A Chromosomal Breakage Syndrome With Profound Immunodeficiency

By Mary Ellen Conley, Nancy B. Spinner, Beverly S. Emanuel, Peter C. Nowell, and Warren W. Nichols

The chromosomal breakage syndromes—ataxia-telangiectasia, Fanconi’s anemia, and Bloom’s syndrome—are associated with growth failure, neurologic abnormalities, immunodeficiency, and an increased incidence of malignancy. The relationship between these features is unknown. We recently evaluated a 21-year-old female with more severe chromosomal breakage, immunodeficiency, and growth failure than in any of the mentioned disorders. As of November 1985, the patient remains clinically free of malignancy. At age 18, the patient’s weight was 22.6 kg (50th percentile for seven years), height was 129 cm (50th percentile for eight years), and head circumference was 42 cm (50th percentile for six months). Laboratory studies demonstrated a marked decrease in both B and T cell number and function. The peripheral blood contained 400 to 900 lymphocytes/μL with 32% T11 cells, 17% T14 cells, and 21% T14 cells. The proliferative responses to phytohemagglutinin (PHA), pokeweed mitogen, and concanavalin A were less than 10% of control. There were 1% surface IgM positive cells, and serum IgG was 185 mg/dL, IgM 7 mg/dL, IgA 5 mg/dL. In lymphocyte cultures stimulated with the T cell mitogens PHA, phorbol ester, and interleukin 2, 55% of the banded metaphases demonstrated breaks or rearrangements. The majority of the breaks involved four fragile sites on chromosomes 7 and 14, 7p13, 7q35, 14q11, and 14q32. These are the sites of the genes for the T cell-antigen receptor and the immunoglobulin heavy chain and are sites of gene rearrangement in lymphocyte differentiation. Epstein–Barr virus stimulated B cells and fibroblast cultures also demonstrated a high incidence of breaks, but the sites were less selective. These findings suggest that the sites of chromosomal fragility in the chromosomal breakage syndromes may be informative and that factors other than the severity of the immunodeficiency or the high incidence of chromosomal damage may contribute to the occurrence of malignancy in the chromosomal breakage syndromes.

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The chromosomal breakage syndromes—ataxia-telangiectasia, Fanconi’s anemia, and Bloom’s syndrome—have several features in common. All are disorders inherited in an autosomal recessive pattern in which spontaneous chromosomal breaks, gaps, and rearrangements are seen in mitogen-stimulated T cells. Although each syndrome can be characterized by distinct clinical and laboratory findings, all have been associated with growth failure, microcephaly, neurologic abnormalities, immunodeficiency, failure to develop secondary sexual characteristics, and an increased incidence of malignancy. The relationship between these features is unclear. It has been postulated that the increased incidence of malignancy may be related to inadequate immune surveillance or immune deficiency. Alternatively, the spontaneous chromosomal breaks may result in an increased rate of mutation, which in turn may result in malignancy.

We recently studied a 21-year-old patient who does not have one of the well-described chromosomal breakage syndromes, who has significant chromosomal instability characterized by spontaneous breaks, gaps, and rearrangements involving multiple chromosomes in T cells, Epstein-Barr virus (EBV)-transformed B cell lines, and cultured fibroblasts. In the T cells, the breaks selectively involve four fragile sites on chromosomes 7 and 14 as has been seen in ataxia-telangiectasia. In the B cell lines and fibroblasts, the abnormalities are less selective. In addition, the patient has profound immunodeficiency involving both B and T cells. At present, the patient has had no malignancies, and there is no family history suggesting an unusual predisposition to malignancy.

CASE REPORT

D.M. was the 5 lb 6 oz product of a full-term uncomplicated pregnancy delivered without problems to a 35-year-old primagravida. Both parents were of eastern European ancestry without consanguinity for at least four generations. The patient was noted to have poor growth and microcephaly within the first three months of life. Between 4 and 6 years of age, the patient developed a chronic cough. Hypogammaglobulinemia was diagnosed at 6 years of age and the patient has been treated with immunoglobulin replacement since that time. At 8 years of age, she was evaluated for short stature. She was noted to be lymphopenic with decreases in both B and T cell numbers; proliferative responses to mitogens were poor. Serum IgG was 120 mg/dL, IgM was 20 mg/dL, and IgA was undetectable. Karyotype analysis was difficult because of poor T cell proliferation but suggested a constitutional abnormality of chromosome 7. The patient was lost to follow-up but continued to receive intramuscular gammaglobulin. In 1983, at age 18, the patient was referred to Children’s Hospital of Philadelphia for evaluation of hypogammaglobulinemia. In the preceding ten years, the patient had had poor growth and a persistent cough but had not required hospitalization for infection or other problems. She had no history of skin rashes, sun sensitivity, telangiectasia, ataxia, or anemia. She had not reached menarche. The patient had completed sophomore year in a school for learning disabled children. Both parents appeared to be of dull normal intelligence; there were no other children. The patient’s maternal grandfather may have died of cancer at age 66. There was no other history of malignancy, unusual infections, or fetal wastage in more than 40 first- and second-degree relatives of the patient and her parents.

On physical examination, the patient was a pale, small, pleasant girl with marked microcephaly. Height was 129 cm (<3rd percentile, 50th percentile for 6-month-old). The skin was pale and doughy without rashes, telangiectasia, or areas of increased}

From the Departments of Pediatrics, Pathology and Laboratory Medicine, and Human Genetics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia.

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Address reprint requests to Dr Mary Ellen Conley, Immunology, Children's Hospital of Philadelphia, 34th & Civic Center Boulevard, Philadelphia, PA 19104.

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or decreased pigmentation. The faces were birdlike, with a prominent, beaked nose and recessed chin. The ears were low, and there was hypoplasia of the dental enamel. The tympanic membranes appeared scarred. The tonsils were small, and there was no cervical lymphoid tissue palpable. Chest examination revealed bilateral rales. There were no murmurs or unusual heart sounds. The liver and spleen were not felt. There was scant straight pubic hair and no breast tissue. Neurologic examination was normal.

On complete blood count, the patient had a hemoglobin of 14.6 g/dL and a hematocrit of 43% with normal red cell indices, WBC was 14,400/μL, with 82% neutrophils, 5% bands, 5% lymphocytes, 1% eosinophils, and 7% monocytes. Platelet count was 575,000/μL. IgG was 185 mg/dL, and IgA was less than 5 mg/dL. Chest x-ray demonstrated bibasilar infiltrates with increased interstitial markings throughout both lung fields and peribronchial thickening. She had bilateral opacification of maxillary and frontal sinuses. Alpha-fetoprotein levels were 185 μg/dL (on gammaglobulin therapy), 1gM was 7 mg/dL, and IgA was less than 5 mg/dL. Chest x-ray demonstrated bibasilar infiltrates with increased interstitial markings throughout both lung fields and peribronchial thickening. She had bilateral opacification of maxillary and frontal sinuses. Alpha-fetoprotein level was normal at 10 ng/mL. Antinuclear antibodies, anti-red cell antibodies, and antibodies to thyroid, smooth muscle, and mitochondria were not present. Somatomedin C was normal. Follicle-stimulating hormone was 40 mIU/mL and luteinizing hormone was 50 mIU/mL. These values are consistent with an ovulatory phase or postmenopausal phase but not normal premenarche.

MATERIALS AND METHODS

Peripheral blood lymphocytes were obtained from heparinized venous blood by Ficoll-Hypaque centrifugation. To assess proliferation in response to standard mitogens, lymphocytes were cultured at 0.5 x 10⁶ cells per milliliter in round-bottom microtiter wells for 72 hours with 5% phytohemagglutinin (PHA) (GIBCO, Grand Island, NY), 5% pokeweed mitogen (GIBCO), or 30 μg/mL concanavalin A (DIFCO, Detroit) and 15% heat-inactivated human serum. Four hours before the cells were harvested, 1 μCi 3H-thymidine was added (expressed as cpm ³H-thymidine). Immunofluorescence staining of lymphocytes was performed as previously described. Cells were enumerated with a fluorescence microscope equipped with epi-illumination (Carl Zeiss, Oberkochen, FRG).

Metaphase chromosomes were obtained from peripheral blood lymphocytes stimulated with PHA or with a mixture of T cell mitogens (PHA, phorbol ester, and interleukin 2⁵). All fragility studies were conducted on cells harvested after 48 hours. To assess bleomycin sensitivity, 30 μg/mL of bleomycin was added 2.5 hours before the end of the culture period. Sister chromatid exchange rates in the presence and absence of mitomycin C (25 ng/mL) were determined by standard methods. X-ray-induced chromosomal aberrations were studied by irradiating lymphocytes at three dose levels—100 rad, 200 rad, and 400 rad—before PHA stimulation. Permanently cell lines from fibroblasts (GM7166) and B lymphocytes (GM7078) were established by the mutant cell repository (Institute for Medical Research, Camden, NJ).

RESULTS

Immunologic studies. The patient has persistent lymphopenia with absolute lymphocyte counts between 400 and 900/μL first noted in early childhood. Lymphocyte enumeration, using indirect immunofluorescence staining techniques, demonstrated marked reduction of all lymphocyte subsets (Table 1). Cells bearing the T cell helper phenotype (T4) were more reduced in number than cells expressing the T cell cytotoxic-suppressor phenotype (T8), resulting in a reversal of the normal T4/T8 ratio (0.8 in the patient compared with 1.6 ± 0.3 in healthy laboratory controls). B cells could be detected with a monoclonal anti-B cell anti-body, B1, or with fluorescein-conjugated antihuman IgM. Further analysis of the B cell phenotype using two-color immunofluorescence staining demonstrated that all of the cells expressing surface IgM also expressed surface IgD; 52% of the B cells could be indirectly stained with the monoclonal antibody B2, which binds to the EBV receptor. In contrast, 89% ± 6% of B cells from controls express the B2 marker. Kappa-lambda ratio was also abnormal. Eighty percent of the patient's B cells expressed kappa and 20% expressed lambda light chain. In healthy controls, B cells are almost equally divided between cells bearing kappa and cells bearing lambda. The patient's lymphocytes did proliferate in response to standard mitogen stimulation, but the response was less than 10% of the control assayed concurrently.

Cytogenetics studies. Chromosome analysis of the pro-band's peripheral T lymphocytes on unbanded chromosomes revealed a high level of spontaneous aberrations, with 38% of the metaphases having abnormalities, including breaks, rings, dicentrics,acentric fragments, and translocations (Table 2). The incidence of aberrant metaphases was even higher in Giemsa-banded material. Of 81 cells karyotyped, 29 had rearrangements involving chromosome 7 or 14. Another 16 cells had rearrangements that appeared to be random but did not involve chromosome 7 or 14, for a total of 55% aberrant metaphases.

In the cells with rearrangements of chromosome 7 or 14, there was clustering of breakpoints at 7p13, 7q35, 14q11, and 14q32 (Fig 1). Among these were three cells with inv(7)(p13q15), four with inv dup(7)(q35q11), three with t(7;14)(q34q11.2), and a small clone of eight cells with t(14;14)(q11q32) (Fig 2). Treatment of the patient's cells with bleomycin during the G2 phase of the cell cycle resulted in an increase in chromosome aberrations, as compared with controls (Table 2). A similar increase has been reported in cells from patients with ataxia-telangiectasia. Comparable studies, with and without bleomycin, were carried out on the patient's parents. As seen in Table 2, there was no increase in spontaneous fragility in the parents, but the data suggest increased sensitivity to bleomycin, as is seen in obligate heterozygote carriers of ataxia-telangiectasia.

<table>
<thead>
<tr>
<th>Table 1. Immunologic Studies</th>
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<tr>
<td><strong>Patient</strong></td>
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<tr>
<td><strong>Absolute Number</strong></td>
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<tr>
<td><strong>%</strong></td>
</tr>
<tr>
<td><strong>Total lymphocytes</strong></td>
</tr>
<tr>
<td><strong>T cells (T4)</strong></td>
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<tr>
<td><strong>T helpers (T4)</strong></td>
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<tr>
<td><strong>T suppressors (T8)</strong></td>
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<tr>
<td><strong>B cells (B2)</strong></td>
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<tr>
<td><strong>Surface IgM</strong></td>
</tr>
<tr>
<td><strong>PHA</strong></td>
</tr>
<tr>
<td><strong>Pokeweed</strong></td>
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<tr>
<td><strong>ConA</strong></td>
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<tr>
<td><strong>Background</strong></td>
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*Expressed as cpm ³H-thymidine.
SITE-SPECIFIC CHROMOSOMAL BREAKAGE

Table 2. Frequency of Aberrations in Unstressed Lymphocytes

<table>
<thead>
<tr>
<th>Total No. of Breaks</th>
<th>Metaphases with Aberrations (%)</th>
<th>Complex Rearrangements</th>
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<tbody>
<tr>
<td></td>
<td>Total Metaphases</td>
<td>Breaks (per cell)</td>
</tr>
<tr>
<td>Patient</td>
<td>73</td>
<td>28(38.3%)</td>
</tr>
<tr>
<td>Mother</td>
<td>101</td>
<td>5(5.0%)</td>
</tr>
<tr>
<td>Father</td>
<td>164</td>
<td>9(5.5%)</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>2(2.0%)</td>
</tr>
</tbody>
</table>

Frequency of aberrations in lymphocytes exposed to bleomycin for 2.5 hours in G2

| Patient             | 88                              | 75(85%)                | 207(2.4)       | 12(14)    | 219(2.5) | 83(94) |
| Mother              | 103                             | 47(46%)                | 125(1.2)       | 0(0-0)    | 125(1.2) | 80(78) |
| Father              | 118                             | 54(46%)                | 114(1.9)       | 11(01)    | 155(1.9) | 66(56) |
| Control             | 100                             | 22(22%)                | 28(28)         | 0(0-0)    | 28(28)  | 26(26) |

The control was studied concurrently with the patient. In controls studied in our laboratory, the range for breaks/cell in unstressed lymphocytes was from 0 to 0.07. When control lymphocytes were stressed with bleomycin, the range was from 0.28 to 0.71 breaks/cell.

Sister chromatid exchange (SCE) studies in the presence and absence of mitomycin C were done on peripheral lymphocytes from the proband and her parents. SCE levels in all instances were within the expected range for our laboratory.

To examine chromosomal fragility in B cells, EBV-stimulated B cell cultures were studied on three occasions in two laboratories. A total of 178 metaphases were examined, and in all instances, there was a high frequency of metaphases with aberrations, ranging from 26% to 50%. Alterations in chromosomes 7 and 14 were found in 12% of banded metaphases but usually involved terminal associations, producing dicentric chromosomes, rather than the nonrandom breakpoints observed in the T cell cultures. SCE studies on the B cell cultures, with or without mitomycin C, were within normal limits for the laboratory.

A fibroblast culture from the patient was established. Of 15 metaphases that were karyotyped, five demonstrated rearrangements (33%), and none of these involved chromosomes 7 and 14. SCE studies were again normal.

The effect of x-irradiation on the cells of the patient and her parents were studied. There was no evidence of increased sensitivity in the parents' peripheral blood T cell cultures or in their long-term B cell lines. The T cells, B cells, and fibroblasts from the patient showed elevated levels of chromosomal breakage on exposure to x-irradiation, but when corrected for spontaneous breakage, the degree of damage was within normal limits. However, there was increased sensitivity to cell killing induced by x-irradiation. At the highest dose (400 rad), mitotic activity was virtually eliminated from the T cell cultures. A more detailed study of the x-ray sensitivity of the patient's fibroblasts, and comparison with the findings in ataxia-telangiectasia, will be published separately.18a

DISCUSSION

Although the patient described in this report has several of the findings seen in all the chromosomal breakage syndromes, she does not have the cardinal features seen in any one of these disorders. She does not have the rash or sun sensitivity seen in Bloom's syndrome, the telangiectasia or ataxia characteristic of ataxia-telangiectasia, or the pancytopenia or bony abnormalities typical of Fanconi's anemia. Her DNA repair defects are most similar to those seen in ataxia-telangiectasia.18 However, her immunodeficiency and spontaneous chromosomal breakage are more profound than that seen in any of the well-described syndromes.

The immunologic defects in each of the chromosomal breakage syndromes are variable and may progress with age. The best documented and most consistent abnormalities are seen in ataxia-telangiectasia in which serum IgA and IgE are usually absent, lymphocyte numbers, particularly T cells, are decreased, and proliferative responses to mitogens are decreased to a mean of 60% of normal. Patients with Bloom's syndrome or Fanconi's anemia may have mildly decreased concentrations of serum immunoglobulins, but proliferative responses to PHA are usually normal.7,8,11 In contrast, our patient has markedly decreased T cell numbers and a proliferative response to mitogens that is less than 10% of control. She also has panhypogammaglobulinemia and low numbers of B cells. The B cells that are present express an immature phenotype similar to that seen in X-linked agammaglobulinemia.14

The degree of chromosomal breakage in each of the three syndromes is also variable and is influenced by culture and
Fig 2. Schematic diagram of chromosomes 7 and 14, with each circle indicating the involvement of a specific site in a chromosomal rearrangement.

assay techniques and the origin of the cells examined. However, there are specific cytogenetic findings typical for each syndrome. Lymphocytes from patients with ataxia-telangiectasia are hypersensitive to x-irradiation and to the radiomimetic agent bleomycin, and often demonstrate cytogenetically abnormal clones, frequently involving chromosomes 7 and 14. Bloom's syndrome is characterized by an increase in quadriradial figures and an increase in spontaneous SCEs. In Fanconi's anemia, there are increased spontaneous breaks and increased breaks and radial figures after exposure to DNA cross-linking agents, such as mitomycin C. By cytogenetic studies, our patient is most similar to patients with ataxia-telangiectasia. She has increased spontaneous chromosome aberrations in lymphocyte cultures, abnormal chromosomes, including translocations and dicentrics, increased sensitivity to bleomycin, and an apparently abnormal clone involving both chromosomes 14. She has normal levels of SCE and does not have increased levels of quadriradial exchange figures. However, there are differences between our patient and patients with ataxia-telangiectasia. EBV-stimulated B cell lines from patients with ataxia-telangiectasia have not demonstrated increased chromosomal breakage, whereas 26% to 50% of the metaphases from our patient's B cell lines were abnormal.

In patients with ataxia-telangiectasia, in our patient, and in other patients in whom the site of chromosomal breakage has been carefully examined, chromosomal breaks in mitogen-stimulated T cells selectively involve four sites. At least three of these four locations—7q35, 14q11, and 14q32—are now recognized as sites of gen rearrangement during lymphocyte differentiation. The genes for the β and α chain of the T cell-antigen receptor are at 7q35 and 14q11, respectively. The immunoglobulin heavy chain gene is at 14q32. Preliminary evidence suggests that the fourth frequently involved fragile site, 7p13, is the site of a gene showing homology to the T cell receptor β chain gene and may be the T cell receptor γ chain gene. One might expect other sites of chromosomal fragility in cell lines not using the antigen receptor genes. Accordingly, chromosome breaks in fibroblast cell lines from our patient and patients with ataxia-telangiectasia do not selectively involve chromosomes 7 and 14. Current tissue culture techniques permit the long-term growth and proliferation of a variety of cell types, including melanocytes, glial cells, and thymic epithelial cells. Cell lines from patients like ours may demonstrate tissue-specific chromosome fragility sites, marking genes of particular importance in that cell line.

Variants of the chromosomal breakage syndromes have been described. These have usually been in patients who demonstrate some but not all of the clinical features of a particular syndrome. Webster et al described a patient similar to ours, an 18-year-old girl with growth failure, chronic pulmonary disease, selective IgA deficiency, lymphopenia with poor proliferative responses to mitogens, and defective DNA repair. However, spontaneous chromosomal breakage was not described. A group from the University of Nijmegen, in The Netherlands, has reported findings on two brothers with microcephaly, growth retardation, mental retardation, café au lait spots, dysgammaglobulinemia, and chromosomal instability, particularly involving chromosomes 7 and 14. These boys may have the same disorder as our patient, although our patient has more profound abnormalities of the immune system on laboratory testing and more profound growth failure. Seemanova et al also described a group of patients with a disorder resembling that seen in our patient. These nine patients had microcephaly with normal intelligence, immunodeficiency, and an increased incidence of malignancy. Although the authors state that chromosome instability did not appear to be a problem, they also note that "chromosome analysis was
repeatedly unsuccessful owing to failure of blast transformation."

The well-described chromosomal breakage syndromes are inherited in an autosomal recessive pattern. Lymphocytes from both parents of our patient appeared to have increased sensitivity to chromosomal damage by bleomycin, suggesting a similar pattern of inheritance. Of interest, the patient’s mother has IgA deficiency (<5 mg/dL) and borderline microcephaly (head circumference 53 cm).

A feature that makes our patient of particular interest is that despite her profound immunodeficiency and her high incidence of chromosomal breakage, she has not developed a malignancy. The increased frequency of malignancy in the chromosomal breakage syndromes, which usually occurs before age 20, has been attributed to both the immunodeficiencies associated with these disorders and the chromosomal breaks. Our patient’s relatively benign clinical course suggests that the equation may be more complex. Mice treated with irradiation at high dose rate develop a greater number of chromosomal breaks in liver cells than do mice irradiated at low dose rate. However, the incidence of hepatoma and ovarian adenoma is higher in animals irradiated at low dose rate. The extensive chromosomal damage after irradiation at high dose rate may render many potential cancer cells incapable of mitosis. A similar phenomenon may be occurring in our patient, with her marked chromosomal fragility eliminating some potentially neoplastic cells, and perhaps also contributing to the severe degree of immunodeficiency and growth retardation. Additional evidence concerning these speculations may be provided by the subsequent history of the small clone of T cells with a t(14;14)(q11;q32) translocation, now present in her peripheral blood (Fig 2). In ataxia-telangiectasia, progression of such clones to T cell leukemia, with additional karyotypic changes, has been described.

The numerous questions raised by the study of this patient indicate the need for more multidisciplinary investigations of such atypical “fragility syndromes.” Not only may additional specific disorders be precisely defined, but also cells from such patients can provide an important contribution that will supplement material from the classic “breakage syndromes” in the study of specific genes and chromosomal mechanisms involved in normal and abnormal growth regulation.

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