub&gt;20&lt;/sub&gt; w</td>
<td>S-Values and Concentrations of &quot;Abnormal&quot; Components</td>
<td>Dates</td>
<td>Tissue</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(g/%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. D.</td>
<td>F</td>
<td>60</td>
<td>γM-glob. Type K</td>
<td>S&lt;sup&gt;0&lt;/sup&gt; 18.0 24.8</td>
<td>8/9/63</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g/% 3.6 1.8 0.8</td>
<td>11/22/63</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4/24/64</td>
<td>Marrow</td>
</tr>
<tr>
<td>B. Y.</td>
<td>M</td>
<td>63</td>
<td>γM-glob. Type L</td>
<td>S&lt;sup&gt;0&lt;/sup&gt; 18.7 27.6 30.4</td>
<td>12/20/63</td>
<td>Blood</td>
</tr>
<tr>
<td>A. T.</td>
<td>F</td>
<td>44</td>
<td>γM-glob. Type K</td>
<td>S&lt;sup&gt;2&lt;/sup&gt; 15.4 23.0</td>
<td>8/14/64</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g/% 5.2 1.4</td>
<td>8/18/64</td>
<td>Blood</td>
</tr>
<tr>
<td>J. B.</td>
<td>M</td>
<td>60</td>
<td>γM-glob. Type K</td>
<td>S&lt;sup&gt;1&lt;/sup&gt; 15.2 26.5</td>
<td>12/28/64</td>
<td>Blood</td>
</tr>
<tr>
<td>J. R.</td>
<td>F</td>
<td>69</td>
<td>γM-glob. Type L</td>
<td>S&lt;sup&gt;0&lt;/sup&gt; 17.8 28.1</td>
<td>1/5/65</td>
<td>Blood</td>
</tr>
<tr>
<td>E. P.</td>
<td>M</td>
<td>56</td>
<td>γM-glob. Type K</td>
<td>S&lt;sup&gt;0&lt;/sup&gt; 18.7 28.2</td>
<td>2/12/65</td>
<td>Marrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g/% 3.0 1.2</td>
<td>2/25/65</td>
<td>Blood</td>
</tr>
<tr>
<td>F. F.</td>
<td>F</td>
<td>63</td>
<td>γG-glob. Type K</td>
<td>S&lt;sup&gt;0&lt;/sup&gt; 6.2</td>
<td>4/19/65</td>
<td>Blood</td>
</tr>
<tr>
<td>C. M.</td>
<td>M</td>
<td>55</td>
<td>γG-glob. Type K</td>
<td>S&lt;sup&gt;1&lt;/sup&gt; 5.7</td>
<td>12/10/63</td>
<td>Blood</td>
</tr>
<tr>
<td>C. S.</td>
<td>M</td>
<td>58</td>
<td>γG-glob. Type K</td>
<td>S&lt;sup&gt;1&lt;/sup&gt; 6.0</td>
<td>5/28/63</td>
<td>Blood</td>
</tr>
<tr>
<td>D. A.</td>
<td>M</td>
<td>49</td>
<td>γG-glob. Type K</td>
<td>S&lt;sup&gt;1&lt;/sup&gt; 6.3</td>
<td>12/17/63</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>g/% 2.7</td>
<td>1/9/64</td>
<td>Marrow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>1/17/64</td>
<td>Blood</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Sex</td>
<td>Age</td>
<td>Type</td>
<td>S%</td>
<td>Date</td>
<td>Condition</td>
</tr>
<tr>
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<td>-------</td>
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<td>-------</td>
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</tr>
<tr>
<td>E. H.</td>
<td>F</td>
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<td>γG-glob.</td>
<td>6.5</td>
<td>5/18/64</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Type K</td>
<td>g%/</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Type K</td>
<td>g%/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. T.</td>
<td>M</td>
<td>48</td>
<td>γG-glob.</td>
<td>6.9</td>
<td>5/26/64</td>
<td>Blood</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Type K</td>
<td>g%/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>L. W.</td>
<td>F</td>
<td>46</td>
<td>γG-glob.</td>
<td>6.4</td>
<td>6/9/64</td>
<td>Marrow</td>
</tr>
<tr>
<td>J. L.</td>
<td>M</td>
<td>53</td>
<td>γG-glob.</td>
<td>6.0</td>
<td>6/9/64</td>
<td>Blood</td>
</tr>
<tr>
<td>L. R.</td>
<td>M</td>
<td>58</td>
<td>γG-glob.</td>
<td>6.7</td>
<td>6/9/64</td>
<td>Blood</td>
</tr>
<tr>
<td>H. B.</td>
<td>M</td>
<td>46</td>
<td>γG-glob.</td>
<td>5.9</td>
<td>8/24/64</td>
<td>Blood</td>
</tr>
<tr>
<td>C. T.</td>
<td>M</td>
<td>56</td>
<td>γG-glob.</td>
<td>5.9</td>
<td>8/24/64</td>
<td>Blood</td>
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<tr>
<td>J. B.</td>
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<td>78</td>
<td>γG-glob.</td>
<td>5.9</td>
<td>8/25/64</td>
<td>Blood</td>
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<tr>
<td>O. B.</td>
<td>F</td>
<td>77</td>
<td>γG-glob.</td>
<td>6.0</td>
<td>8/25/64</td>
<td>Blood</td>
</tr>
<tr>
<td>T. C.</td>
<td>M</td>
<td>60</td>
<td>γA-glob.</td>
<td>5.6</td>
<td>1/15/65</td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Type K</td>
<td>S%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. D.</td>
<td>M</td>
<td>65</td>
<td>γA-glob.</td>
<td>5.7</td>
<td>4/10/65</td>
<td>Blood</td>
</tr>
<tr>
<td>M. C.</td>
<td>F</td>
<td>45</td>
<td>γA-glob.</td>
<td>6.7</td>
<td>1/18/65</td>
<td>Blood</td>
</tr>
</tbody>
</table>

a) Penicillamine (D,L- or D-forms); b) Melphalan; c) Chlorambucil; d) Urethane; e) Prednisone; f) Hydrocortisone; g) Busulfan; h) Protein Concentration (g%).
RESULTS

There was considerable variation in the response of the lymphocytes in tissue culture. On the whole, growth was good in patients with γM-MG, but considerable variation was observed in the γG-and γA-MG patients, in some of whom more than one tissue culture was necessary to prepare karyotypes. In many of these, mitoses were infrequent. In some instances, the chromosomes tended to stick together, resulting in inadequate spreading of the chromosomes.

In all three types of monoclonal gammopathies, nonspecific abnormalities, including poor matching due to differences in the degree of coiling, deletions or translocations of chromatin material, polyplody and chromatid or iso-chromatid breaks or gaps were observed. Endoreduplication was seen occasionally. Some cells had one or two pairs of homologous chromosomes lying side by side in the metaphase plate, suggesting the possibility of individual endoreduplication22 of these chromosomes.

Abnormal chromosomes in the AB size range or larger were reported by others1-9 in association with primary macroglobulinemia (Waldenström), variously described as metacentric (M),2 (m),3,7 submetacentric (M,m),3 (m),4 (sm),5 or subterminal (sm).1,6,8 Similar abnormalities were observed in five of seven patients with γM-MG, in all three with γA-MG, and in seven of 14 with γG-MG (Fig. 1). Affected cells made up 2 per cent to 30 per cent of the cells studied. These MG chromosomes occurred as supernumerary chromosomes in four of the five γM-MG patients, in two of three γA-MG patients and in only one of the seven γG-MG patients. The other six γG-MG patients exhibited this abnormality in pseudodiploid cells. The patient with γG-MG and chronic myelocytic leukemia (C.S.) exhibited, in addition to normal diploid cells, some cells the only abnormality of which was the presence of Ph1 chromosomes (Fig. 2a), and other cells possessing an abnormal chromosome indistinguishable from the MG-chromosomes, with or without a Ph1 chromosome (Fig. 2b). The MG chromosomes occurred both as supernumerary chromosomes and in pseudodiploid cells.

Additionally, abnormalities in the most metacentric of the smallest group C chromosomes (No. 12) were prominent in all of the γM-MG patients, in two of the γA-MG patients, and in eight of the γG-MG patients. These abnormalities consisted of missing chromosomes, the presence of one or more extra chromosomes or structural abnormalities of the chromosomes of this pair.

Definite evidence of the establishment of stable abnormal cell lines was lacking in most cases; however, a relatively stable pseudodiploid cell line was observed in a γG-MG patient (D. A.) with only one normal No. 12 chromosome and an extra submetacentric (m) chromosome, a little larger than the group E chromosomes (Fig. 3). This extra chromosome probably represented the other No. 12 chromosome with a deletion of about half of the long arms. This patient had had localized plasmacytomas 7 years and 2½ years prior to the present study. These tumors had been treated with local x-radiation.

One γM-MG patient (J. B.) exhibited severe Coombs’-positive hemolytic
anemia, transiently controlled with prednisone. Two attempts to culture chromosomes were unsuccessful during the period when hemolysis was suppressed, but a third attempt was successful after hemolysis reappeared.

Another γM-MG patient (H.D.), who had a severe cold antibody type hemolytic anemia due to an extremely high titer (1:262,000) of cold agglutinins, was an XO/XX mosaic. She was phenotypically normal and had borne two apparently healthy children. This patient exhibited none of the stigmata of Turner's syndrome, but four of seven 45-chromosome cells were XO cells, and approximately half of the 46-chromosome cells were XO cells containing an MG chromosome (Fig. 4). On three occasions, buccal smears revealed that 5 per cent or less of the cells were positive for Barr bodies.*

Studies were made of cells both from cultures of peripheral blood and from direct preparations of marrow in eight of the 24 patients, and from a 48-hour marrow culture of a ninth: four of the patients with γM-MG, four with γG-MG, and one with γA-MG. In one patient with γM-MG and in two

*We are grateful to Dr. Julian Chen, Department of Pathology, University of Texas Medical Branch. Galveston, Texas for performing these studies.
Fig. 2.—Cells from patient with γG-monoclonal gammopathy and chronic myelocytic leukemia: (A) Showing a Ph¹ chromosome-containing cell. (B) Cell containing both a Ph¹ chromosome and an MG-chromosome (m).
with γG-MG, 2 per cent to 23 per cent of the cells analyzed from direct marrow preparations contained an MG chromosome (Table 2). One of the latter was the patient with chronic myelocytic leukemia and γG-MG. Eighteen per cent of the cells studied in the marrow preparations possessed abnormal chromosomes in the AB range or larger. The marrow exhibited no increase in plasma cells, while marrows from the other two patients whose cells contained MG chromosomes were pleomorphic and exhibited ample numbers of normal marrow elements. No mitoses were present in preparations from direct harvests of marrow from two patients with γG-MG who had complete, or nearly complete, replacement of normal marrow elements by lymphoid elements, plasmablasts, and plasmacytes (Table 2).

The abnormalities observed in these 24 patients are summarized in Table 3. Since it is impossible to classify the MG chromosomes consistently with any specific group or pair by reason of size, morphology, or origin, they were omitted from statistical evaluation. The criteria used for the selection of abnormal cells, upon which these data are based, were the absence of a chromosome, the presence of one or more extra chromosomes in a pair or group, unequivocal deletions or translocations, or other major structural changes, excluding chromatid gaps and other minor abnormalities. The sign rank test for paired observations was used to evaluate the statistical significance of the abnormalities by chromosomal groups and pairs. Significantly more abnormali-
ties were present in group C than in any of the other groups (p < 0.05) due to frequent involvement of pair 12. Abnormalities in pair 12 were significantly increased in comparison with those in each of the other pairs (p < 0.05). Indeed, the abnormalities in pair 12 alone were significant when compared with the total abnormalities in each of the other groups (p < 0.05). Other sensitive foci suggested by the statistical data were pairs 5 and 20, and possibly pair 22.

**Discussion**

Although chromosomal abnormalities have been described in patients with primary macroglobulinemia (Waldenström),¹ ² no definite or consistent abnormalities have been previously reported in myeloma. In 1961, Bottura et al.¹ identified a large subterminal (sm) extra chromosome in cells of a patient with primary macroglobulinemia (Waldenström), and German et al.² described a very large metacentric (M) supernumerary chromosome in another case of primary macroglobulinemia (Waldenström). Since then, nine additional cases,³ ⁴ showing a supernumerary chromosome in the AB size range or larger, varying somewhat in centromere position, have been reported. The percentages of affected cells in these patients ranged from approximately 4 per cent⁵ ⁶ to 50 per cent ¹ ⁶. In one instance,⁵ a metacentric chromosome (m), larger than any of the normal complement, and a somewhat smaller submetacentric (sm) chromosome were found in cells of the same culture.
Preliminary reports by the present authors\textsuperscript{26,27} bring to 16 the total number of cases of $\gamma$M-MG exhibiting the abnormal chromosomes. Benirschke\textsuperscript{3} called the abnormal “marker” chromosomes the “W”-chromosomes. Bottura et al.\textsuperscript{1} suggested that if the chromosomal abnormalities observed in their cases were confirmed consistently in primary macroglobulinemia (Waldenström), “they would provide, for the first time, a morphological basis of a genetic kind for a biochemical defect in man,” German et al.\textsuperscript{2} cited the lack of uniformity in the morphologic characteristics of the abnormal chromosomes as probable evidence against a constant chromosomal aberration in this disease and raised the possibility that a similar portion of a specific chromosome, possibly the long arms of a No. 2, might conceivably be involved. Patau\textsuperscript{23} in a brief interpretative account, suggested that a No. 2 chromosome might well be the origin of the “W”-chromosome and postulated certain types of breakage and reunion which could account for the variable morphology.

Heni and Siebner\textsuperscript{7} found a supernumerary submetacentric (m) chromosome, in size between chromosome pairs 2 and 3, in marrow cells from a patient with primary macroglobulinemia (Waldenström). Thymidine labeling studies indicated that the abnormal chromosome in this case labeled similarly to the group A chromosomes. Elves and Israels\textsuperscript{5} published a report of three patients manifesting either a metacentric (m) chromosome larger than any other in the cell or a submetacentric (sm) chromosome in the AB size range, or both. One of these patients had macroglobulinemia, and two had increased concentration of $\gamma$-globulin. These authors postulated linkage between the chromosomal defects and the protein-producing potentiality of the cells carrying them and suggested that the genetic defect may appear several years prior to the appearance of macroglobulinemia, conferring upon the cell the ability to produce the abnormal globulin in response to an appropriate stimulus.

Ferguson and Mackay,\textsuperscript{6} in 1963, described three cases of primary macroglobulinemia (Waldenström), one of which demonstrated an abnormal chromosome similar in morphology to that described by Bottura,\textsuperscript{1} in approximately 50 per cent of the cells.

In 1959 Baikie et al.\textsuperscript{24} and in 1961 Richmond et al.\textsuperscript{25} found no chromosomal abnormalities in five patients with untreated myeloma. During 1963, reports of chromosomal studies in nine patients with myeloma were published.\textsuperscript{10-13} Five of these manifested varying degrees and types of chromosomal aberrations. In two of five untreated patients, Bottura\textsuperscript{10} demonstrated variable chromosomal abnormalities. One patient had one aneuploid cell with a structurally abnormal chromosome and several fragments. The other patient had two distinct clones of cells: one, a normal diploid line, the other having 45 chromosomes with one group C chromosome consistently missing. The patient described by Castoldi et al.\textsuperscript{11} had two lines of hypodiploid cells in marrow cultures, with variable karyotypic aberrations, but demonstrated the presence of a metacentric (m) medium sized aberrant chromosome in all cells. These authors suggested that the chromosomal abnormalities were reminiscent of the chromosomal imbalance often associated with malignant growth. Lewis et al.\textsuperscript{12,13} encountered abnormal chromosomal findings of a
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Table 2.—Showing the Cellular Composition of the Marrow Samples Studied Morphologically, the Mitotic Activity in the Cytogenetic Preparations, and the Cells Showing MG Chromosomes or Abnormalities of Pair 12

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
<th>Myel. etc.</th>
<th>Eryth.</th>
<th>Lymphs</th>
<th>Plasma</th>
<th>Date</th>
<th>Mitoses*</th>
<th>Cells Showing Mitoses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. D.</td>
<td>4/24/64</td>
<td>37.9</td>
<td>41.7</td>
<td>19.9</td>
<td>0.5</td>
<td>1/12</td>
<td>2.4</td>
<td>4.9</td>
</tr>
<tr>
<td>B. Y.</td>
<td>4/8/64</td>
<td>58.5</td>
<td>20.5</td>
<td>15.2</td>
<td>5.8</td>
<td>4/9/64</td>
<td>0.22</td>
<td>0.0</td>
</tr>
<tr>
<td>A. T.</td>
<td>8/18/64</td>
<td>Preparations too faded to count.</td>
<td></td>
<td></td>
<td></td>
<td>2.75</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>J. R.</td>
<td>2/25/65</td>
<td>23.6</td>
<td>8.3</td>
<td>58.7</td>
<td>9.4</td>
<td>3.10</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C. S.</td>
<td>12/13/63</td>
<td>93.9</td>
<td>1.6</td>
<td>4.4</td>
<td>0.1</td>
<td>1/9/64</td>
<td>0.2</td>
<td>18.8 12.5</td>
</tr>
<tr>
<td>E. H.</td>
<td>5/6/64</td>
<td>45.2</td>
<td>22.1</td>
<td>19.8</td>
<td>12.9</td>
<td>0.35</td>
<td>0.0</td>
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</tr>
<tr>
<td>L. D.</td>
<td>5/15/64</td>
<td>43.7</td>
<td>11.9</td>
<td>20.9</td>
<td>23.5</td>
<td>0.2</td>
<td>23.0 7.7</td>
<td></td>
</tr>
<tr>
<td>L. W.*</td>
<td>5/26/64</td>
<td>1.7</td>
<td>0.7</td>
<td>45.8</td>
<td>51.8</td>
<td>*6/11/64</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>T. C.</td>
<td>1/28/65</td>
<td>34.1</td>
<td>32.5</td>
<td>22.0</td>
<td>11.4</td>
<td>1/27/65</td>
<td>0.67</td>
<td>0.0 2.9</td>
</tr>
<tr>
<td>S. D.</td>
<td>4/8/64</td>
<td>2.2</td>
<td>0.1</td>
<td>2.6</td>
<td>95.1</td>
<td>4/16/64</td>
<td>0.0</td>
<td>0.0 0.0</td>
</tr>
</tbody>
</table>

*There were no mitoses in the direct harvest of marrow from this patient. The reported percentage of mitoses is based on preparations from a 48-hour culture of the marrow.

a. Percentage of mitoses encountered in cytogenetic preparations, based upon the evaluation of 4000 nuclei.
b. Cell types expressed in per cent, based upon the evaluation of 1000 nucleated cells.
c. Percentage of cells counted and analyzed on cytogenetic preparations.

Table 3.—Data on Frequency of Chromosomal Abnormalities in 235 Karyotypes from 24 Patients with 3 Types of Monoclonal Gammopathies

<table>
<thead>
<tr>
<th>Groups</th>
<th>A*</th>
<th>B*</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pairs</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>X</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>Number of cells</td>
<td>5</td>
<td>14</td>
<td>8</td>
<td>7</td>
<td>20</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Per cent of total</td>
<td>2.1</td>
<td>6.0</td>
<td>3.4</td>
<td>3.6</td>
<td>8.5</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Group totals</td>
<td>27</td>
<td>27</td>
<td>82</td>
<td></td>
<td></td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>and per cent</td>
<td>11.5%</td>
<td>11.5%</td>
<td>34.9%</td>
<td>11.1%</td>
<td>10.6%</td>
<td>11.9%</td>
<td>8.5%</td>
</tr>
</tbody>
</table>

*Excluding MG Chromosomes.

variable nature in three cases of myeloma. In contrast to the hypoploidy found by Bottura and Castoldi et al., the abnormalities in these three cases involved hyperploid cells. One of the cases lacked a normal cell line completely. Valentini et al. in 1964 made brief reference to their failure to find "constant and typical" karyotypic changes in plasmacytomas. Of interest is the study of Fjelde et al. in 1962 of transplantable murine plasma cell leukemias. In chromosome preparations of tumor tissue, four abnormal cell lines were found. Three of these carried marker chromosomes resembling the MG-chromosomes herein described.

In all nine reported myeloma patients, the diagnosis was based on clinical findings, and the serum proteins were evaluated by total serum protein and paper electrophoresis only. In all of our patients, the "abnormal" proteins were characterized by determination of total serum proteins, paper electrophoresis, immunoelectrophoresis, and analytical ultracentrifugation (Table 1). Since the protein abnormalities were not always accompanied by classical clinical manifestations of myeloma or macroglobulinemia, the designations of γG-, γA-, and γM-monoclonal gammopathies (MG) have been employed.

Our findings of large abnormal extra chromosomes, in the AB size range or larger, in varying percentages of cells in five of seven γM-MG patients, are substantially in accord with those previously reported. In one patient, however, this abnormality occurred consistently in pseudodiploid cells rather than as a supernumerary element. Our observations in γG- and γA-MG patients are at variance with those previously described in myeloma. Aberrant chromosomes similar to those associated with γM-MG were present in over half of our patients. In six of seven γG-MG patients these abnormal chromosomes were present in pseudodiploid cells, while in two of three γA-MG patients they were supernumerary. The difference between the occurrence of the abnormal chromosomes as supernumeraries in γM- and γA-MG or as constituents of pseudodiploid cells in γG-MG appears to be significant (p < 0.05). Since the aberrant chromosomes in the AB size range or larger were observed in all three types of monoclonal gammopathies, and since they were variable in both size and centromere position, we have used the term "MG chromosomes" for convenience in referring to them in this presentation. The terminology suggested by Levan et al. has been used parenthetically throughout the manuscript, wherever reference has been made to specific chromosomes. In coordinating Levan's terminology with that used in the review of the literature, r and d values were calculated from the published illustrations. The majority of MG chromosomes in our own material fell in the m and sm range, although a few large M chromosomes were encountered.

In addition to the MG-chromosomes, a high incidence of abnormalities was noted in the smallest group C chromosomes, interpreted as No. 12, in nearly all of the γA- and γM-MG patients and in over half of the γG-MG patients. These were heterogeneous abnormalities and included missing or extra chromosomes in this size range as well as structural abnormalities. In compiling the data for Table 3, karyotypes of all cells, whether broken or not, were
included to avoid bias in deciding whether or not a cell was broken and to 
evaluate the possible predilection of specific sites for chromosome loss as a 
result of mitotic instability, etc. In 34.9 per cent of cells, group C was involved 
in these abnormalities, and anomalies of pair 12 involved 22.5 per cent of 
the cells. These statistically significant data suggest the existence of certain 
chromosomal foci of abnormalities in all three types of monoclonal gammop-
athies. Abnormalities involving pair 5 in 8.5 per cent of these abnormal 
cells and pair 20 in 8.9 per cent are less impressive but still statistically 
significant.

Failure to note the abnormalities in pair 12 in previous reports may have 
been due to several factors. In the first place, previous studies dealt with 
individual case reports or a small series of cases. In our series, these defects 
were not easily recognized except in the patient in whom a distinct abnormal 
cell line was established (Fig. 3). The frequent involvement of a few cells 
with variable defects in this area of the karyotype in many patients was 
impressive. Second, the methodology employed is a determinant. Attention 
has been centered on aneuploid cells in the search for abnormal chromosomal 
patterns associated with monoclonal gammopathies. As a result, it seems 
probable that insufficient consideration has previously been given to the 
possibility that 46-chromosome cells may be pseudodiploid. For this reason, 
microscopic analysis of all cells counted was initiated, selecting for photography 
and karyotyping all cells in which microscopic matching was not readily 
accomplished. In general, microscopic matching was much easier in those 
patients without abnormalities than in those possessing abnormal cells. Third, 
there may be reluctance to attempt matching of group C chromosomes because 
of inherent difficulties. Despite the risk of assuming complete accuracy, 
matching attempts within this group—based upon general morphologic 
considerations such as size, coiling patterns, etc.—seemed justified. Fourth, 
it must be considered that in all the reported studies in myeloma, marrow 
cells alone were examined before treatment, whereas similar studies of the 
macroglobulinemia patients were made on peripheral blood cultures in some 
instances and on direct marrow aspirated in others. Chromosomal analysis 
of cells harvested from blood cultures of patients with \( \gamma \)A- and \( \gamma \)G-MG may 
account for the difference between our findings and those of others.

Our data obtained from the study of myeloma patients differ from those 
previously reported. Bone marrow only was studied by investigators pre-
viously reporting chromosomal abnormalities in myeloma. The abnormalities 
herein described primarily involved cells from phytohemagglutinin-stimulated 
cultures of peripheral blood lymphocytes, except for those present in the 
patient with \( \gamma \)G-MG and chronic myelocytic leukemia. In this patient (C. S.), 
as well as in one \( \gamma \)M-MG patient (H. D.) and in one other \( \gamma \)G-MG pa-
tient (L. D.), similar cells were observed in direct preparations of marrow. 
The heterogeneity of the marrow in the latter two patients, and our inability 
to identify, morphologically, cells in mitosis in the cytogenetic preparations, 
make it presently impossible to classify the cells exhibiting abnormal karyo-
CHROMOSOMAL ABERRATIONS COMMON TO γ TYPES OF MG

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types. Abnormal cells from the peripheral blood (29.4 per cent), and possibly from the marrow (18.8 per cent) probably were myeloid precursors in the case of the patient with γG-MG and chronic myelocytic leukemia (C. S.). Peripheral blood was cultured without phytohemagglutinin stimulation and harvested in 48 hours. The marrow was characteristic of chronic myelocytic leukemia. To date this patient has developed neither a plasmacytoma nor any other tumor which might account for the monoclonal gammopathy.

The relationship between the lymphocyte and the plasma cell, normal or malignant, is controversial. The variable aneuploidy previously reported in the marrow of myeloma patients may represent karyotypic deviation sometimes observed in advanced malignancy, whereas the abnormalities in lymphoid cells of myeloma patients may represent earlier and more basic changes, possibly directly or indirectly related to causation of the disease.

Three of the patients in this series are to be considered individually. The γG-MG patient (D. A.) who developed an abnormal cell line, apparently involving a deletion of the long arms of a pair 12 chromosome, is of particular interest in view of the long clinical course of the disease and the two previous courses of local x-radiation to areas of skeletal involvement by plasmacytomas. Among possible explanations for this abnormal cell line, three are particularly cogent. An abnormal cell line could have been present prior to the onset of the disease. The abnormality may have been due to x-ray damage to a sensitive chromosomal focus. A third possibility is that the abnormal cell line may reflect fortuitous involvement of this area by the x-ray damage, producing a cell line which developed because of optimal survival conditions. In 23 other patients, the failure to demonstrate a similar significant abnormal cell line militates against the first suggestion. Evidence relevant to the second and third suggestions is not available.

The γM-MG patient (H. D.), in whom XX/XO mosaicism was present, is of special interest. While approximately half of her 46-chromosome cells were normal, the other half were XO-cells carrying the MG-chromosomes. Since all of the 46-chromosome cells possessing the MG-chromosomes were XO-cells, their occurrence was basically comparable to their presence as supernumeraries in cells with a normal complement of sex chromosomes. This patient was phenotypically normal and had borne two healthy children. Evidence of fertility and a relatively normal phenotype are not incompatible with XX/XO mosaicism. An elevated titer of cold agglutinins (1:32,000 to 1:262,000) was present and 3½ years after the diagnosis of primary macroglobulinemia (Waldenström) of the cold agglutinin type was established, she developed Hashimoto's thyroiditis with an antithyroglobulin antibody titer of 1:10,000. It may be significant that an older sister has rheumatoid arthritis. The constellation of X-chromosome aberration, autoimmune disorders, and chromosomal anomalies has been noted previously. Relationship between autoimmunity and the production of chromosomal aberrations has been postulated by Fialkow. Sparks and Motulsky observed Hashimoto's thyroiditis in two patients with gonadal dysgenesis and isochromosome-X. Fialkow and
Gartler demonstrated that extracts of human leukocytes induced hyperploidy in homologous fibroblast cultures. The most marked responses were produced by extracts from two patients with chromosomal abnormalities and thyroid antibodies. One of the two extracts which produced marked responses in autologous fibroblast cultures was from a patient with XX/XO mosaicism and thyroid autoantibodies.

The simultaneous occurrence of a monoclonal gammopathy and myelocytic leukemia has been reported. Our patient (C. S.) with such a combination had both γG-MG and Ph1-positive chronic myelocytic leukemia. Cells from both direct marrow preparations and peripheral blood cultures with MG-chromosomes were recognized, and abnormalities in the pair 12 chromosomes were prominent. These abnormalities occurred in both Ph1-positive and Ph1-negative cells. The mitosing cells harvested from cultures of peripheral blood may be assumed to be young myeloid elements because only 48-hour cultures without PHA were used.

It seems probable that the chromosomal abnormalities, comparable to those seen in patients with monoclonal gammopathy, occurred in young myeloid cells rather than transformed lymphoid cells. Subsequent efforts to culture this patient's blood cells and marrow have repeatedly failed, probably due to the administration of busulfan.

Systematic evaluation of the influence of treatment upon these chromosomal abnormalities has not been undertaken. In a patient with γA-MG (T. C.), whose blood was obtained on the third day of a course of 1-sarcolysin treatment, abnormalities similar to those observed in the other patients were present but were accompanied by other profound disturbances of the karyotypes. Twelve days later, without further treatment, these chromosomal aberrations had almost disappeared from the marrow. Only two of 34 cells were grossly abnormal.

The significance of the chromosomal abnormalities in patients with three types of monoclonal gammopathies is uncertain. The variety of aberrations in a single patient and the variable morphology of MG-chromosomes from patient to patient militates against a simple causal relationship between any one of the chromosomal defects and the etiology of the monoclonal gammopathies. Indeed, morphologically similar abnormalities may accompany disorders other than monoclonal gammopathies. In a patient undergoing myeloblastic transformation of long-standing polycythemia rubra vera, subterminal (st) and submetacentric (m) giant chromosomes and other abnormalities were identified. A patient with Ph1-positive chronic myelocytic leukemia had very large subterminal (st) chromosomes, similar to the MG-chromosomes in Bottura's case of macroglobulinemia, which occurred primarily in pseudodiploid cells. Subsequent studies in the patient with chronic myelocytic leukemia revealed this chromosome to be present in all leukemic cells, and chromosomes from either pair 2 or pair 12 were absent in all these cells. Serum protein studies on this patient have, as yet, revealed no abnormalities. "Marker" chromosomes resembling the MG-chromosomes...
were reported in a case of acute myeloblastic leukemia and two cases of acute lymphoblastic leukemia.35 The similarities of abnormal chromosomes in these various conditions do not imply identity, and, conversely, available data do not prove the MG-chromosomes to be true marker chromosomes in the sense that they could be considered to be pathognomonic of monoclonal gammopathy. One might postulate that some unidentified defect or defects, common to all three types of monoclonal gammopathies, contribute to the formation of the MG-chromosomes.

**Summary and Conclusions**

1. Chromosomal patterns in 24 patients with γG-, γA- and γM-type monoclonal gammopathies (MG) are described.
2. Significant chromosomal abnormalities were observed in all three types of monoclonal gammopathies. Abnormal chromosomes in the AB size range, or larger (MG-chromosomes), were present in five of seven patients with γM-MG, in three of three patients with γA-MG and in seven of 14 patients with γG-MG. Abnormalities in the smallest group C chromosomes (pair 12), consisting of missing chromosomes, extra chromosomes, or structural anomalies, were noted in all γM-MG patients, in two with γA-MG and in eight with γG-MG.
3. The literature dealing with chromosomal aberrations in primary macroglobulinemia (Waldenström) and myeloma has been reviewed, and the various abnormalities have been discussed.
4. These chromosomal abnormalities suggest a common denominator for the three types of monoclonal gammopathies, without implying either etiologic or pathognomonic specificity.

**Summario in interlingua**

1. Es describite le configurationes chromosomal in 24 patientes con gammopathias (MG) monoclonal del typos γG, γA, e γM.
2. Significative anormalitates chromosomal esseva observate in omne le tres typos de gammopathia monoclonal. Chromosomas anormal in le region dimension de AB o plus (chromosomas MG) esseva presente in cinque de septe patientes con γM-MG, in tres de tres patientes con γA-MG, e in septe de 14 patientes con γG-MG. Anormalitates in le plus micre chromosomas de gruppo C (par 12), consistente de chromosomas absente, de chromosomas supernumerari, o de anormalitates structural esseva notate in omne patientes con γM-MG, in duo con γA-MG, e in octo con γG-MG.
3. Es revistate le litterature concernite con aberrationes chromosomal in macroglobulinemia primari (de Waldenström) e myeloma, e le varie anormalitates es commentate.
4. Iste anormalitates chromosomal suggestiona le presentia de un denominator commun pro le tres typos de gammopathia monoclonal, sed isto non significa que ille denominator possederea specificitate etiologic o pathognomonic.
ADDENDUM

Since the original submission for publication of this paper, four additional cases of primary macroglobulinemia (Waldenström) and two cases of \( \gamma \)-G-myeloma have been reported. In these patients, large, metacentric (M,m), submetacentric, and supernumerary chromosomes in the group A size range were observed (M,sm) in cells from blood and/or marrow.

REFERENCES


Chromosomal Aberrations Common to Three Types of Monoclonal Gammopathies

ELSIE W. HOUSTON, STEPHAN E. RITZMANN and WILLIAM C. LEVIN