Chromosomes in the Spinal Fluid:
Evidence for Metastatic Origin of Meningeal Leukemia

By Renato Mastrangelo, Wolf W. Zuelzer, Peter S. Ecklund and Ruby I. Thompson

It is well known that the blastic cells in acute leukemia often show numerical or other aberrations from the normal karyotype, which permit their characterization as stem lines. While these may undergo further evolution, they generally remain identifiable during consecutive relapses throughout the course of the disease, thus constituting evidence for the persistence of an autonomous cell population. Thus far, chromosomal studies have been restricted to bone marrow and cultured blood cells. Except for a single report demonstrating the Ph chromosome in myeloblasts in the spinal fluid of a patient entering the blastic phase of chronic granulocytic leukemia, the cytogenetic aspects of leukemic cells in the central nervous system have not been investigated. Under current therapeutic conditions, between one third and one half of the children with acute leukemia show involvement of that system at some time. The origin of the leukemic cells in the spinal fluid has been the subject of speculation. The demonstration of a chromosomal constitution common to these cells and the leukemic bone marrow population would greatly strengthen the evidence for their metastatic character. This report describes the cytogenetic findings in the spinal fluid of nine children with acute leukemia.

Case Material

The patients were children ranging from seven months to 10 years in age in whom the diagnosis of acute leukemia was made on the basis of blood and bone marrow findings, and who had been followed for periods of 7–63 months. The leukemia was classified as lymphoblastic ("stem cell") in seven cases, and as myeloblastic and monocytic in one case each. Spinal fluid was obtained during periods of symptomatic involvement of the nervous system, in several instances after previous similar episodes for which intrathecal methotrexate and radiation therapy had been given. The shortest interval between a previous episode and the time when the spinal fluid was studied was four months. The patients...
![Table 1: Summary of Cytogenetic Findings; Modal No. + Markers](image)
Table 1. Continued

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Genome</th>
<th>At Diagnosis</th>
<th>Bone Marrow</th>
<th>Relapse</th>
<th>Spinal Fluid Months After Diagnosis</th>
<th>Bone Marrow Subsequent Relapses</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.</td>
<td>46, XX</td>
<td>46, XX, Bq−, range 46-53</td>
<td>46, XX, Bq−</td>
<td>46, XX, Bq−, ?3C+, D+, E+, C−, F+, mar1+</td>
<td>mar1−Bpo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>No specimen</td>
<td>53, a single metaphase (no markers)</td>
<td>53, XX, A+, 2C+, D+, 2G+, mar1+</td>
<td>mar1−Dp+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>46, XY</td>
<td>46, XY, B−, C−, mar1+, mar2+</td>
<td>46, XY, B−, E−, F−, C+, 2mar1+</td>
<td>mar1&gt;F&lt;E group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>45, XX, C−</td>
<td>46, XY, B−, D−, F−, 2mar1+, mar2+, mar #16s</td>
<td>46, XY, B−, 2C−, D+, G+, mar2+</td>
<td>mar2−Bq+ mar #16s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>46, XX</td>
<td>46, XX</td>
<td>46, XX, Bq−</td>
<td>46, XX, Bq−, C+, D−</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>46, XX</td>
<td>46, XX</td>
<td>55, XX, ?6C+, 2D+, G+</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Chicago Conference: Standardization in Human Cytogenetics. Birth Defects: Original Article Series, Vol. II, No. 2, December, 1966. To characterize the size of a marker chromosome, its length as compared to its nearest normal-appearing chromosome is indicated by the symbols >, <, and ~. The number symbol (#) is used to differentiate specific numbered chromosomes from supernumerary chromosomes.
had also received various other antileukemic agents. All but one (case eight) were in apparent hematologic remission.

**METHOD**

The method is a modification of the "direct" bone marrow method.\(^1\) From 2–3 ml of freshly drawn spinal fluid is placed in complete tissue culture medium (MEM)\(^*\) instead of a basic salt solution. After incubation for one hour at 37°C, colcemid is added to give a concentration of 0.2μg./ml. After another hour of incubation the specimen is centrifuged and the supernatant fluid discarded. 5.0 ml of 0.075 M KCl is added. After seven minutes at 37°C two drops of Carnoy's fixative is added. After mixing and centrifuging, the specimen is fixed in three changes of Carnoy's solution. Slides are prepared by rapid flame drying and stained with Giemsa at pH 7.0. Satisfactory preparations were obtained when the spinal fluid cell count was 200/mm\(^3\) or higher. Chromosomes in 50–75 cells were counted and suitable metaphases (as a rule 10–20) were karyotyped.

**RESULTS**

In every case in which satisfactory relapse bone marrow preparations were available for comparison, good correspondence between the cytogenetic findings in the spinal fluid and initial or in some cases subsequent bone marrow populations were demonstrable. In two instances (cases 1 and 2, Table 1) the initial marrow cells and the cells in the spinal fluid obtained 26 months and seven months later, respectively, had identical modal numbers and marker

---

\(^*\)"Chromosome Medium 1A without Phytohemagglutinin" from Grand Island Biological Company.
Fig. 2a.—Case 3. Representative spinal fluid karyotype 21 months after diagnosis.  
Fig. 2b.—Representative bone marrow karyotype 22 months after diagnosis. Note karyotype identical with that shown in Fig. 2a, except for additional supernumerary chromosome.

chromosomes. In case 1, the marrow cells subsequently underwent further clonal evolution, as shown by the development of a second marker and a progressive increase of the modal number from 52 to 55. In case 2 the marker was particularly striking in that it consisted of an acentric fragment which maintained a fixed association with a D group chromosome (Fig. 1).

In case 3, similar evidence of clonal evolution first became apparent in the spinal fluid which showed a marker (Fig. 2 A) not seen in the initial bone marrow, but noted in a single marrow metaphase during partial relapse two months earlier. The initial modal number in the bone marrow was 46. The spinal fluid cells at 21 months showed a mode of 49 (Fig. 3). Subsequent marrow specimens during relapses at 22 and 24 months showed a virtually identical karyotype with the modal number 50 and the marker (Fig. 2 B).
In case 4, the modal number both in the marrow and 25 months later in the spinal fluid was 46. The initial marrow had shown four different markers, each of which was occasionally found in single cells during remission at 13 and 22 months. These and an additional fifth marker were seen in various combinations in the spinal fluid cells.
Case 5 exhibited a Bq - chromosome in the initial bone marrow. The same chromosome was frequently found in single marrow cells during remission over a period of almost two years. This chromosome was present in spinal fluid cells 23 months and 27 months after onset of the disease. Both the initial marrow and the spinal fluid cells showed a mode of 46 with a range from 46–53 and 46–51, respectively. The spinal fluids also showed a marker in the hyperdiploid cells.

In case 6, no initial marrow specimen was available. Spinal fluid cells 28 months after diagnosis had a modal number 53 and a marker (Fig. 1) which persisted in two subsequent episodes of meningeal leukemia, as did the modal number (Fig. 4). As yet, no marrow relapse has been observed in this patient, but interestingly enough, a single 53 cell was found during remission in a bone marrow obtained at 35 months.

In case 7, some spinal fluid cells carried a marker one seen in the marrow at
diagnosis. In this specimen, obtained seven months after diagnosis, a marker two was also seen (Fig. 5 A) and this marker simultaneously appeared in the bone marrow cells (Fig. 5 B).

In case 8, the initial marrow population was bimodal 45/46 without markers. The spinal fluid at 15 months showed only 46 cells. Approximately 40 per cent of these carried a Bq — chromosome.

Case 9 lacked a relapse bone marrow preparation for comparison. The spinal fluid cells 63 months after diagnosis showed a modal number 55. Figure 1 shows a composite of the markers seen in bone marrow and spinal fluid in the group as a whole.

**DISCUSSION**

Whatever the cause of the malignant transformation, the nature of acute leukemia as an autonomous neoplasm is no longer in serious doubt. The leukemic cell population in the bone marrow often exhibits the cytogenetic characteristics of a single stem line which may show further clonal evolution in the course of time. Such findings are consistent with a clonal origin of the leukemic cells from a malignant mutant. If this concept is valid, it is a priori likely that leukemic involvement of the central nervous system is metastatic, rather than the result of an autochthonous new growth, a possibility suggested by the demonstration that the choroid plexus and the leptomeningeal mesenchyme of the human embryo has the potential for hemopoiesis. The identity or similarity of the chromosomal constitution of the leukemic populations in the spinal fluid with those in the bone marrow constitutes direct evidence for a common origin and thus for the metastatic nature of meningeal leukemia. In view of the nonrandom effects of certain drugs and viruses on the chromosomal apparatus observed *in vitro*, the possibility cannot be completely excluded that the unknown leukemogen might produce identical karyotypic abnormalities in bone marrow and spinal fluid cells and that the latter might thus be of separate, local origin. It would be difficult, however, to explain on this basis the finding at both sites of certain large markers, which by their size indicate their formation as the result of translocations, for while breakage of chromosomes and formation of markers due to deletions might be nonrandom, the recombination of the fragments should be largely a matter of chance. An even stronger argument derives from the observation of identical clonal evolution in spinal fluid and bone marrow elements in the course of the disease, for such parallelism of secondary changes would be difficult to explain if two independent cell populations were involved.

The findings are therefore interpreted as evidence for the metastatic origin of the leukemic cells in the spinal fluid and, by extension, in other, less accessible extramedullary sites such as kidney and gonads. By the same token these observations militate against a multicentric origin of human acute leukemia.

**SUMMARY**

The cytogenetic findings in the spinal fluid of nine children with acute leukemia are presented. Identity or similarity of the chromosomal constitution...
of the leukemic populations in the spinal fluid and in the bone marrow was observed. The findings are presented as direct evidence for the metastatic nature of meningeal leukemia.

ACKNOWLEDGMENTS

The authors wish to acknowledge the valuable technical assistance of Mrs. Laura Brown and Mrs. Geraldine Dabney.

REFERENCES

Chromosomes in the Spinal Fluid: Evidence for Metastatic Origin of Meningeal Leukemia

RENATO MASTRANGELO, WOLF W. ZUELZER, PETER S. ECKLUND and RUBY I. THOMPSON