Serum Monoclonal Immunoglobulin Bands in Undifferentiated Lymphomas of Burkitt and Non-Burkitt Types

By Ian Magrath, David Benjamin, and Nick Papadopoulos

Using an improved electroimmunofixation technique that combines the sensitivity of high resolution agarose gel electrophoresis with the specificity of immunoprecipitation, we have demonstrated monoclonal immunoglobulin bands in the serum of patients with undifferentiated lymphomas of Burkitt and non-Burkitt types. Monoclonal bands were detected in the serum of 12 of 21 patients with extensive tumor, and 1 of 10 patients with minimal tumor. All of the bands were identified as IgM of a single light chain class. Such bands were not detected in the serum of patients with lymphoblastic lymphoma (7) or African Burkitt’s lymphoma (6). There was disappearance of the bands after therapy and reappearance at relapse. These findings, coupled with previously reported in vitro information, indicate that undifferentiated lymphoma cells secrete immunoglobulin of IgM isotype. Therefore, such monoclonal bands may be of potential value as tumor markers.

SERUM MONOCLONAL immunoglobulin bands have been described in a variety of disease states as well as asymptomatic individuals, but are most consistently associated with lymphoid malignancies exhibiting pronounced plasmacytoid differentiation such as lymphoplasmacytoid lymphomas and myeloma.

In recent years, the major subdivision of lymphocytes into T and B cells has been shown to hold for lymphoid malignancies. It is perhaps surprising that monoclonal immunoglobulin bands are detected in the serum of only about 5% of patients with lymphomas, since 70%-80% express surface immunoglobulin. This may reflect either a lack of differentiation of the majority of lymphomas, with a corresponding failure to secrete immunoglobulins, or the insensitivity of currently available techniques or both.

Burkitt’s lymphoma has been characterized as a B-cell neoplasm by virtue of its expression of surface immunoglobulin (SIg). We have recently shown that both fresh tumor cells and cell lines derived from American Burkitt’s lymphoma and undifferentiated lymphomas, in contrast to African Burkitt’s lymphoma, secrete quite large amounts of IgM into the medium (up to 1 µg/ml/10^6 cells in 48 hr). Recently, monoclonal bands were reported in acute leukemias with L3 (Burkitt) morphology, but until now, serum monoclonal immunoglobulin bands have not been described in Burkitt’s lymphoma or other undifferentiated lymphomas.

Using an improved, high resolution electrophoresis technique, we have examined serum samples from young patients with lymphomas, particularly American Burkitt’s lymphoma, for the presence of monoclonal immunoglobulin bands that would be predicted to occur from our findings with Burkitt’s lymphoma cell lines. In this article we describe the detection and identification of such bands, and provide evidence that they are the product of the tumor cells themselves.

MATERIALS AND METHODS

Patients less than 35 yr of age with a histologic diagnosis of malignant lymphoma, who had been investigated and treated at the Pediatric Oncology Branch (POB) of the National Cancer Institute or at the Lymphoma Treatment Centre of the Uganda Cancer Institute, Kampala, were included in this study. In the POB patients, histologic material had been reviewed by a panel of experienced hematopathologists; the diagnosis of review was used for this study. Patients were clinically staged and treated as previously described. Sera from patients with undifferentiated lymphomas of Burkitt type and non-Burkitt type were studied; patients with both high (stages C, D) and low (stages A, B, AR) tumor burdens being included. Sera from patients with lymphoblastic lymphomas were also studied; the majority of these had extensive mediastinal lymphomas.

Immunologic studies, as previously described, have been performed on many of these lymphomas. Although tumor tissue was available in all cases, we have found that undifferentiated lymphomas (Burkitt and non-Burkitt) invariably expressed surface immunoglobulin, while all of the lymphoblastic lymphomas tested by us formed E-rosettes with sheep erythrocytes and failed to express surface immunoglobulin.

The African patients were clinically staged and treated at the Lymphoma Treatment Centre in Kampala according to previously described protocols. The patients included in this study were selected purely on the basis of the availability of a pretreatment serum sample, and extensive disease.

Serum Samples

Serum is routinely obtained before treatment in POB patients. Blood was allowed to clot at room temperature and the serum frozen within a few hours at -70°C. Serum from Africa was stored for up to 2 yr at -20°C, and thereafter (6-8 yr) at -70°C. The longest period of storage was 8 yr, and the shortest a few months. The majority of the sera examined have been stored for 1-4 yr.
Serum Protein Electrophoresis

Fractionation of serum proteins was performed by a high resolution agarose gel electrophoresis technique originally developed\(^1\) and recently improved to detect weak but discrete homogeneous γ-globulin bands that are undetectable by the routine protein electrophoresis techniques employed in most hospital clinical chemistry laboratories.\(^4\)

The main features that distinguish this electrophoretic technique from other methods are: (1) the low concentration of agarose (0.5%), which allows free migration of large protein molecules and minimizes distortion of electrophoretic patterns; (2) the low concentration of the barbital buffer (0.05 M) that reduces electrophoresis to 12 min; and (3) the placement of the serum sample in a narrow slit, which minimizes diffusion of the protein fractions and results in sharp definition of the protein bands.

In the present study, questionably present or extremely weak "mini" bands were excluded from consideration. The data presented are based only on easily discernible, discrete bands in the γ-globulin region.

To identify the bands, immunofixation was combined with electrophoretic separation. After electrophoretic demonstration of a monoclonal band, identification at the site of the band was accomplished by placing a cellulose acetate strip soaked in monospecific antibody against heavy chains or light chains directly on top of the band on the unstained gel. The antibodies were allowed to diffuse into the gel for 15 min at 37°C. An immunoprecipitation reaction occurred only when homologous antigens and antibodies were present. The slide was then immersed in saline overnight, so that the rest of the proteins were washed out, and the insoluble precipitate remaining was stained with amido black as described in the electrophoresis procedure.\(^1\)

Serum Immunoglobulin Levels

In American patients, serum was routinely obtained for determination of immunoglobulin levels prior to the commencement of therapy. IgM, IgA, and IgG levels were determined by nephelometry at 355 nm using an immunoprecipitation reaction. Serum immunoglobulin determinations were all carried out on fresh rather than stored serum.

RESULTS

Thirty-one American patients with undifferentiated lymphomas were studied. They ranged in age from 2 to 35 yr. Twenty-four were males (M:F = 3:1). Twenty-two had extensive (stage C or D) disease, and 10 had small tumor burdens (stages A, B) or completely resected abdominal tumor (stage AR). Seven patients with lymphoblastic lymphoma were studied (age range, 14–22 yr; M:F ratio, 5:2), and six patients with African Burkitt’s lymphoma (age range, 3–12 yr; M:F ratio, 2:1). The incidence of serum monoclonal immunoglobulin bands in the various groups of patients prior to treatment is shown in Table 1. With one exception, such bands were observed only in American patients with extensive undifferentiated lymphomas, 12 of whom had monoclonal bands. The exceptional patient was also an American child with undifferentiated lymphoma in whom all overt tumor had been resected. Following therapy, he manifested plasma biochemical changes consistent with an “acute tumor lysis” syndrome,\(^1\) suggesting that widespread infiltrative disease undetected at surgery or by staging studies was present. When the American undifferentiated lymphomas with extensive disease were subdivided into Burkitt’s lymphoma and non-Burkitt’s lymphoma, no difference in the frequency of monoclonal bands was discernable between the groups (Table 1). No discrete monoclonal bands were observed in the serum of seven patients with lymphoblastic lymphoma (only one of whom had localized disease) or six with African Burkitt’s lymphoma (five of whom had stage C or D disease). All the latter patients exhibited diffuse, polyclonal hypergammaglobulinemia.

Table 1. Occurrence of Monoclonal Immunoglobulin Bands According to Histologic Diagnosis and Stage of Disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Total Bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>American undifferentiated</td>
<td></td>
</tr>
<tr>
<td>lymphomas</td>
<td></td>
</tr>
<tr>
<td>Stages A, B, AR</td>
<td>10</td>
</tr>
<tr>
<td>Stages C, D</td>
<td>21</td>
</tr>
<tr>
<td>Burkitt’s</td>
<td>13</td>
</tr>
<tr>
<td>Non-Burkitt’s</td>
<td>8</td>
</tr>
<tr>
<td>Lymphoblastic lymphomas</td>
<td></td>
</tr>
<tr>
<td>Stage A</td>
<td>1</td>
</tr>
<tr>
<td>Stages C, D</td>
<td>5</td>
</tr>
<tr>
<td>African Burkitt’s lymphoma</td>
<td></td>
</tr>
<tr>
<td>Stage A</td>
<td>1</td>
</tr>
<tr>
<td>Stages C, D</td>
<td>5</td>
</tr>
</tbody>
</table>

Identification of Monoclonal Bands and Comparison With Immunoglobulin Secretion by Cell Lines

In all 13 patients whose serum contained monoclonal bands, the bands were sufficiently pronounced to permit identification by immunofixation, at least with regard to heavy chain class. The results of one such study are shown in Fig. 1. In all cases, the paraprotein was identified as IgM. In five cases, an associated kappa chain was identified, in three a lambda chain, and in five patients the light chain type could not be identified (Table 2). In six of these patients, a continuous cell line established from the tumor cells was available for study, and in a seventh patient, fresh tumor cells were studied. In each case, the cells secreted IgM. In five secreted IgM kappa, one secreted IgM lambda, and in the fresh tumor, the identity of the light chain was not established. In four cases (three kappa and one lambda) light chain type had been determined both in the immunoglobulin secreted by the cell line and in the serum monoclonal band. In every case there was consistency between the light chain types present in the cell line and in the serum (Table 2).

Serial Studies

In six patients whose serum showed a monoclonal band at presentation, serial sampling of serum was
undertaken to determine the relationship between the presence of the immunoglobulin band and clinical course. In all six patients, the monoclonal bands rapidly became undetectable after chemotherapy. In all five patients who relapsed, recurrence of tumor was associated with the reappearance of the same monoclonal band that was initially present (Fig. 2). In two patients, the abnormal immunoglobulin bands reappeared prior to the diagnosis of relapse, in one case 3 mo before relapse was confirmed.

Serum Immunoglobulin Levels

Serum immunoglobulins were measured on a total of 40 patients with undifferentiated lymphomas and 7 patients with lymphoblastic lymphoma. As a group, patients with undifferentiated lymphomas tended to have low IgG and IgM levels at presentation, 25% and 17%, respectively, being below the lower limit of the normal reference interval. Only three patients had abnormally low IgA levels. Seventy-five percent of all immunoglobulin levels (G, A, and M) were below the midpoint of the reference interval. Median values in mg/dl were: IgG, 786 (reference interval 650–1600), IgA, 137 (reference interval 65–415), IgM 133, (reference interval 50–320). Patients with low IgG more frequently had low IgM—5 patients had both low IgG and IgM. Further, of 4 patients with an IgA level below the reference interval, 3 also had low IgG levels, and the fourth patient had an IgG level at the lower limit of normal. In 2 patients, all 3 immunoglobulins were below normal levels. There was no correlation between clinical stage or prognosis and immunoglobulin levels or between the presence of a monoclonal band

Table 2. Comparison of Immunoglobulins Secreted by Cell Lines or Fresh Tumor Cells, Serum Monoclonal Bands, and Serum Immunoglobulin Levels

<table>
<thead>
<tr>
<th>Patient</th>
<th>Monoclonal Band</th>
<th>Immunoglobulin Secreted In Vitro</th>
<th>Serum Immunoglobulin Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.K.</td>
<td>M</td>
<td>M - *</td>
<td>936</td>
</tr>
<tr>
<td>J.D.F.</td>
<td>Mx</td>
<td>Mx</td>
<td>336†</td>
</tr>
<tr>
<td>W.M.</td>
<td>MA</td>
<td>Mx</td>
<td>1,344</td>
</tr>
<tr>
<td>M.C.</td>
<td>MX</td>
<td>MX</td>
<td>656</td>
</tr>
<tr>
<td>D.S.</td>
<td>Mx</td>
<td>Mx</td>
<td>896</td>
</tr>
<tr>
<td>J.L.</td>
<td>Mx</td>
<td>Mx</td>
<td>920</td>
</tr>
<tr>
<td>W.B.</td>
<td>MA</td>
<td>MA</td>
<td>256†</td>
</tr>
<tr>
<td>R.G.</td>
<td>Mx</td>
<td>Mx</td>
<td>1,100</td>
</tr>
<tr>
<td>S.T.</td>
<td>M-</td>
<td>Mx</td>
<td>610†</td>
</tr>
<tr>
<td>P.R.</td>
<td>M-</td>
<td>Mx</td>
<td>576†</td>
</tr>
<tr>
<td>M.S.</td>
<td>M-</td>
<td>Mx</td>
<td>576†</td>
</tr>
<tr>
<td>C.A.</td>
<td>M-</td>
<td>Mx</td>
<td>736</td>
</tr>
<tr>
<td>D.J.</td>
<td>M-</td>
<td>—</td>
<td>114</td>
</tr>
</tbody>
</table>

*Fresh tumor cells. Remaining data in this column are derived from cultured tumor cell lines.
†Abnormally low serum immunoglobulin level.
‡Abnormally high serum immunoglobulin level.
and abnormal immunoglobulin levels (Table 2). Immunoglobulin levels in patients with lymphoblastic lymphoma were quite normal, except for one patient in whom the IgM level was low.

DISCUSSION

Electrophoretically homogeneous protein bands that extend over a very narrow region of the \( \gamma \)-globulin zone under standard conditions have been clearly shown to be monoclonal. They express a single heavy and light chain class, and where tested, also a single idiotype.\(^{16}\) Using standard techniques, such bands are found in about 1\% of adults below 70 yr.\(^{2,17}\) In a recent survey of 600 normal sera of healthy employees in this institution, using the improved agarose gel technique, about 5\% contained monoclonal \( \gamma \)-globulin bands of various intensities.\(^{14}\) This indicates that the agarose technique is more sensitive than previously used techniques, an indication borne out by direct comparison. Monoclonal bands may also be associated with lymphoreticular malignancy, particularly diseases in which the predominant cell type is of the B-cell series and well differentiated, including myeloma, lymphoplasmacytoid neoplasms, and less commonly, chronic lymphocytic leukemia. Monoclonal bands, particularly IgA, have also been occasionally observed in T-cell neoplasms, such as the Sézary syndrome.\(^{18}\) Such bands have not been observed in lymphomas in which the predominant cells are undifferentiated B cells, a group that includes Burkitt’s lymphoma. Similarly, although African Burkitt’s lymphoma cells or their derived cell lines express surface immunoglobulin, nearly always IgM,\(^ {19,21}\) most investigators have found negligible amounts of IgM in the supernatant of such cell cultures, indicating little or no secretion of immunoglobulins by these cells.\(^ {8,21}\) We have recently demonstrated, however, that cell lines of American undifferentiated lymphoma origin secrete quite large quantities of IgM—significantly more than African lines—and have also shown this to be true for fresh or cryopreserved tumor cells. The secreted IgM is monoclonal on the basis of light-chain type and two-dimensional polyacrylamide gel electrophoresis.\(^ {8}\) Thus, monoclonal immunoglobulin bands ought to be detectable in the serum of such patients if sufficiently sensitive techniques are used.

Using a recently improved electrophoretic technique in combination with immunofixation, we have now been able to demonstrate the presence of such immunoglobulin bands in the serum of 57\% of our patients with extensive (stage C or D) undifferentiated lymphoma. In none of these patients was a band demonstrated by cellulose acetate protein electrophoresis. In patients with small or completely resected tumors (stages A, B, AR), we were able to detect only one such band in 10 patients. The bands identified by immunofixation were all IgM and of either kappa or lambda light chain type. Where a tumor cell line or fresh tumor cells from the same patient had also been studied, there was concordance of light chain expression between cells and serum in all cases. Serial examination of serum from six of our patients who manifested immunoglobulin bands at presentation demonstrated a clear relation-
ship to the patients' clinical status, becoming undetectable after chemotherapy and reappearing at relapse.

It is not clear why only a little more than half the patients with extensive undifferentiated lymphoma had monoclonal bands. There was no correlation with the histologic diagnosis—approximately equal numbers of patients with Burkitt's and non-Burkitt's lymphomas manifested such bands—or with the site of disease. It is possible that the electrophoretic method used was not sensitive enough to pick up monoclonal immunoglobulins that were present in lesser amounts in the remaining patients or that some of the tumors did not secret immunoglobulin. It is also possible that the rate of detection was reduced by using cryopreserved serum, although a monoclonal band was detected in one serum after 8 yr of storage.

The finding of a correlation of the presence of a monoclonal band with tumor burden and clinical course, coupled with the correspondence of the heavy and light chain classes with those secreted by the same patient's fresh tumor cells or tumor-derived cell line, indicates that the monoclonal bands are immunoglobulins secreted by the tumor cells. The significance of this with regard to the origin of the tumor cells is speculative, but this finding may be of considerable practical value in the management of patients with undifferentiated lymphomas, since it provides a specific tumor marker. Further studies will be necessary to determine the diagnostic value and sensitivity as a marker of residual or recurrent tumor. The presence of such bands in CSF, ascitic, or pleural fluid should also be sought; the detection of CSF bands in particular may provide a means of monitoring CNS disease. It may prove possible to further increase the sensitivity of the electrophoresis technique or to detect abnormal immunoglobulin fragments in the serum of patients with undifferentiated lymphomas. Free light chains, for example, are secreted, sometimes in quite large amounts, by our tumor-derived cell lines. It is of note that serum immunoglobulin M levels were not noticeably increased by the presence of the weak monoclonal bands. Discrete serum immunoglobulin bands were not detected in lymphoblastic lymphoma, which is of T-cell origin, or in African Burkitt's lymphoma. The latter showed diffuse polyclonal gammapathy only, consistent with the normal African pattern. The absence of monoclonal bands in African Burkitt's lymphoma is consistent with in vitro data, but more patients must be studied before it can be confirmed that there is a difference in this regard between African and American Burkitt's lymphoma.

In some of the sera examined, two weak monoclonal bands were detected. This occurred in two of the patients studied serially, and an identical pattern was seen at presentation and relapse. In one of these cases studied by immunofixation, one band was IgM kappa, but the second could not be identified. The nature of this additional band, therefore, remains unknown.

The finding of monoclonal immunoglobulin bands in patients with undifferentiated lymphomas by an agarose gel technique when more widely used methods have failed to reveal them calls for a reevaluation of the frequency of monoclonal bands in other types of lymphoma. It indicates that the incidence of abnormal serum immunoglobulin bands may be higher than has been previously described.

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REFERENCES

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