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Somatic Rearrangement of Chromosome 14 in Human Lymphocytes

(leukemia/lymphoproliferation/ataxia-telangiectasia)

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ABSTRACT **Ataxia-telangiectasia is a rare genetic disorder associated with immune deficiency, chromosome instability, and a predisposition to lymphoid malignancy. We have detected chromosomally anomalous clones of lymphocytes in eight patients with this disorder. Chromosome banding disclosed that the clones are consistently marked by structural rearrangement of the long arm (q) of chromosome 14. A translocation involving 14q was found in clones obtained from seven of the eight patients whereas a ring 14 chromosome was found in a clone obtained from the other. These findings as well as data obtained by others for patients with ataxia-telangiectasia suggest that structural rearrangement of 14q is the initial chromosomal change in lymphocyte clones of patients with this disorder.**

Chromosomes of lymphocytes from one of the patients were studied before *and* after the onset of chronic lymphocytic leukemia. Before leukemia was diagnosed, the patient had a lymphocyte clone with a 14q translocation. This clone appears to have given rise to the leukemic cells.

We hypothesize that structural rearrangement of 14q is directly related to abnormal growth of lymphocytes and that it may be a step toward the development of lymphoid malignancies. Increasing evidence, provided by others, for the nonrandom involvement of 14q in African-type Burkitt's lymphoma and other lymphoid neoplasms further strengthens this hypothesis.

Early in this century, Boveri postulated that cancer represented the escape of a cell from the normal regulation of proliferation. The mechanism of escape was postulated to be through changes in the chromosome constitution within the nucleus of the cell (1). Interest in this idea has waxed and waned. Recently some workers, such as Burnet, taking the same general view, suggest that cancer is the end-result of a "sequence of somatic mutations expressed in the capacity of a cell to proliferate without experiencing normal morphogenetic control" (2). The nature of the somatic mutations, however, has remained largely conjectural.

One detectable somatic mutation in cancer is the Philadelphia chromosome found in patients with chronic myeloid leukemia (3, 4). Further evidence for tumor-specific chromosome changes has been found in meningiomas, some of which lack a chromosome 22 (5), and in African-type Burkitt's lymphomas, the majority of which have an extra band in the long arm of chromosome 14 (6).

We have studied lymphocytes from patients with ataxia–telangiectasia (A–T), a rare autosomal recessive condition associated with chromosome instability (7) and a predisposition to lymphoid malignancy (8). Previous longitudinal studies of benign lymphocytes in a patient with A–T, showed a clone marked by a translocation involving both chromosomes 14 (9). We have now detected similar clones of chromosomally marked lymphocytes in seven other patients with this disorder.

The clones consistently show rearrangement of the long arm (q) of chromosome 14. The break points in this chromosome are within a specific region, and there is no obvious loss or gain of chromosome material. One patient with a translocation clone, whom we studied, developed chronic lymphocytic leukemia. It is intriguing to note that the leukemic cells appear to stem from the preexisting translocation clone.

These and related data from other laboratories suggest that the 14q rearrangement in A–T lymphocyte clones may be a consistent chromosome finding. We here report our observations to date and postulate that (*i*) the 14q rearrangement may be a key premalignant change in A–T lymphocytes and (*ii*) structural rearrangement of 14q may be a change which is a common denominator in many kinds of premalignant and malignant clones of lymphoid cells in humans.

MATERIALS AND METHODS

Patients. All patients had well-documented A–T with progressive cerebellar ataxia, oculocutaneous telangiectases, immunodeficiency, and pedigrees consistent with autosomal recessive inheritance (10). None of the patients were known to be related to one another, except for cases S and T, who are sisters.

Cell Culture and Preparation. Lymphocytes from heparinized blood obtained from patients with A–T, were cultured with phytohemagglutinin. A culture time of 72 hr was selected because previous studies had shown that A–T lymphocytes, which typically react poorly to mitogens, respond maximally at about 72 hr and usually provide sufficient metaphases for analysis (11). Cells in metaphase were accumulated by adding colchicine 1.5–2 hr before harvest; they were treated with hypotonic KCl (0.075 M), fixed in methyl alcohol:acetic acid (3:1), dropped onto glass slides, and air dried.

Staining, Photography, and Chromosome Analysis. Slides were stained with quinacrine mustard for Q-banding (12) or by the acetic-saline-Giemsa method for G-banding (13). Cells were photographed with a Zeiss photoscope equipped for light and fluorescent microscopy with an HB 200 mercury light source. Photographic enlargements (2400×) were scored for chromosome number, breaks, and morphology. Karyotypes were arranged according to the Paris Conference (14).

Abbreviation: A–T, ataxia–telangiectasia; A–T lymphocyte· lymphocyte from patient with A–T; A–T clone, two or more lymphocytes marked by identical chromosome changes from patient with A–T.

TABLE 1. *Lymphocyte clones in with ataxia-telangiectasia patients*

Case	Chromosomes banded	Age (years) At first study	Age (years) At most recent study	Lymphocyte clones Karyotype	Lymphocyte clones Percent* at first study	Percent* at most recent study	Ref.
A	−	38	—	45,XX,−D,t(D;D)	100	—	15
B	−	17	—	45,XY,−D,Dq+	30	—	16
C	−	16	—	46,XY,Dq+	65	—	16
D	−	16	16	46,XY,Dq+	80	86	17
E	−	10	—	46,XY,t(Dq+;?)	5	—	18
F	−	10	11	46,XX,t(Dq−;Cq+)	8	10	19
G	−	21	22	46,XX,Dq−	10	12	19
H	−	11	12	46,XY,t(Dq−;Cq+)	6	12	19
I	−	6	—	46,XX,t(Dq−;Cq+)	5	—	19
J	−	10	—	47,XY,+C	7	—	19
K	−	7	8	46,XX,t(Dq−;Dq+)	8	16	19
L	−	14	—	47,XX,+C	6	—	19
M	−	15	16	46,XX,t(Dq−;Dq+)	8	10	19
N	+	16	—	46,XY,t(14q−;6q+)	—	—	—
O	+	8	—	46,XX,r14	60	—	—
P	+	19	23	46,XY,t(14q−;14q+)	1	80	20
Q	+	17	20	46,XX,t(14q−;7q+)	0	3	20
R	+	10	—	46,XY,t(14q−;7p+)	2	—	20
S	+	25	27	46,XX,t(14q−;Xq+)	80	69	21
T	+	16	18	46,XX,t(14q−;14q+)	1	38	21
U	+	7	—	46,XY,14q+	29	—	—
V	+	24	31	46,XX,t(14q−;14q+)	21	100	—

The D group includes pairs 13–15. The symbols "p" and "q" refer to the short and long arms, respectively. The symbol "t" stands for translocation. Cases Q and R are mentioned in the addendum to ref. 9. Cases S and T are mentioned in a book chapter (21).

* Based on various numbers of cells (range 10–100).

RESULTS

Proliferation of lymphocytes marked by a chromosome 14 translocation was first observed in clones obtained from a patient with A–T (case P) in whom the percent of lymphocytes containing the translocation increased from 1% to greater than 80% during a 4-year period (9). The patient died of progressive pulmonary disease and never manifested malignancy during life or at autopsy.

Lymphocyte clones with comparable chromosome markers have now been detected in a number of patients with A–T. Data on the original patient, seven others whom we have studied, and 14 additional patients reported from other centers, are presented in Table 1.

The clones all involve rearrangement of a D-group chromosome (chromosomes 13–15) except possibly in two cases (J, L) in which differential staining was not done so that D-chromosome involvement remains a possibility. In cases (N–V) where precise chromosome identification was done, all clones were marked by a structural rearrangement of the long arm of chromosome 14. Several examples (cases P–T) are shown in Fig. 1.

The structural rearrangements were clear-cut translocations with two exceptions: an elongated chromosome 14 in case U, in which the origin of the extra material is unknown; and a ring 14 in case O, which presumably arose following partial deletion of the ends of the short and long arms of a no. 14 chromosome.

We analyzed the translocations in six cases (P–T, V) and found the breakpoints in chromosome 14 to be clustered in the q12 band (Fig. 2). Loss or gain of chromosome material was not detectable by measurement (22). The other chromosome participating in the translocation process is most frequently the no. 14 homolog. The 6, 7, and the X chromosomes were identified as the other participants.

The proportion of lymphocytes containing a chromosome 14 rearrangement varied from case to case. There is generally an increase with time in the percentage of lymphocytes carrying the chromosome 14 translocation in patients who have been studied longitudinally (Table 1).

Additional evidence that the phenomenon represents a dynamic process is the frequent emergence of a sub-population of lymphocytes containing the original 14 translocation plus one or more additional chromosome rearrangements. For example, in case S, a subclone was found in which the remaining proximal portion of the 14 was translocated to a chromosome 22, forming a dicentric chromosome. A striking increase in dicentric chromosomes within an A–T translocation-marked clone has been noted previously (9), but no satisfactory explanation can be proposed at this time.

One of the patients with A–T listed in Table 1 (case V) has chronic lymphocytic leukemia. In 1967, prior to the clinical onset of leukemia, chromosome studies of lymphocytes showed a minor population (29/136 = 21% of metaphases) with a 14;14 translocation identical in morphology to that in our first case. The leukemic lymphocytes have the same translocation. In addition to the original 14;14 translocation, the leukemic cells contain other chromosome rearrangements (Fig. 3). The modal number of chromosomes is 41 (range 39–42) at the present time.

DISCUSSION

Chromosome Rearrangements in A–T Lymphocytes. The pattern of chromosome changes in A–T lymphocyte clones is clearly nonrandom. The changes tend to involve chromosome

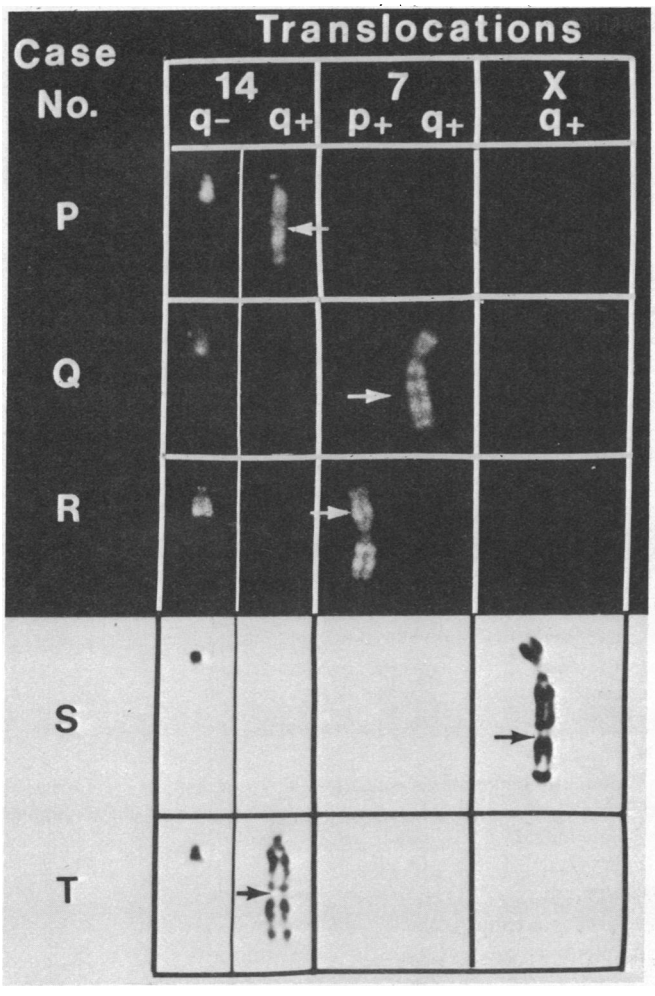

FIG. 1. Translocation chromosomes in lymphocyte clones from cases P–T with ataxia–telangiectasia. Chromosomes in cases P–R were banded with quinacrine mustard, whereas those in cases S and T were Giemsa-banded. Note that each case involves a translocation of the long arm of chromosome 14, leaving an abbreviated no. 14 (left hand column). The arrows indicate the breakpoints.

14. Chromosome 14 is not extra or missing, but is rearranged. The rearrangement, usually a translocation, involves the long arm of chromosome 14. The breakpoints in chromosome 14, which permit the translocations, are in the q12 band, whereas those in the recipient chromosomes are at or near the end of the chromosomes.

Only a few exceptions to date have been observed in A–T clones. The breakpoints and the extent of the deletion are not yet known in the ring 14 chromosome. The two clones with extra C chromosomes have not yet been studied with banding, and it is not possible to identify the chromosomes involved in these other cases.

The Specificity of Chromosome 14 in A–T Lymphocytes. Nonrandom rearrangement of chromosome 14 in A–T lymphocyte clones must reflect either preferential breakage and rearrangement at the 14q12 band and/or a selective advantage accorded to lymphocytes with rearrangements involving this chromosome region.

It is known that many patients with A–T show increases in lymphocyte chromosome breakage at times in the course of their disease (7, 23), which provide ample opportunity for

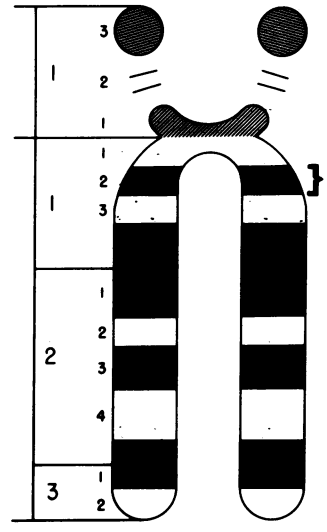

FIG. 2. Diagrammatic representation of the banding pattern on chromosome 14; bracket shows the location of the breakpoints in band 14q12.

the occurrence of translocations and other rearrangements. The elevated breakage throughout the karyotype cannot by itself explain the nonrandom involvement of 14q12, since neither we nor others have noted this band to be a "hot spot" or inherently fragile. Also, no evidence at present suggests the affinity of any environmental agent, such as a virus, for the 14q12 band.

Somatic crossing over at this region with the terminal regions of chromosomes 6, 7, 14, and X could perhaps reflect the persistence of ancient homologies within the genome. There is, however, also no evidence for or against this alternative explanation at present.

Selection and Clonal Proliferation. Chromosome rearrangements occur in cultured lymphocytes from normal persons. It is interesting that these rearrangements tend to involve the long arm of chromosome 14 and that the breakpoints cluster in the 14q12 band (24–26), as has been observed in the A–T lymphocyte clones.

We think it highly likely that chromosome rearrangements occurring in the 14q12 band may accord a selective advantage to lymphocytes, particularly in disorders such as A–T. The advantage may derive from increased rate of cell division or prolonged cell lifespan. An increased mitotic rate, concomitant with decreased chromosome breakage, has been observed in A–T translocation lymphocytes (9).

Lymphocyte proliferation can be conceptualized in terms of population genetics. A chromosome rearrangement in a lymphocyte may be disadvantageous, neutral, or advantageous. The cell with a deleterious rearrangement will either die (or persist without dividing); the cell with a neutral rearrangement will be proportionally represented in the general lymphocyte population; whereas, the cell with an advantageous rearrangement will spawn a clone of increasing proportions. Relaxation of the proliferative control mechanisms may also permit a lymphocyte clone to grow with comparative vigor.

Chromosome 14 Rearrangement and Malignancy in A–T. A key question regarding rearrangement of chromosome 14 in A–T lymphocytes is whether these cells have a predisposition

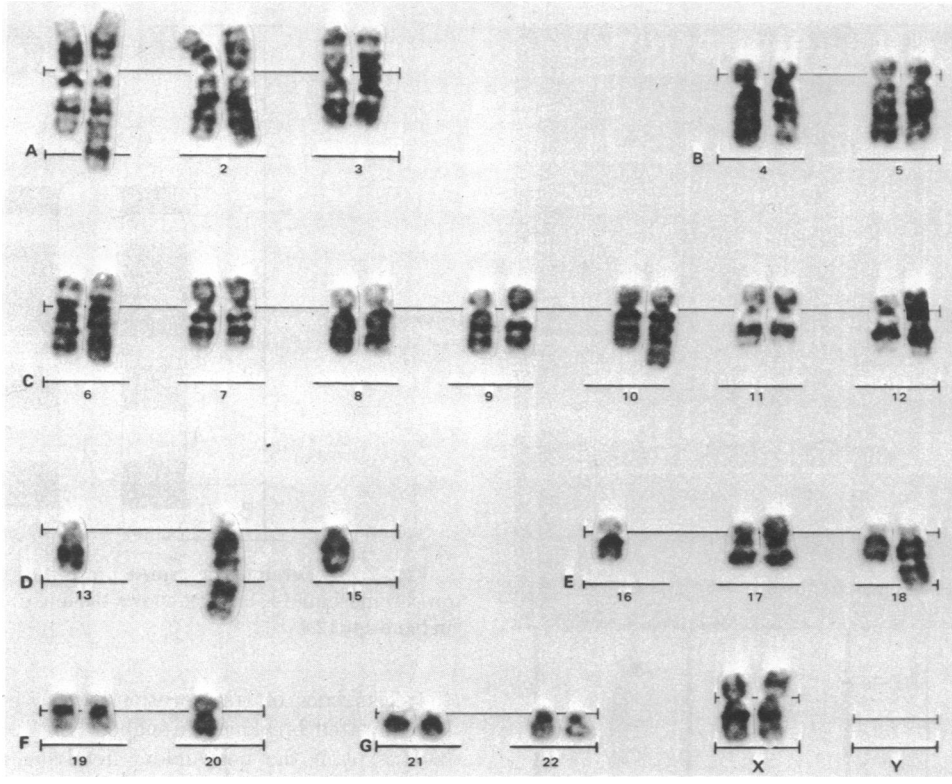

FIG. 3. A representative karyotype from case V who has chronic lymphocytic leukemia as well as A–T. In addition to the original 14;14 translocation detected in lymphocytes in 1968 prior to the clinical onset of leukemia, the patient's lymphocytes now show a modal number of 41 chromosomes due to a series of other chromosome changes. These include a 1;13 and 15;18 translocation, extra material on a no. 10 chromosome, and absence of a no. 16 and a no. 20 chromosome. The karyotype is Giemsa-banded.

to malignancy. Prime evidence bearing on this matter is still scant. It includes one A–T patient (Table 1, case V) in whom we had the opportunity to analyze chromosomes from lymphocytes both before and after the onset of chronic lymphocytic leukemia. The preleukemia study in 1968 was of suboptimal quality but all analyzable metaphases showed a Dq−;Dq+ translocation. Subsequent restaining to produce banding of the same metaphases disclosed that the translocation involved both no. 14 chromosomes. The patient's lymphocytes now show this same translocation plus a series of additional rearrangements (Fig. 3).

Other relevant evidence has been recently reported from Russia (19). A patient with A–T and "lymphoreticular neoplasia" had a translocation (Dq−;Cq+) in lymphocytes. Data on the frequency of chromosomally marked lymphocyte clones in patients with A–T, on the frequency with which these clones persist, and on the frequency with which they may become malignant can only be obtained after more patients are studied longitudinally.

The Philadelphia Rearrangement and Malignancy. Certain parallels exist between the chromosome 14 rearrangement in A–T lymphocytes and the Philadelphia chromosome in myeloid cells. The Philadelphia chromosome (no. 22) is part of a rearrangement, usually with chromosome 9 (4). There is unequal translocation of material with shortening of the 22 and proportionately lengthening of the long arm of chromosome 9. Whether the Philadelphia translocation is reciprocal is not yet established. In any event, preliminary evidence indicates that it may predate the clinical onset of disease (27). A clone of bone marrow cells is marked in an irrevocable manner and once found, the Philadelphia chromosome presumably never entirely vanishes from the marrow of a patient with chronic myeloid leukemia (28). The large majority (about 90%) of patients with chronic myeloid leukemia have this characteristic chromosome rearrangement (29).

Position Effect and Morphogenetic Control. Cell function and behavior may be altered through the loss of genes by chromosome deletion or by gene dosage through chromosomal duplication. It is more difficult to propose a way in which chromosome rearrangements which do not entail perceptible loss or gain of material could alter cell function. In mice (30) and other organisms (31), the term "position effect" has been employed to describe a comparable situation. Until an understanding of the underlying biochemical mechanisms is acquired, we will not know whether position effect in humans involves gene repression or the expression of genes not previously expressed.

Cell growth and behavior may be affected by the Philadelphia rearrangement in myeloid cells and by the chromosome 14 rearrangement in A–T lymphocytes. It follows that there may be genetic information pertaining to myeloid cell growth on chromosome 22 and/or 9, whereas similar information pertaining to the growth of lymphoid cells may be situated on chromosome 14, perhaps in the neighborhood of band q12. The nature of this information is purely conjectural at this point.

Chromosome Changes in Myeloid and Lymphoid Proliferative Disorders. Aside from the Philadelphia rearrangement in chronic myeloid leukemia, other consistent chromosome changes have been reported in myeloid proliferative disorders. In the acute phase of chronic myeloid leukemia, there is often

clonal evolution with addition of a no. 8 chromosome, an extra Philadelphia chromosome, a no. 17 or an isochromosome 17 (32, 33); in cases of acute myeloid leukemia an 8;21 translocation was noted (34–36); an abnormally small chromosome 20 was seen in six patients with polycythemia vera (37), and trisomy 8 was observed in cases of acute leukemia, chronic myelogenous leukemia, and polycythemia vera (36, 38). Rarely, if ever, has mention been made of chromosome 14 in myeloid neoplasias.

Contemporary banded chromosome data are beginning to accumulate from studies of various lymphoid proliferative states. In the current context, the most provocative findings involve African-type Burkitt's lymphoma. Manolov and Manolova, studying biopsies and cell cultures from patients with Burkitt's lymphoma, found 10 of 12 with an extra band on the long arm of chromosome 14 (6). Identical results in Burkitt cell lines have been reported (39, 40), but the origin of the extra material on 14q remains uncertain. One patient with multiple myeloma and one with plasma cell leukemia have had banded chromosome studies and an extra band on 14q was noted in both (41). An extra band on 14q was described also in one patient with Hodgkin's disease and one with lymphocytic lymphoma (42). Chromosome 14 was involved in a translocation with chromosome 11 in a primary culture from a patient with acute lymphoblastic leukemia (43) and a familial translocation of chromosome 14 was reported in another patient with acute lymphoblastic leukemia (44). The frequent rearrangement of chromosome 14 in human lymphoproliferative disorders suggests that the specificity of the initial chromosome aberration is related to the type of cell characteristic of these human neoplasms.

Chromosomes and Cancer. What role do chromosome changes play in the malignant process? A century-long debate has taken place on this matter. Are chromosome changes the cause of cancer or are they simply meaningless epiphenomena? The adoption of either of these polar views appears unwarranted given our present state of ignorance. Answers will need to come from numerous different investigative approaches. For the time being, the most profitable view may be that chromosome changes in neoplastic cells are an integral step in the neoplastic process itself.

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