The Production of γG-Globulin by Lymphocytes in Chronic Lymphocytic Leukemia

Immunocytologic Investigation of a Case

By Charles N. Gamble and Hunter O. Cutting

Evidence from numerous sources indicates that the immunoglobulins are synthesized by plasma cells or their immediate precursors.2-11 Additional evidence from two different observations suggests that lymphocytes can also contribute to the formation of gamma globulin. One of these observations is the finding of small numbers of gamma globulin containing cells variously described as small lymphocytes,8 “small” antibody-containing cells,9 small lymphocytic plasma cells,10 lymphocytoid plasma cells,11 and mediumsized and large lymphocytes12 in immunocytologic studies utilizing the fluorescent antibody technic. The other observation is the very rare occurrence of the excessive production of a monoclonal gamma globulin in association with the neoplastic proliferation of lymphocytes in chronic lymphocytic leukemia and lymphosarcoma in the absence of plasma cell proliferation.5,13-16 Recently, we encountered such a case of chronic lymphocytic leukemia associated with the excessive production of a monoclonal γG-globulin. Immunocytologic studies demonstrated the presence of gamma globulin within the cytoplasm of the proliferating young and mature lymphocytes, and these cells were interpreted as the cells of origin of the monoclonal serum protein.

Case History

The patient, R. L., a 66-year-old retired Chief Petty Officer, was admitted to the United States Naval Hospital, San Diego, California, on April 17, 1963, with acute urinary retention of 3 days duration. Except for symptoms of partial bladder neck obstruction during the previous year, he had enjoyed excellent health his entire life. Physical examination revealed enlargement of the prostate gland, moderate generalized lymphadenopathy, and slight enlargement of the spleen and liver. A diagnosis of chronic lymphocytic leukemia was made following examination of peripheral blood and bone marrow. An anomalous serum protein was found by paper electrophoresis following an unexpected total serum protein determination of 9.4 Gm. per cent. A retropubic prostatectomy was performed, and pathologic examination revealed nodular hyperplasia and a diffuse stromal infiltrate of lymphocytes. The patient has been seen periodically to the present time and

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has remained asymptomatic and required no therapy. Physical examination has remained unchanged, except for a moderate increase in peripheral lymphadenopathy and splenomegaly. During this period of observation, hemoglobin values have ranged between 11.4 and 12.8 Gm. per cent and leukocyte counts between 25,000 and 42,000/cu. mm. Differential leukocyte counts have shown a persistent lymphocytosis in the range of 90 per cent. Numerous other laboratory tests have been negative or normal. Specifically, the patient's urine has been repeatedly negative for protein and Bence-Jones protein; direct and indirect Coombs tests have been negative, and a skeletal survey failed to reveal any bony lesions.

Special Studies

Characterization of the Anomalous Serum Protein

The initial task in investigating this case immunohematologically was to characterize the anomalous serum protein. Paper electrophoresis of the serum had shown the protein to be present in the gamma region and to be extremely homogeneous or monoclonal in character and to account for 55 per cent of the total serum protein (fig. 1). Ouchterlony gel diffusion with varying dilutions of the patient's serum and normal human serum against rabbit antihuman gamma globulin further accentuated the high concentration of the anomalous component and showed its precipitation with antihuman gamma globulin. The monoclonal character of the protein was suggested by its sharp separation from the residual normal gamma globulin in the patient's serum (fig. 2). Immunelectrophoresis of the patient's undiluted serum against antihuman gamma globulin revealed a localized arc in continuation with


**Fig. 3.**—Serum immunoelectrophoresis showing the anomalous protein as a sharply localized arc (arrows) in continuation with the precipitin line of \( \gamma \)G-globulin.

**Fig. 4.**—Ultracentrifugation pattern of serum. A: At 24 minutes showing only a trace of 19S globulin. B: At 88 minutes showing a marked increase in 7S globulin (arrow).

the precipitin line of \( \gamma \)G-globulin. This arc showed \( \beta_2 \) mobility and a characteristic prominent convexity toward the antibody trough, indicating marked antigen excess. The sharp localization of the arc further indicated the monoclonal character of the protein (fig. 3). Ultracentrifugation analysis of the patient's serum revealed only a trace of 19S globulin, while 7S globulin accounted for 48 per cent of the total serum protein and the 4S component accounted for 52 per cent (fig. 4). Dr. Henry Kunkel of the Rockefeller Institute examined the patient's serum with specific antisera and identified the anomalous serum component as a \( \gamma \)G-globulin with type K light chains and tentatively identified the heavy chains as being intermediate between subgroups \( \text{Ge} \) and \( \text{Ne} \) and Gm type \( \text{a} - \) \( \text{b} - \) \( \text{f} - \).21

**Hematologic Studies**

Following characterization of the anomalous serum protein, the patient was investigated further hematologically. A second bone marrow aspiration was performed to obtain material for detailed cytologic and immunofluorescent studies. Sections of clotted blood from the aspirate revealed cellular particles which were markedly hypercellular due to a diffuse infiltration by small and
medium-sized lymphocytes (fig. 5). Of considerable interest and importance was the finding that plasma cells were not increased in numbers and in many fields were difficult to find. When encountered, they were noted in a perivascular location contrasting to the diffusely scattered cells of the plasmocytic neoplasias. A differential cell count done on concentrated smears of the bone marrow revealed a decrease in normal hematopoiesis with a marked increase in lymphocytes; 4.4 per cent of the cells were reticular lymphocytes, 49.5 per cent were medium-sized lymphocytes, and 33.2 per cent were small lymphocytes (table 1). A differential cell count of the peripheral blood obtained at the same time as the bone marrow revealed a marked lymphocytosis with a similar distribution of lymphocyte cell types as encountered in the bone marrow (table 1). The cell types were classified on the basis of size, appearance of the cytoplasm, nuclear cytoplasmic ratios, nuclear chromatin pattern and nucleolar prominence. They were designated as hematopoietic reticular cells, reticular lymphocytes, medium-sized lymphocytes and small lymphocytes, us-

**Table 1.—Differential Cell Count**

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Bone Marrow (%)</th>
<th>Peripheral Blood (%)</th>
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<tbody>
<tr>
<td>Normoblasts</td>
<td>6.2</td>
<td>0</td>
</tr>
<tr>
<td>Myeloblasts and leukoblasts</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neutrophiles and precursors</td>
<td>5.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Eosinophiles and precursors</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Basophiles and precursors</td>
<td>0.2</td>
<td>0</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>87.1</td>
<td>90.6</td>
</tr>
<tr>
<td>Reticular lymphocytes</td>
<td>4.4</td>
<td>9.8</td>
</tr>
<tr>
<td>Medium lymphocytes</td>
<td>49.5</td>
<td>46.4</td>
</tr>
<tr>
<td>Small lymphocytes</td>
<td>33.2</td>
<td>34.4</td>
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</tbody>
</table>

*1000 cells counted.*
Fig. 6.—Concentrated bone marrow smear. Hematopoietic reticular cell (H), reticular lymphocyte (R), medium-sized lymphocyte (M), and small lymphocyte (S) within a single field. Wright's stain (×1350).

Immunocytologic Studies

Concentrated smears of bone marrow and buffy coat of peripheral blood were also obtained for fluorescent antibody studies. Following fixation in ether-alcohol, the smears were stained with fluorescein-conjugated antihuman gamma globulin (fluor. anti-HGG) and examined by ultraviolet microscopy. Controls consisted of fluor. anti-HGG absorbed with the patient's serum, fluor. anti-HGG absorbed with human gamma globulin, and fluorescent antihuman serum albumin. In addition, smears from the buffy coat of normal blood, marrow and buffy coat of a patient with liver disease and hypergamma-globulinemia, and the marrow and buffy coat of a patient with chronic lymphocytic leukemia with hypogammaglobulinemia were stained with the same reagents. In the patient's marrow, 6.6 per cent of the lymphocytes and plasma cells showed specific fluorescence, while approximately half this number fluoresced in the peripheral blood (table 2A). A differential count of these fluorescent cells revealed that the cytoplasm of all three types of lymphocytes fluoresced as well as the cytoplasm of occasional plasma cells which were present (table 2B). The reticular lymphocytes were characterized by fairly abundant cytoplasm showing moderately intense fluorescence (fig. 7). Medium-sized lymphocytes were the most abundant fluorescent cells and showed a narrower fluorescent cytoplasm (fig. 8). Approximately one-quarter of the fluorescent cells were small lymphocytes with a very narrow rim of cytoplasm which fluoresced only faintly (fig. 9). The occasional plasma cells encountered revealed a fluorescence similar in intensity to the medium-sized and reticular lymphocytes (fig. 10).

Cryostat sections of a biopsy specimen of an enlarged posterior cervical
Table 2A.—Number of Fluorescent Cells per 100 Lymphocytes and Plasma Cells*  

<table>
<thead>
<tr>
<th></th>
<th>Bone Marrow</th>
<th>Peripheral Blood</th>
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<tr>
<td></td>
<td>6.6</td>
<td>3.5</td>
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</table>

*1000 cells counted.

Table 2B.—Differential Count of Fluorescent Cells*  

<table>
<thead>
<tr>
<th></th>
<th>Bone Marrow (%)</th>
<th>Peripheral Blood (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticular lymphocytes</td>
<td>15.0</td>
<td>26.0</td>
</tr>
<tr>
<td>Medium lymphocytes</td>
<td>55.0</td>
<td>44.0</td>
</tr>
<tr>
<td>Small lymphocytes</td>
<td>23.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>7.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*100 cells counted.

Lymph node were also stained with fluor. anti-HGG and the previously mentioned control sera. Hematoxylin- and eosin-stained sections revealed effacement of the normal architecture of the node by a diffuse infiltrate of lymphocytes. Irregular eosinophilic hyaline material was present throughout the substance of the node and fluoresced brilliantly following staining with fluor. anti-HGG (fig. 11). This material probably represents a form of para-amyloid, but further studies to define it more exactly have not been carried out. Of considerably greater interest and significance to the cellular origin of the anomalous monoclonal protein was the finding of single fluorescent cells, small groups of fluorescent cells and large groups of fluorescent cells with the morphology of reticular lymphocytes, medium lymphocytes and small lymphocytes in the lymph node sections. The single fluorescent cells were relatively large and brightly fluorescent and appeared to represent single reticular lymphocytes or single hematopoietic reticular cells (fig. 12). The small groups of fluorescent cells appeared to be made up of reticular lymphocytes and medium lymphocytes admixed with occasional small lymphocytes (fig. 13). Finally, the large groups of fluorescent cells seemed to consist of an admixture

Fig. 7.—Reticular lymphocytes. A: Wright’s stain showing characteristic morphology in the light microscope. B: Fluorescent antihuman γ-globulin (fluor-anti-HGG) stain showing moderately intense fluorescence of relatively abundant cytoplasm. (× 1350).
PRODUCTION OF γG-GLOBULIN

Fig. 8.—Medium-sized lymphocytes. A: Wright’s stain. B: Fluor-anti-HGG stain showing a relatively narrow cytoplasm of moderately intense fluorescence. (× 1350).

Fig. 9.—Small lymphocytes. A: Wright’s stain. B: Fluor-anti-HGG stain showing a very narrow rim of only faintly fluorescent cytoplasm. (× 1350).

of all three cell types and because of this were not sharply defined in the hematoxylin and eosin sections (fig. 14). These small and large groups of fluorescent cells were interpreted as proliferating clones and as the cellular sites of origin of the anomalous serum protein.

Electron Microscopy

A portion of the buffy coat of the peripheral blood was osmium-fixed, Vestopal-embedded, and examined in the electron microscope.25 Cells corresponding to small, medium-sized and reticular lymphocytes were encountered. Of interest was the fact that the cytoplasm of some of these cells contained many more endoplasmic reticulum profiles and associated ribonucleoprotein granules than are found in the cytoplasm of normal lymphocytes.26 These cells appear to be similar to the lymphocytes containing massive amounts of rough endoplasmic reticulum described by Zucker-Franklin in the macroglobulinemia of Waldenstrom.27 No cells with an extensive tubular endoplasmic reticulum characteristic of cells producing protein for export were found.
Fig. 10.—Mature plasma cell. A: Wright’s stain. B: Fluor-anti-HGG stain. (× 1350).

Fig. 11.—Lymph node. A: Hematoxylin and eosin stain showing irregular hyaline material scattered throughout the node. B: Fluor-anti-HGG stain showing intense fluorescence of the hyaline material. (× 130).

Fig. 12.—Lymph node. Singly scattered large cells. A: Hematoxylin and eosin. B: Fluor-anti-HGG. (× 660).
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Fig. 13.—Lymph node. Small groups of cells composed of reticular, medium-sized and small lymphocytes. A: Hematoxylin and eosin. B: Fluor-anti-HGG. (x 330).

Fig. 14.—Lymph node. Large groups of cells consisting of an admixture of all three cell types. A: Hematoxylin and eosin. The outline of the cell group (arrows) is poorly defined due to the admixture of numerous small lymphocytes. B: Fluor-anti-HGG stain. Specific cytoplasmic fluorescence sharply defines the group. (x 130).

DISCUSSION

Of the neoplastic proliferative disorders associated with excessive production of the immunoglobulins, multiple myeloma is by far the most common and is usually associated with $\gamma$G-globulin and less commonly with $\gamma$A-globulin. The macroglobulinemia of Waldenstrom in which the anomalous serum protein is a $\gamma$M-globulin accounts for 10 per cent or less of cases showing electrophoretically homogeneous serum protein peaks. Recently, a new entity, heavy chain disease, has been reported in which $\gamma$G-globulin-heavy chains are present in excess in patients serum and urine. Reported cases of other types of lymphoproliferative malignancies associated with an anomalous monoclonal serum protein are rare. Morphologically these cases are usually classified as reticulum cell sarcoma, lymphosarcoma of the lymphocytic type and chronic lymphocytic leukemia. In the majority of these cases the proliferation of reticulum cells and lymphocytes is associated
with increased numbers of plasma cells which are believed to be the cells of origin of the abnormal serum proteins. In very rare cases, no plasmacellular proliferation is identified and the protein is considered to have arisen from the neoplastic lymphocytes.\textsuperscript{5,13-16}

Clinically and morphologically the present case appears to represent a case of chronic lymphocytic leukemia in which a small per cent of the proliferating lymphocytes have the capacity to produce γG-globulin. It differs morphologically from the "textbook" case of chronic lymphocytic leukemia characterized by a "monotonous" pattern of mature lymphocytes in exhibiting an increased number of young forms. Hematopoietic reticular cells, reticular lymphocytes and medium-sized lymphocytes are, however, found in small numbers in many cases of chronic lymphocytic leukemia\textsuperscript{22} and in occasional cases are as prominent as in the present case.

The relatively small percentage of proliferating cells containing gamma globulin (table 2A, table 3) indicates that the neoplastic process also involves lymphocytes which apparently do not have the capacity to produce gamma globulin and that these latter cells make up the bulk of the cells present. This suggests that normally only a small number of cells classified as lymphocytes are capable of forming gamma globulin and may explain the rarity of anomalous serum proteins associated with strictly lymphocytic proliferative disorders. An alternative explanation for the relatively small number of cells containing gamma globulin is the deletion of the capacity to produce gamma globulin in the majority of the proliferating cells through mutation early in the course of the neoplastic process.

As in the case of plasma cells in which younger cells classified as plasmablasts and proplasmocytes appear to be the most active in protein synthesis,\textsuperscript{2} the younger cells in this case, the reticular lymphocytes and medium-sized lymphocytes appeared to contain the most gamma globulin (table 3). Of considerable interest was the presence of faint fluorescence of the cytoplasm of a significant percentage of small lymphocytes (table 3). The finding of gamma globulin in these mature cells and the lack of any apparent morphologic relationship with the occasional plasma cells present indicates a population of gamma globulin containing cells distinct from plasma cells.

Finally, the small and large groups of fluorescent cells noted in the sections of lymph node suggest a family of gamma globulin producing cells arising from one parent cell. This finding fits well with the "one clone—one protein" hypothesis to explain the monoclonal character of the anomalous serum protein.

**Summary**

A case of chronic lymphocytic leukemia associated with the excessive production of a monoclonal γG-globulin has been investigated by immunofluores-
Production of γG-globulin

cent technics. Gamma globulin was demonstrated within the cytoplasm of young and mature lymphocytes and these cells were interpreted as the cells of origin of the monoclonal protein. The finding of gamma globulin within the cytoplasm of mature small lymphocytes in the absence of any apparent morphologic relationship with the occasional plasma cells present was interpreted as indicating a population of gamma globulin forming cells distinct from plasma cells, and suggested that a small number of lymphocytes may also contribute to the formation of immunoglobulins under normal conditions. The distribution of the fluorescent lymphocytes in sharply defined small and large cells groups within a lymph node supports the concept of the clonal origin of single molecular species of gamma globulin.

SUMMARIO IN INTERLINGUA

Un caso de chronic leucemia lymphocytic associate con un production excessive de monoclonal globulina γG esseva investigate per technicas a immunofluorescentia. Globulina gamma esseva demonstrate intra le cytoplasma de lymphocytos juvene e matur, e iste celulara esseva reguardate como le cellulara de origine del proteina monoclonal. Le constatation de globulina gamma intra le cytoplasma de micre lymphocytos matur in le absentia de omne apparente relation morphologic con le presentia occasional de plasmocytos esseva reguardate como indication que il existeva un population de cellulara formante globulina gamma a parte le plasmocytos. Isto suggestionava que un micre numero de lymphocytos contribue possibilemente etiam al formation de immunoglobulinas sub conditiones normal. Le distribution del lymphocytos fluorescentic in nettemente definite gruppos de micre e grande cellulara intra un nodo lymphatic supporta le conception del origine clonal de simple species molecular de globulina gamma.

ACKNOWLEDGMENTS

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