Chromosome Markers and Evidence for Clone Formation in Lymphocytes of a Patient with Sézary Syndrome

Jan C. Liang, Mary Esther Gaulden, and James H. Herndon, Jr.

ABSTRACT

Cytogenetic studies of 222 metaphase lymphocytes stimulated by phytohemagglutinin were carried out on a patient diagnosed clinically as having Sézary syndrome. Twenty-two cells (10%) contained 42 to 100 chromosomes. The remaining 200 cells contained 46 chromosomes and revealed evidence of clone formation; 45 were apparently normal diploid cells, but 155 were pseudodiploid with at least one long submetacentric marker in each cell. This marker was shown to have a consistent banding pattern from cell to cell. Of the 25 pseudodiploid cells karyotyped, there were other types of markers present. Normal chromosomes 2 and 17 were missing in all 25 karyotypes. There were seven sets of two cells, each with an identical karyotype, suggesting subclonal formation. Many of the phytohemagglutinin-stimulated nondiploid white blood cells had one or more nuclear protrusions. Cytogenetic examination of peripheral lymphocytes may be of value in diagnosing and following the course of this disease.

INTRODUCTION

The Sézary syndrome, first described by Sézary and Bouvair (25) in 1938, is a rare disease characterized by chronic erythroderma and the presence of so-called Sézary cells in the peripheral blood and affected skin. The morphological characteristics of these cells have been described by Taswell and Winkelmann (28). Crossen et al. (7) first showed that Sézary cells behave like lymphocytes rather than histiocytes or monocytes as suggested by other investigators (4, 24). The cells do not normally divide but can be stimulated to divide in vitro by the mitogen PHA. Other features provide strong evidence that Sézary cells represent abnormal but partially functional T-lymphocytes (6).

Chromosome abnormalities in the peripheral lymphocytes of a Sézary patient were first reported by Crossen et al. in 1971 (7). Two types of abnormal cells were found: hypotetraploid cells with a large metacentric chromosome (designated as "A" marker); and hypertetraploid cells with a large submetacentric chromosome (designated as "B" marker). So far, only 26 cases of Sézary syndrome have been investigated cytogenetically (1, 5-8, 15, 16, 31), and in 17 of them, chromosomes strikingly similar to one or both of the A and B markers of the patient of Crossen et al. have been the most frequently observed markers.

In the present paper, we present data on a Sézary syndrome patient, some of whose cytogenetic abnormalities resemble those that have been described and some of which have not previously been reported.

CASE HISTORY

The patient was a white male born in 1901. He began having cutaneous symptoms in 1952 to 1953 and was treated with unspecified doses of X-rays (60 kVp) to the right ear and hairline and to both palms. In 1960, he developed a scaly dermatitis on the extensor surfaces of the arms and legs; pain and swelling developed in the small joints of the hands and feet. The diagnosis of psoriasis complicated by psoriatic arthritis was made. After topical medications had given little relief, systemic medication was prescribed, including methotrexate (10 mg p.o. once a week for 4 weeks in April 1968) and hydroxyurea (amounts and exact time of administration not known).

In 1974, he developed marked cracking and splitting of his palms and soles. In January 1975, his WBC rose to 17,300 (normal range, 4,800 to 10,500) with 68% lymphocytes. An internist tentatively diagnosed chronic lymphocytic leukemia but prescribed no treatment. In May 1976, he developed generalized redness with induration especially marked over the legs. By this time, his WBC had risen to 36,500.

In September 1976, Dr. J. H. Herndon, Jr., examined the patient and tentatively diagnosed mycosis fungoides. The patient received 3 injections of methotrexate (25, 50, and 75 mg, respectively) at approximately 2-week intervals. A skin biopsy at this time revealed a dense polymorph dermal infiltrate including large histiocytes with atypical nuclei along with many small cells of mixed type. There were moderate exocytosis of small round cells and several clumps of such cells within the lower epidermis (Pautrier’s microabscesses). In late October 1976, his WBC had risen to 89,000 with 86% lymphocytes, all of which were determined to be T-lymphocytes (40% rosetted with sheep RBC, and the remainder were positive for the ζ-surface antigen). In addition, electron microscopy of thin sections of the patient’s cell buffy coat showed that all lymphocytes contained the cerebriform nuclei characteristic of Sézary cells. It was thus possible to make a positive diagnosis of Sézary syndrome.

After entering a program of intermittent leukapheresis in February 1977, the patient improved clinically and the WBC remained low. He was treated twice weekly from February to April, once a week during May and June, and intermittently in July. In August 1977, his skin condition again worsened in spite of a WBC of 6000. At this time, nitrogen mustard was applied topically to the whole body (10 mg in 50 ml water) once daily for approximately 3 weeks. In late April 1978, he was given whole-body topical electron beam therapy (60 MeV, a...
developed progressively severe edema and disability and died of a myocardial infarction in January 1979.

MATERIALS AND METHODS

Leukocyte-rich samples of blood obtained April 19 and June 28, 1977, immediately following leukapheresis were cultured in A-1 chromosome medium (Difco Laboratories) containing PHA for 72 hr. Colcemid (final concentration, 0.1 µg/ml) was added to the cultures for the final 1.5 hr. Cells were then harvested, treated with 0.075 M KCl, and fixed in absolute methanol:glacial acetic acid (3:1). The slides were air dried (12) and either uniformly stained with Giemsa or treated by Seabright’s trypsin method (23) for G-banding. Analyzed by eye were 222 metaphases (113 from the first sample and 109 from the second sample). Karyotypes were prepared for 25 uniformly stained cells and one G-banded cell.

RESULTS

Because there were no significant differences in the cytogenetic observations in the 2 blood samples analyzed, the results have been combined. Nuclear protrusions, which according to Atkin and Baker (3) are indicative of long marker chromosomes, were frequently found on the nuclei of the PHA-stimulated, nondividing, WBC of this patient (Fig. 1).

The distribution of cells with respect to chromosome number is given in Table 1. Two hundred cells had 46 chromosomes, and the remaining 22 cells were aneuploid to hypotetraploid, with 42 to 100 chromosomes. Of the 200 with 46 chromosomes, 45 (23%) were apparently normal diploid cells, and one each of normal chromosomes 2 and 17 was noted in all cells containing an M1 chromosome marker (M1) appearing in each cell.

The M1 marker has a consistent banding pattern from cell to cell, 3 of which are shown in Fig. 2, inset. One distinct band on the short arm near the centromere and 3 prominent bands on the long arm characterize this marker. The origin of M1 is not obvious, but the fact that one normal chromosome 2 is missing in all cells containing an M1 suggests that the latter may be derived from a chromosome 2. Its exact origin can be determined only by comparing a number of cells.

The banding pattern of the B marker of Crossen et al. (7) has not been previously described. Because the M1 chromosome of our patient appeared to resemble the unbanded B marker, the 2 marker chromosomes were compared for arm ratios (p:q, short:long) and length relative to those of the normal chromosome 1. Both the arm ratios, 0.37 for the B marker and 0.39 for the M1, and the relative lengths, 1.13 for the B marker and 1.11 for the M1 chromosome, are remarkably similar.

Six markers in addition to the M1 were observed in this cell. It should be noted that clear banding was obtained in a normal subject’s lymphocytes processed at the same time. There were some repetitive patterns of chromosome loss; e.g., the absence of one each of normal chromosomes 2 and 17 was noted in all 25 karyotypes. Also, either a chromosome 1 or 3 or both were absent; in 3 cells, both of the normal chromosomes 3 were absent. The presence of 7 sets of 2 cells with an identical karyotype suggests subclonal formation. Cells with ring chromosomes, dicentrics, and minutes that have been reported in some Sézary patients (5, 16, 31) were not observed in our patient.

DISCUSSION

This paper reports the first case in which nuclear protrusions have been observed in a Sézary syndrome patient. Since Ruddle (21) first described nuclear protrusions on interphase nuclei of an established line of pig kidney cells, they have been observed on nuclei of both tumor and nontumorous cells by other investigators (2, 11, 14, 27). It is believed that nuclear protrusions are indicative of the presence of long marker chromosomes (3). This proved to be true for the present case.

Most cases of Sézary syndrome previously reported were of the large-cell type, characterized by a near-tetraploid chromosome complement (7, 16, 31). A small-cell type with pseudodiploid or hyperdiploid chromosome number was first described by Lutzner et al. (16). Since the majority of the cells in our patient have a diploid-range chromosome number, our patient’s disease appears to be of the small-cell type.

The B marker of Crossen et al. (7) is by far the most frequent one that has been found in Sézary patients (7, 16, 31). As noted above, it is quite similar to the M1 chromosome in our patient. The presence of the M1 always accompanied the absence of one normal chromosome 2, which suggests that the marker may be derived from a chromosome 2. Its exact origin can be determined only by comparing a number of cells with clear banding patterns.

The difficulty we experienced in obtaining good banding of the chromosomes of Sézary cells has also been reported in 15 of the 19 patients to whose chromosomes banding methods have been applied (1, 5, 8, 16, 31). In some patients, clear bands could be obtained on chromosomes of the apparently normal cells but not on those of the heterodiploid cells containing markers (1, 8, 16). Although the reason for the refractoriness of the chromosomes of some Sézary cells to banding is not obvious, the fact that 6 groups of investigators, including us, have observed this unusual phenomenon suggests that it may be real. We were not able to explore it further with our patient because additional blood samples could not be obtained.

Consistent loss of one chromosome 17 was noted in the aberrant cells of our patient as well as in 3 other Sézary

<table>
<thead>
<tr>
<th>Cells</th>
<th>No. of chromosomes</th>
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<tbody>
<tr>
<td>200(90)*</td>
<td>46</td>
</tr>
<tr>
<td>7 (3)</td>
<td>42-45</td>
</tr>
<tr>
<td>4 (2)</td>
<td>47-48</td>
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<tr>
<td>11 (5)</td>
<td>60-100</td>
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Table 1

Chromosome complements in 222 lymphocytes of a Sézary syndrome patient

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patients (16, 31) and in 7 mycosis fungoides patients (9). Both of these lymphoproliferative syndromes are now regarded as cutaneous T-cell lymphomas. Frequent loss or gain of one or more E-group chromosomes has been reported in patients with various kinds of lymphomas (17, 26). It has been hypothesized by Spiers and Baikie (26) that chromosome 17 abnormalities play a special role in the genesis and/or evolution of neoplasia, particularly in lymphoid and reticuloendothelial tissues. In this connection, it is of interest that the gene for thymidine kinase, which regulates nucleic acid metabolism, is located on the chromosome 17 (20). Aberrations in chromosome 17, either in structure or number, may provide a selective growth advantage which promotes neoplasia (17, 18).

Aneuploidy has been reported in all seven patients with established small-cell Sézary syndrome who have been examined cytogenetically (16, 31). The relationship between aneuploidy and prognosis of AML has been investigated by Sakurai and Sandberg (22) in 88 patients. The mean survival time of those patients with only aneuploid metaphases in their bone marrow was 1.2 months, that of those with both diploid and aneuploid metaphases was 7.2 months, and that of those with only diploid metaphases was 9.1 months. The results of our study and those of others show that all the Sézary patients have at least some fraction of cells with an apparently normal karyotype. The long benign course of this disease may be due to normal functioning of these cells. All types of aneuploidy have been found in the Sézary patients studied thus far: pseudodiploidy (our study and Ref. 16); hyperdiploidy (16, 31); and hypodiploidy (8). In a study of 170 adults with acute leukemia (including 143 with AML), Trujillo et al. (29) found that the mean survival time varied with the type of ploidy. For hyperdiploid cases, it was 49 weeks; for the pseudodiploids, it was 36 weeks; and for the hypodiploids, it was only 13 weeks. Whether the aneuploidy found in the Sézary syndrome will have the same prognostic significance as that in AML still remains to be determined when additional data are available.

Evidence for clone formation has been reported in only 4 out of 26 Sézary patients who have previously been examined cytogenetically (1, 7, 16, 31). In our patient, the fact that all 155 pseudodiploid cells had the same marker, M1, is evidence for but not proof of their derivation from an initial clone with this abnormal chromosome. The 7 sets of 2 cells each with an identical karyotype suggest subclone formation in this patient.

Methotrexate, hydroxyurea and X-rays, which were given to the patient prior to our study, have been reported to produce chromosome breaks and other structural abnormalities (13, 19, 30). Although we cannot rule out the possibility that these mutagens may have produced in stem cells all of the chromosomal abnormalities reported in this paper, the fact that some strikingly similar marker chromosomes have also been found in untreated patients (16, 31) renders this possibility less probable. Banded chromosome studies on additional Sézary patients prior to treatment are needed to help clarify this point.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. Some nuclear protrusions seen on the lymphocytes of Sézary syndrome patient. × 3750.

Fig. 2. A G-banded karyotype of a Sézary cell. Inset, 3 M1 chromosomes from 3 other pseudodiploid cells. × 9700.
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