Leukemia and Lymphoma in Ataxia Telangiectasia

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Ataxia telangiectasia (A-T) is a progressive neurologic disorder that has also been described, variously, as either an immunodeficiency disorder, a chromosomal instability disorder, or a DNA repair disorder. These alternative descriptions are an indication of the pleiotropic effects that the A-T gene exerts in the homozygous state. One of the most important features of A-T is the increased predisposition to particular types of leukemia and lymphoma. Previous reports have suggested that approximately 10% of all A-T homozygotes develop a malignancy, and a 70-fold and 250-fold excess for leukemias and lymphomas, respectively, has been reported. Because A-T can be diagnosed early in childhood, it is possible to identify cells in the preclinical phase of leukemogenesis. This, in turn, offers the possibility of following the genetic changes associated with the development of some leukemias in this disorder.

The aim of the present review is to compare the features and frequency of the leukemia/lymphoma types observed in A-T patients with those in non-A-T patients and to describe possible factors contributing to the development of these tumors. As a result of important changes in the classification and diagnostic methods for leukemias and lymphomas in recent years, we can now attempt to answer, more accurately, such questions as the following: (1) Is the spectrum of leukemias/lymphomas in A-T different from that seen in the non-A-T population? (2) Do the tumors that are present in A-T patients show a phenotype distinct from those in non-A-T patients? (3) Is the distribution by age and sex of these tumors in A-T the same as in non-A-T patients? (4) Is there heterogeneity for predisposition to leukemia/lymphoma in A-T? (5) What is the relationship of either the immunodeficiency or increased radiosensitivity in A-T to the leukemia/lymphoma predisposition in these patients? (6) Is there a common underlying mechanism for the development of particular tumors in both A-T and non-A-T individuals?

Clinical Features of A-T

A-T has a birth frequency of about 1 in 300,000.\(^1\) The major neurologic features result from cerebellar degeneration and include progressive cerebellar ataxia presenting in infancy, abnormal eye movements, and dysarthria.\(^1\) The cerebellar ataxia is initially truncal, but within 5 years peripheral coordination also becomes affected. Dystonia and athetosis are seen in the majority of patients. All patients show apraxia of horizontal and vertical sacchadic eye movements. Other important features include defects in both cell-mediated and humoral immunity, thymic hypoplasia, hypogonadism, growth retardation, and the presence of telangiectasia, particularly of the bulbar conjunctiva. A high level of serum α-fetoprotein is a consistent feature in A-T that is a valuable aid in the diagnosis of the disorder.

Patients have a primary immunodeficiency and A-T is the single largest disorder represented in the Immunodeficiency Cancer Registry.\(^2\) The major clinical consequence of immunodeficiency in A-T patients is an increased likelihood of developing infection. Indeed, severe infection remains an important cause of death in these patients. However, the degree of immunodeficiency in A-T varies from patient to patient, with some patients showing no increased tendency to infections.\(^6\)

With respect to humoral immunity, some but not all A-T patients show deficiencies in IgA, IgG2, and IgE.\(^7\) In addition, all patients appear to have a poorly developed or absent thymus, and both helper and cytotoxic T-cell activity is reduced.\(^8\) Waldmann et al\(^8\) suggested that low levels of some classes of Ig result from failure of the switch process from IgM production to IgA, IgG, and IgE.\(^8\) Carbonari et al\(^9\) reported that A-T patients have reduced numbers of T-cell bearing α/β T-cell receptors (TCR), whereas the numbers of T cells with γ/δ TCR are normal or slightly elevated in these patients.

Heterogeneity in A-T

Clinical heterogeneity in a disorder may reflect an underlying genetic heterogeneity—or the influence of modifying genes. In the case of A-T, not all patients might be equally predisposed to the development of a particular cancer if the disorder was genetically heterogeneous. The age span of...
greater than 40 years over which A-T patients can develop leukemia is an important indicator of genetic heterogeneity.\(^3\)

However, clinically, A-T has been regarded as a fairly homogeneous disorder in terms of the final clinical features shown by patients. The diagnostic sine qua non remains early onset progressive cerebellar ataxia with later onset ocular telangiectasia.\(^1\) However, there is evidence of clinical heterogeneity in terms of the age of onset of cerebellar symptoms, the rate of progress of symptoms, and their severity at defined ages.\(^1\) One of the most striking variations in the presenting features, although clinically less important, is the degree of telangiectasia. Within a single family affected individuals can have quite differing degrees of telangiectasia; therefore, it is likely that other genes may affect the severity of this particular feature. Similarly, there is clear heterogeneity in the presentation of immunodeficiency or rather the propensity to infection, not only between individuals in different families but also within families.\(^1,6\)

The clinical heterogeneity has recently been emphasised by the finding that a group of patients can be identified who show a later onset or slower rate of progress of cerebellar degeneration. These patients are seen in approximately 16% of A-T families. They also show a smaller increase in the cellular radiosensitivity that is associated with the majority of A-T patients.\(^1,12\)

There is a further reason for being aware of any possible clinical heterogeneity in this disorder. Although A-T is classified as an autosomal recessive disorder, this may not be the case in all families. There is a well-founded relationship between the birth frequency of an autosomal recessive disorder and the proportion of parents of patients who show consanguinity. The expected level of parental consanguinity in A-T with a birth frequency of 1 in 300,000 is 10%. In a study of families in the UK, a much lower rate was observed.\(^4\) An explanation for this may be that A-T is not always a recessive disorder. One possibility is that some patients have a new dominant mutation. Another explanation for the low consanguinity rate is that the A-T phenotype is more common than we realize.

The A-T gene was mapped to chromosome 11q22-23\(^5\) in 1988. The linkage and recombination evidence pointed to the existence of a single A-T locus between the markers A4 (D11S1819) and A2 (D11S1818) at this position.\(^14\) The maximum location score was obtained for a position approximately 1 cM proximal to D11S384/D11S535.\(^15\) The A-T gene was recently cloned from the region between D11S384 and D11S535.\(^16\) It codes for a major 12-kb transcript present in various tissues and cell types. There may be several additional transcripts resulting from alternative splicing. Mutations are widely scattered throughout the 5.9-kb cloned cDNA available so far; most lead to premature truncation of the protein product and the remainder are small in-frame deletions. There is significant homology of the A-T gene product with several yeast and mammalian phosphatidylinositol 3-kinases that are involved in signal transduction, meiotic recombination, and cell cycle control. These homologies are consistent with the known pleiotropic effects of the A-T mutation that lead to defects in signal transduction, cellular differentiation, cell cycle control, and an abnormal response to DNA damage.

Genetic complementation indicating the presence of four complementation groups in A-T has previously been reported,\(^17,18\) although the finding of a single A-T gene makes the existence of these groups more difficult to interpret. There is little evidence for locus heterogeneity, although one family with two affected cousins that we have studied showed no clear evidence that the A-T gene in this family was on chromosome 11q22-23.\(^19\)

**CYTOGENETICS OF A-T**

In A-T patients of any age, approximately 10% of all T lymphocytes show the presence of translocations and inversions mainly involving chromosomes 7 and 14 at specific breakpoints. The chromosome rearrangements include inv(7)(p13q35), t(7;7)(p13;q35), t(7;14)(p13;q11), t(7;14) (q35;q11), t(14;14)(q11;q32), t(14;14)(q11;q32), and also t(X;14)(q28;q11). With the exception of 14q32 and Xq28, these breakpoint sites are the locations of TCR genes and, in some instances, the translocations have been confirmed to occur within them.

The presence of specific translocations at this frequency in A-T patients is a useful aid to the diagnosis of the disorder, although it should be emphasized that translocations of these types do occur spontaneously in non-A-T individuals, but at a much lower frequency. Estimates for non-A-T individuals vary, but these are approximately 5 to 6 × 10\(^{-5}\) for all t(7;14) translocations, 2 to 3 × 10\(^{-4}\) for inv(7), 3 to 6 × 10\(^{-5}\) for inv(14), and 0.6 × 10\(^{-4}\) for t(14;14).\(^20-22\) Interestingly, the proportions of the different chromosome translocations and inversions appear to be different between A-T and non-A-T patients.\(^23\) In A-T patients, inv(7) is the commonest rearrangement, rather than the t(7;14) translocations most frequently seen in normal individuals. It is estimated that A-T patients have an approximately 40-fold increase in translocations involving both chromosomes 7 and 14 compared with non-A-T patients,\(^21\) but the increase for inv(7) is even greater.

A single A-T patient will often show the presence of a number of T lymphocytes each with a different translocation and patients may have 2% to 3% of cells with the same translocation.\(^22\) Whether these small numbers of cells with the same translocations are clonal or are independent translocation events is not known. However, translocation cells can proliferate to produce large clones of more than 90% of stimulated T lymphocytes. In this case, clonality can be confirmed, eg, by analysis of TCR gene rearrangements. Other translocations, in which the breakpoints do not involve immune system genes\(^25,26\) and do not produce large clones, are also seen, albeit less frequently.

There appears to be two types of chromosome translocation in T lymphocytes from A-T patients involving TCR genes.\(^27\) One type appears to involve the sites of two TCR genes, eg, \(\beta\) and \(\gamma\) chains in both inv(7)(p13q35) and t(7;7)(p13;q35). \(\alpha\) and \(\beta\) chain genes in t(7;14)(q35;q11) and \(\alpha\) and \(\gamma\) in t(7;14)(p13;q11). These rearrangements are commonly observed in A-T cells but remain as small clones. The second type, which is rare, appears only to involve either the \(\alpha\) or \(\beta\) chain genes along with a second nonimmune system gene, eg, inv(14)(q11q32), t(14;14)(q11q32), and...
The presence of spontaneously occurring chromosome translocations involving breakpoints in TCR genes in A-T lymphocytes suggests a defect in some form of recombination. There is also a 10- to 100-fold increased frequency of TCR hybrid genes formed by interlocus recombination in A-T lymphocytes compared with normals. However, hybrid TCR genes are reported to develop into malignant tumors some years later, after additional genetic changes (see below). Translocations, particularly those involving the sites of TCR genes in T cells, are, therefore, the characteristic form of chromosome abnormality in A-T patients. Clonal translocations may be associated with both B and T lymphocytes and both may occur simultaneously in the same A-T patient.

Translocations in B cells resulting from the breakage and fusion of two Ig genes might be expected to arise in an analogous way to T-cell translocations involving two TCR genes. Such a case has been reported for a t(2;14) translocation observed in a lymphoblastoid cell line (LCL) isolated from an A-T patient. If there was a general predisposition to rearrangement in B lymphocytes, then t(14;22) translocations, eg, involving IgH and IgK, might also be expected. The fact that these have not been observed may be a consequence of their rarity or that the t(2;14) translocation is preferentially formed in A-T cells.

B lymphocytes from A-T patients isolated by T-cell rosetting and activated with Staphylococcus Aureus Cowan I organism also showed high levels of apparently random chromosome rearrangement. A t(2;14)(p11;q32) translocation was observed in short-term cultured B lymphocytes from two sisters with A-T. One sister was shown to have both a small B-cell clone that remained small (<10% of B cells) over a period of about 12 years and also a t(X;14) T-cell translocation that was present in all phytohemagglutinin (PHA)-stimulated T cells. In an LCL derived from this patient, the t(2;14) translocation was shown to be of a second type, with one breakpoint within IgH and the other on 2p11 occurred outside and proximal to IgK, with respect to the telomere, in an unknown gene. This type of translocation may be the equivalent to the T-cell translocation involving both the TCRα and a second unknown locus which has tumorigenic potential. Her sister was also shown to have both a small t(2;14) translocation in her B lymphocytes over a period of 12 years and a large t(14;14) translocation in her T cells (unpublished observations). There is some evidence, therefore, that the second type of t(2;14) translocation is present consistently, albeit at low levels, in B lymphocytes from some A-T patients at least. However, in these two sisters that we studied, neither showed evidence for a large expansion of clonal B lymphocytes, suggesting that these translocation B cells may show a smaller proliferative capacity compared with translocation T cells.

Chromosome abnormalities, in the form of clonal translocations, have been observed in fibroblasts from A-T patients. These translocations are not consistent between patients and probably represent selection in vitro of abnormal cells. No examples of translocations involving chromosomes 7 and 14 or Ig genes have been observed in A-T fibroblasts.
in A-T, but an inv(14) has been observed in one patient 11 years of age (Table 1).

In contrast with A-T children, it is clear now that young adult A-T patients have a particular predisposition to T-cell prolymphocytic leukemia (T-PLL) that is first preceded by the development of a large translocation clone in their peripheral T cells.

The main characteristics of T-PLL in non-A-T patients include a median age of diagnosis of about 69 years and a high WBC count (ranging up to \(1,000 \times 10^9/L\)) in this initial series of patients. Approximately half of the patients had lymphadenopathy, 40% showed hepatomegaly, 73% showed splenomegaly, and about 25% showed skin infiltrates. Membrane markers show that, in most cases, tumors are CD4+ CD8- (65%), some are CD4+ CD8+ (21%), a few are CD4- CD8-, and all are CD7+. None of the patients reported by Matutes et al. showed the presence of the early thymocyte surface markers CD1a (OKT6) or TdT, although about half were positive for CD38, which is present on more mature cells. T-PLL in non-A-T patients is therefore a post-thymic (TdT+) T-cell proliferative disease commonly observed as a very aggressive disease, with the median survival being only months. A proportion of tumors described in the literature as the T-cell form of CLL are likely in fact also to be T-PLL.

Table 1. Leukaemia/Lymphoma in A-T Patients in the UK

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>Clinical Disease</th>
<th>Chromosomes Rearranged in Tumor</th>
<th>Year of Tumor Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (AT5BI)</td>
<td>27/M</td>
<td>T-PLL</td>
<td>Complex inv(14)</td>
<td>1984 [30,31]</td>
</tr>
<tr>
<td>2* (AT8BI)</td>
<td>43/F</td>
<td>T-PLL</td>
<td>Complex t(X:14)</td>
<td>1990 [45,46]</td>
</tr>
<tr>
<td>3</td>
<td>23/M</td>
<td>B-cell centroblastic lymphoma</td>
<td>?</td>
<td>1993</td>
</tr>
<tr>
<td>4*</td>
<td>15/F</td>
<td>T-ALL</td>
<td>?</td>
<td>1995</td>
</tr>
<tr>
<td>5*</td>
<td>12/F</td>
<td>B-cell lymphoma</td>
<td>?</td>
<td>1989</td>
</tr>
<tr>
<td>6*</td>
<td>11/F</td>
<td>T-ALL</td>
<td>Complex inv(14) t(7;19)</td>
<td>1991</td>
</tr>
<tr>
<td>8*</td>
<td>7/M</td>
<td>T-ALL</td>
<td>?</td>
<td>1990</td>
</tr>
<tr>
<td>9 (AT1BI)</td>
<td>7/M</td>
<td>Lymphoma</td>
<td>?</td>
<td>1974 [44]</td>
</tr>
<tr>
<td>10</td>
<td>7/M</td>
<td>T-cell lymphoma (intracranial)</td>
<td>?</td>
<td>1991</td>
</tr>
<tr>
<td>14</td>
<td>4/M</td>
<td>ALL B or T?</td>
<td>?</td>
<td>1984 [42]</td>
</tr>
<tr>
<td>16</td>
<td>2/M</td>
<td>T-cell lymphoma</td>
<td>?</td>
<td>1990</td>
</tr>
<tr>
<td>17*</td>
<td>2/M</td>
<td>T-ALL</td>
<td>?</td>
<td>1980</td>
</tr>
</tbody>
</table>

Patients no. 4 and 8 are siblings. Patients no. 12 and 13 are siblings. * Families included in the linkage study of McConville et al. 15

Is the immunophenotype of T-PLL associated with A-T patients different from that of non-A-T patients? Tumors with the immunophenotype CD1- CD2+ CD3+ CD4+ CD8+ or single positive CD4+ or CD8+ have been reported in A-T patients. Of the 11 patients in Table 2, 6 are either CD8+ or CD4+CD8+ double positives and only 1 is a single CD4+. This finding compares with a reported 65% of non-A-T patients who were CD4+CD8-. Although these are small numbers, there may be an excess of CD8+ and CD4+CD8+ tumors in A-T patients.

Some interesting points arise from a comparison of the B-cell tumors observed in A-T and non-A-T individuals. In the recent UK data shown in Table 1, 3 A-T patients (1 adult and 2 children) were shown to have B-cell tumors. Although they are a relatively small proportion of all the leukemia/lymphoma in this series of patients, the frequency of B-cell tumors in A-T patients is still clearly increased compared with non-A-T patients. Previously published data also indicated an increased predisposition to B-cell tumors in A-T patients, although the use of B-cell surface markers was not in wide use at the time of that publication. The report of Spector et al. showed that the median age for non-Hodgkin’s lymphoma and all leukemia in A-T children (<16 years of age) was 9 years in both cases, which is different from...
the age range of approximately 2 to 9 years in which the majority of cases of common ALL occurs in non--A-T patients.\textsuperscript{52,53} The three known cases of B-cell tumor among the UK patients (Table 1) were 9, 12, and 23 years of age, respectively. It appears that the pre--B-cell leukemias that are most frequent in non--A-T children are much less common in A-T patients. This might suggest that the B-cell tumors in A-T are affecting an older age group or are of a different histologic range. One can conclude from the present limited series that there is certainly a clear predominance of T-cell tumors, although the proportions of T- to B-cell tumors in A-T patients is still uncertain.

Hodgkin’s lymphoma has also been reported in A-T patients, and the lymphocyte depletion subtype, rare in children less than 10 years of age, has been seen (Table 1).\textsuperscript{10}

**PRIMARY CHROMOSOME TRANSLocations ASSOCIATED WITH THE DEVELOPMENT OF LEUKEMIA AND LYMPHOMA IN PATIENTS WITH A-T**

Are the chromosome translocations associated with T-ALL and T-PLL different in A-T and non--A-T patients? Although there is uncertainty over T-ALL, there is sufficient information to show that the translocation breakpoints appear to be the same for T-PLL. Table 3 shows the chromosomal analysis of published cases of T-cell tumors in A-T patients.

In non--A-T patients, the primary chromosomal changes associated with childhood T-ALL involve a wide range of translocations, including t(1;14), t(7;19), t(7;9), t(11;14), t(10;14), etc, in which a TCR gene is involved.\textsuperscript{65} These translocations appear to involve controlling or master genes at the top of the regulatory cascade, and the tumor phenotype may follow the primary translocation event quickly, with few additional chromosomal changes.\textsuperscript{66} Nothing is known, in detail, about the translocations associated with childhood T-ALL in A-T patients. However, patient no. 6 (Table 1) with T-ALL showed the presence of an inv(14) and t(7;19) translocation. There are also 2 further patients in Table 3 with T-ALL, but with a mixed thymic and mature cell phenotype. Table 3 also includes the chromosomal analysis of a lymphoma occurring in a 10-year-old boy.\textsuperscript{71}

In contrast, T-PLL in non--A-T patients does not show the same variety of translocations involving the TCR genes but is most frequently characterized by the presence of inv(14), t(14;14), or t(X;14) rearrangements.\textsuperscript{46,48,65} These 3 chromosomal rearrangements are also associated with T-PLL in A-T patients. Most of the detailed chromosomal and molecular analysis in T-cell tumors in A-T patients has been undertaken on T-PLL/T-CLL in young adult patients. An analysis of the translocation clones associated with the development of T-PLL (or T-CLL) has been possible in A-T patients because of the prior diagnosis of A-T and the subsequent serial sampling of patients’ blood to follow the expansion of chromosomally marked clones.

There is evidence for the evolution of large T-cell translocation clones in A-T patients.\textsuperscript{30,45,55,57} The primary translocations that have been studied include t(X;14)(q28;q11), inv(14)(q11q32), and t(14;14)(q11q32) (Tables 2 and 3) and all have been reported to undergo malignant transformation\textsuperscript{30,32,45} in older A-T patients. Because all these chromosome rearrangements have been observed alone in the initial
rejoining, irrespective of whether this is in A-T or non-A-T patients. The primary chromosome rearrangement is usually the same type of translocation, t(7;14)(q35;q32), has also been observed in T-cell tumors of 2 non-A-T individuals (Lv and Pt, respectively) and from 4 non-A-T patients, the TCR breakpoint was at 14q11 (TCRα) and 7q35 (TCRP), and 7p14 (TCRγ). In T-CLL (Table 3) from patient Lv (non-A-T) and patient C (Table 3), the TCR breakpoint was in TCRα, 7q35 (TCRP), and 7p14 (TCRγ). Virgilio et al7' and Sherrington et al7' have ordered these seven breakpoints relative to each other by chromosome walking. The breakpoints that have been cloned from T-cell tumors from A-T patients (bold lettering) or non-A-T individuals (normal lettering). A total of 450 kb of this genomic region, approximately 10 Mb centromeric to the IgH locus, has been covered by chromosome walking. Vertical arrows represent positions of breakpoints that have been cloned from T-cell tumors from A-T patients (bold lettering) or non-A-T individuals (normal lettering). A total of 450 kb of this genomic region, approximately 10 Mb centromeric to the IgH locus, has been covered by chromosome walking. Vertical arrows represent positions of breakpoints that have been cloned from T-cell tumors from A-T patients (bold lettering) or non-A-T individuals (normal lettering). A total of 450 kb of this genomic region, approximately 10 Mb centromeric to the IgH locus, has been covered by chromosome walking.
techniques and produced a genomic map of 450 kb of the germline TCL1 locus. They showed that the breakpoints were present in two clusters separated by approximately 160 kb. A further 14q32 breakpoint, in a (t(14;14)(q11;q32) A-T translocation, has recently been mapped just centromeric to the breakpoint in the non-A-T patient. Pt.4 These breakpoints are summarized in Fig 1 and indicate the great value of studying the A-T translocation clones. Virgilio et al5 have now identified the TCL1 gene in the 160-kb region between the two clusters of breakpoints.

Just as different chromosome translocations can occur by breakage on either side of the c-myec oncogene in Burkitt’s lymphoma,7 so the breakpoint at 14q32 in T-PLL can occur on either side of the TCL1 gene. The 14q32 breakpoints in the inv(14) inversion are clustered at a position centromeric to the TCL1 gene, irrespective of whether they are from A-T or non-A-T patients, which results in juxtaposition of TCR Ca 3’ of TCL1. In simple t(14;14)(q11;q32) translocations, the breakpoints are in the cluster telomeric to TCL1, again irrespective of whether they are from A-T or non-A-T patients and TCR Ca is juxtaposed 5’ of TCL1. Activation of TCL1, presumably controlled by TCR regulatory elements, can therefore occur whether TCR is upstream or downstream of TCL1.25,26 Occasionally, the breakpoint in the 14q+ chromosome from the t(14;14) translocation occurs centromeric to TCL1, ie, the same as is seen for inv(14) inversions. These are only found in A-T patients. One possible explanation that has been put forward for this abnormal position of the breakpoint in these translocations is that there is an inserted duplication associated with the translocation.25 Inverted duplications at the site of the 14q32 translocations, as well as deletions,31,32 may be a feature of A-T patients and their presence may be related to the putative defect in recombination in A-T cells.

Virgilio et al5 have recently identified a 1.3-kb transcript for TCL1. They suggest that expression of TCL1 in tumors is associated with either t(14;14)(q11;q32) or inv(14)(q11q32) rearrangements, after the demonstration of high levels of transcripts of TCL1 in a leukemic cell line (SUPT11) and in 2 cases of T-PLL, respectively. TCL1 is expressed preferentially in cells of the lymphoid lineage by both immature B and T cells, but mature B and T cells in the circulation do not normally express TCL1. The high level of expression of TCL1 in leukemic cells with t(14;14) or inv(14) but not in leukemic cells with other chromosome rearrangements suggests that the gene is deregulated by translocation to the TCRa/b locus.

After 14q32, the chromosomal region most often translocated to the TCRa chain region in A-T patients with T-PLL/T-CLL is Xq28 (2 of 8 patients in Table 3). The t(X;14)(q28;q11) translocation is very rarely observed in T-cell tumors in non-A-T patients.67-70 Two A-T patients with t(X;14)(q28;q11) and T-PLL have been reported,45,46,77-79 and a further A-T patient with the t(X;14) who has not yet developed a tumor.79 Molecular cloning of the t(X;14) breakpoint from the tumor cells of A-T patients D and E (Table 3) showed that the breaks on Xq28 are within one of two genes that are transcribed in opposite directions from a CpG island located approximately 70 kb telomeric of the factor VIII gene.45,80 (Fig 2). The two genes c5.1A and c5.1B are separated by approximately 300 bp. The Xq28 break in T cells from a further A-T patient (Dol) (Fig 2) with a large non-malignant clone was found to lie within the first exon of c6.1B.70 Only 3 bp downstream of the breakpoint in patient E (Fig 2). Smaller transcripts of the c6.1B gene were observed in T cells from this patient. The Xq28 breakpoints of two non-A-T individuals with T-PLL were shown to be within the c6.1A gene.70,77 (Fig 2). The c6.1B gene is transcribed in all 5 patients that have been analyzed at the molecular level. Of the two genes, therefore, it is more likely that c6.1B plays a role in the clonal expansion of T cells.

Four families of transcripts have been identified from c6.1B/MTCPI (mature T-cell proliferation-1).70 Two of these, A1 and B1, showed the presence of potential open reading frames. The A1 and B1 transcripts encoded 68 and 107 amino acid polypeptides, respectively. The 1.2-kb A1 transcript is expressed at relatively high levels in lymphoid tissues, skeletal muscle, and heart, whereas the 2-kb B1 splice form only accounted for a small percentage of transcripts. The A1 transcripts encoding 68 amino acids gave an 8-kD protein (p8). An anti-p8 antisera showed overexpression of the 8-kD protein in leukaemic cells with a t(X;14)(q28;q11) translocation and immunofluorescence showed that the 8-kD protein was located in the cytoplasm,91 more specifically in the mitochondria.92

Fu et al93 have recently reported the important finding that
The TCL1 protein has considerable sequence similarity to the product of the B1 transcript containing 107 amino acids encoded by c6.1B/MTCP1. At present, there have been no reports of the location of 107 amino acid protein product using antibody.

Both types of translocation involving TCL1 and MTCP1 are observed in A-T and non-A-T T-PLL, but, at the same time, it is interesting to recall the much older median age for diagnosis of T-PLL in non-A-T patients. The abnormal expression of genes at either 14q32 or Xq28, by translocation, is not by itself sufficient for leukemogenesis, because translocation clones can exist for many years in the absence of tumor development. Additional genetic changes are required.

ADDITIONAL CHROMOSOME CHANGES IN LEUKEMIA/LYMPHOMA IN A-T AND NORMAL PATIENTS

In A-T patients, T-PLL appears to develop over a period of years after the progressive selection of consecutively developing clones, with each additional subclone adding one or more further genetic rearrangements to its predecessor, eventually giving rise to the leukemic clone. There is, therefore, the potential to identify a sequence of gene expression important in the development of such a tumor.

In each of 2 patients in whom we studied the appearance of a translocation T-cell clone [containing an inv(14) in patient C and t(X;14) in patient D, respectively] and who both eventually developed T-PLL, there was no lymphocytosis as a result of proliferation of the major translocation clone. The primary translocation allowed proliferation from a particular T stem cell to take over the T-cell compartment but did not allow the cell to escape from control over an absolute cell number. Regulation of total cell numbers was therefore maintained. Some lymphocytosis was noted only after the appearance of further genetic changes (translocations); in 1 patient (patient D), consecutive changes involving chromosomes 11, 16, 19, and 22 were associated with decreasing doubling times. An important result of an early additional genetic change, therefore, is to allow the cell to escape control over absolute cell numbers in the circulation. Figures 3 and 4 show the increase in both WBC and lymphocyte numbers for the 2 patients we studied. Figure 4, in particular, illustrates the steady and irreversible lymphocytosis that began at about 4 years (50 months) before leukemia was diagnosed. This period of 4 years was the time during which the various subclones emerged. The figure also illustrates the shorter doubling time of tumor cells, of about 2 months, in the last few months and the dramatic final increase in numbers of tumor cells.

Studies on one of these patients (patient D) in particular showed that consecutive development of subclones from preceding clones occurs with several subclones coexisting at any single sample time. Each has a different proliferative capacity. Some clones are outgrown by new subclones with a faster doubling time. Some clones can proliferate to very high proportion of the population before being outgrown by yet more aggressive cells.

It is clear from Table 3 that the same complement of translocations is not seen between tumors from different patients, although several tumors have particular translocations in common. In patient C, the presence of inv(14), i(8q), 6q- chromosomes in the final clone has also been observed in another A-T patient. However, an i(8q)-containing clone in an A-T patient who subsequently developed T-PLL was equally outgrown by other clones (unpublished observation). An i(8q)-containing clone was also outgrown in another leukemic A-T patient. It is likely, therefore, that several combinations of additional chromosome rearrangements in different subclones can produce malignant phenotypes that may or may not themselves be outgrown by more aggressive subclones, but any one of which might possibly prove fatal to the patient. At present, the identities of the genes involved in changes additional to the primary translocation are not known. It is also not known whether the order of occurrence
of additional genetic changes is important or whether it is the total accumulation of changes that is sufficient to allow tumorigenesis.

The great genetic complexity associated with the malignant phenotype is a feature of these tumors (Table 3) and is shown by patient D in Fig 5, although it is not known how much of this is necessary or sufficient to produce the tumor. Although the initial chromosome translocation confers a proliferative advantage, further mutational events over a period of time are required to give tumor transformation. This clonal evolution may occur in different ways, eg, either as a sequential development of subclones from previous ones, as shown in Fig 4, or as the appearance and competition between apparently unrelated subclones containing the primary translocation (unpublished observations). It is notable that, once the initial translocation cell develops complex subclones, about 5 years lapse before the tumor is diagnosed.

It is not clear if there are any subtle differences in the genetic changes, additional to the primary translocation, that distinguish T-PLL in A-T and non-A-T patients, respectively. The variety of chromosomal changes makes this difficult to analyze, but the presence of inv(14), i(8q), 6q−, for example, seen in A-T patients, has also been reported relatively frequently in non-A-T patients with T-PLL.28,64

**OTHER POSSIBLE FACTORS AFFECTING B- AND T-CELL TUMOR DEVELOPMENT IN A-T PATIENTS**

The potential for additional factors influencing leukemia/lymphoma development in A-T has been examined. These factors include (1) the type and degree of immunodeficiency in these patients and (2) the level of genetic heterogeneity.

B-cell tumors may arise in individuals with either inherited or induced primary immunodeficiencies, and Epstein-Barr virus infection may be an important cofactor in the development of lymphoma in these patients.5 Spector et al attempted to assess whether the status of immunologic function differed between A-T patients who had cancer and those that did not develop malignant disease. They postulated that, if immunodeficiency is a susceptibility factor for tumor development in A-T, then those patients with more severe immunodeficiency might be more likely to develop lymphoid tumors. However, there appeared to be no difference in the severity and type of immunodeficiency between A-T patients with tumors and those without tumors. In addition, the nature of the immunodeficiency seemed to make no difference to the type of tumors developed by A-T patients. Spector et al suggested that the increased likelihood of developing a malignancy and the immunodeficiency are independent consequences of the same basic defect in A-T patients.

The wide age range over which leukemia develops in A-T patients provides an indication of genetic heterogeneity in A-T.10 The nature of the precise mutations in A-T patients may be important in influencing which type of tumor develops. This will be known once all the mutations in a population of A-T patients have been typed. However, for the present, some indication of the probable importance of particular mutations can be gauged from observations on families. Concordance for the development of ALL lymphoid tumors and stomach cancer has been reported in AT sibling pairs.1,10 In
the UK data (Table 1), there is also concordance for the development of T-ALL in 2 siblings 7 and 15 years of age (Table 1, patients no. 4 and 8) and for the development of T-cell lymphoma in 2 siblings 5 and 6 years of age (Table 1, patients no. 12 and 13).

In older A-T patients, one family showed 2 affected siblings, both of whom had large translocation clones that developed into a T-cell tumor in adulthood. In 1 sibling, this was described as T-PLL. In a UK family with 2 affected siblings, both again showed the presence of a large translocation T-cell clone and 1 developed T-PLL in adulthood (Table 1, patient no. 2). In a second UK family with 2 affected siblings, 1 developed a large T-cell clone and subsequently T-PLL (Table 1, patient no. 1), although the other sibling had no evidence of a large clone and died in adulthood from complications after a respiratory infection. The evidence for concordant development of large cytogenetically abnormal clones suggests that there is preferential development or growth of particular translocation cells in an individual. There also appears to be concordance within families for the development of both large translocation clones and T-PLL.

The observation of concordance of tumor type in A-T siblings supports the contention that there is variation in genetic predisposition to tumor development within the A-T population. It is unlikely, therefore, that all A-T patients have the same probability of developing any form of T-cell tumor, but rather predisposition in any single family will be to a particular tumor type and will result from the presence of a particular molecular defect that, in turn, will depend on the presence of particular A-T mutations. A corollary of this is the question of whether all A-T patients with the same tumor type, eg, T-PLL, share precisely the same genetic defect.

Is it possible that the lower age distribution for T-PLL development in A-T patients compared with non-A-T patients has some causal significance? Because the initiating translocation involves a TCR gene, the most likely time for its appearance is during TCR gene rearrangement, early in the development of the thymus (although it also seems likely that T-cell maturation continues to occur in the adult but at a reduced rate). This must be true of both A-T and non-A-T individuals. The age range in which T-PLL occurs in the non-A-T population is 33 to 91 years, compared with a range of 26 to 43 years in A-T (excluding the 2 mixed phenotype patients [B and F] and patient A with an unusual T-ALL: Table 3). There is, therefore, a clear overlap between the two groups. One interpretation is that the younger age of occurrence in A-T patients is not due to some additional special feature of A-T cells but due to the increased frequency of T-PLL together with the shorter life span of A-T patients. Alternatively, if there are several molecular defects in A-T, with some more severe than others, that can give rise to tumors of different types and at different ages, this may contribute to the different age distribution for these tumors in A-T and non-A-T patients. It is interesting that, of the 11 published cases of T-PLL/T-CLL and T-ALL with mature phenotype in A-T patients, 9 are younger than the lower age limit for the age range of T-PLL in non-A-T patients (Table 2).

OTHER TUMORS IN PATIENTS WITH A-T

Although the major excess of tumors in A-T is leukemia/lymphoma, other tumors are also increased compared with non-A-T patients. Information on other tumor types in A-T patients is confined to a small number of epidemiologic studies in the United States, United Kingdom, and Norway; data in the Immunodeficiency Cancer Registry, and sporadic case reports. Nonlymphoid tumors, mainly carcinomas, represent about 13% to 22% of all malignancies in A-T patients.

A contribution to the different ratio of lymphoid to epithelial cell tumors observed in A-T patients compared with the normal population is probably the lower average life span of A-T patients. In general, nonlymphoid tumors reported by Spector et al were found in older A-T patients with an average (median) age of 17 years.

Spector et al reported a female excess for solid tumors. This was due to ovarian, uterine, and stomach tumors. A smaller excess of liver cancer was observed in both male and female patients. The largest difference in nonlymphoid cancers in A-T patients compared with the general population was the frequency of stomach cancer, which was greater than 7 times the observed level in female patients in the general population. In their study, Morrell et al did not report an increased frequency of stomach cancer but did see one tumor each of the ovary and uterus and two tumors of the liver.

Both Spector et al and Morrell et al reported tumors of the parotid glands. The presence of only one breast cancer was observed in both male and female patients. The largest difference in nonlymphoid cancers in A-T patients compared with the general population was the frequency of stomach cancer, which was greater than 7 times the observed level in female patients in the general population. In their study, Morrell et al did not report an increased frequency of stomach cancer but did see one tumor each of the ovary and uterus and two tumors of the liver.

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Multiple tumors were observed in 5% to 15% of patients in the study by Spector et al and 4% in the study by Morrell et al. This is very important because it suggests that the A-T gene in the homozygous state may play a general role in the predisposition to cancer in many cell types.

TUMORS IN A-T HETEROZYGOTES

Although A-T is a rare disease, carriers of the A-T gene are estimated to be about 1% or more of the US population. Given this high frequency, it is important to establish whether the A-T gene contributes to the increased risk of cancer in heterozygotes, because it would be numerically more important in cancer predisposition in the general population. There are no obvious clinical features that distinguish A-T heterozygotes from the rest of the population.

There are three reports indicating that female relatives of A-T patients have an excess risk of breast cancer. The estimated relative risk of breast cancer in A-T heterozygotes is estimated to be 3.9-fold. The proportion of sporadic breast cancer cases due to the A-T gene is estimated to be quite significant at between 1% to 13%, with a best estimate of 3.8%, using an A-T gene frequency of 0.5%. A recent intriguing result has shown loss of heterozygosity in patients...
with sporadic breast cancer, on 11q22-23, although over a wide region that includes the A-T gene. The association between heterozygosity for the A-T gene and familial predisposition to breast cancer has been examined by Cortessis et al. and Wooster et al., who performed genetic linkage analysis in breast cancer families by using markers linked to the A-T gene on chromosome 11q22-23. No evidence was found for linkage between breast cancer and these markers. After adjusting for the probability that each family was linked to the BRCA1 gene on chromosome 17q, the LOD scores for all markers were negative.

These observations on linkage are in apparent conflict with the available epidemiologic data from the United States, Britain, and Norway, which suggest that a proportion of breast cancer cases may be carriers of the A-T gene. However, a relative risk of 8 for breast cancer in heterozygotes gives a cumulative risk of breast cancer of about 24% by the age of 60. This risk associated with A-T is substantially less than the risk of 55% suggested by segregation analysis for breast cancer susceptibility genes in general based on the Cancer and Steroid Hormone Study. In addition, multiple case families used in linkage studies are more likely to be associated with a highly penetrant gene than one such as A-T that is of low penetrance for breast cancer. Nevertheless, the absence of linkage in these studies suggests that the contribution of the A-T gene to familial breast cancer is only minor. It also gives credence to the possibility that any association between a single A-T gene and an elevated risk of breast cancer depends on other factors such as environmental agents.

There is no significant association between any other cancer type and A-T heterozygosity, although a number of possible associations are suggested by the studies of Swift et al and Morrell et al. The notable absence in A-T heterozygotes of a significant excess of lymphomas and leukemias, the tumors most frequently seen in A-T homozygotes, cannot be explained.

**TUMORS IN OTHER DISORDERS SHOWING SOME FEATURES OF A-T**

Several other chromosome instability syndromes show an increased susceptibility to different malignancies. However, a distinction can be made between the type of chromosome abnormality associated with A-T patients and those associated with, eg, Fanconi’s anemia (FA) and Bloom’s syndrome (BS). In both FA and BS, the abnormalities in lymphocytes are mainly chromatid gaps, breaks, and interchanges, and BS is characterized by the presence of high levels of sister chromatid exchanges. The types of tumor seen in FA include acute leukemia and liver cancer. BS patients are remarkable for the variety of histologic tumor types that they show, including acute leukemia, lymphoma, Hodgkin’s disease, breast cancer, large bowel cancer, and tumors of the tongue, larynx, oesophagus, lung, cervix, and skin.

Patients with the Nijmegen breakage syndrome (NBS) show microcephaly, short stature, a “bird-like” face, and cafe-au-lait spots. In common with A-T, these patients have an immunodeficiency, a similar increase in translocations involving chromosomes 7 and 14 in the peripheral T cells, and the same cellular phenotype as A-T patients with respect to increased radiosensitivity. NBS patients have been allocated to one of two complementation groups, V1 or V2, using radioresistant DNA synthesis as an indication of complementation. Twins were described by Curry et al. with features of both A-T and NBS, but the relationship of this unusual phenotype to both A-T and NBS remains unclear.

Patients with NBS also show an increased susceptibility to lymphoid tumors. Nine tumors have been described in 30 NBS patients. Of these, 8 were lymphomas (including B-cell tumors and 2 T-cell tumors) and 1 was a glioma. No leukemias have so far been reported. No inv(14)(q11q32) inversions have been observed in the lymphocytes of NBS patients, and t(14;14)(q11;q32) translocations are rare. Consistent with the lack of large clones with these rearrangements is the absence of T-PLL, although the age range of NBS patients with tumors, it should be noted, was only 2 to 19 years. Curiously, there is a report of an 9-year-old NBS patient who had an t(X;14)(q28;q11) translocation in 30% of his lymphocytes and who developed lymphoma (quoted in Hsieh et al).
served in T lymphocytes. Particular chromosome translocations in T cells, involving a break in a TCR gene, are characteristic in individuals with T-ALL or T-PLL in non-A-T patients. The majority of T-cell tumors in A-T are T-ALL and T-cell lymphoma, about which virtually nothing is known chromosomally, and the assumption is that the increased number of translocations leads to the increased level of these tumors. In older T patients, the expansion of specific translocation T-cell clones has been followed to the point to which they develop into T-PLL. All the evidence, therefore, suggests that the A-T mutation in the homozygous state allows a large increase in production of translocations formed at the time of V(DJ) recombination, and this leads to the increased predisposition to leukemia.

The general increased predisposition to T-cell tumors compared with B-cell tumors in A-T patients may be related to a preferential occurrence of translocations in T cells. Relatively little is known about translocations in circulating B lymphocytes in normal indivduals, but A-T siblings have been shown to have clonal chromosome rearrangements of both B and T cells, simultaneously, although in these siblings the T-cell clones occupied all the T-cell compartment and the B-cell clones were small. An important inference from these facts is that the A-T defect preferentially affects immune system gene recombination in T cells rather than B cells. Recent evidence suggests that the V(DJ) recombination machinery is not identical or is not regulated identically in T- and B-cell progenitors. This finding is consistent with the hypothesis that V(DJ) rejoining in the majority, at least, of A-T patients may be preferentially deficient in T cells compared with B cells giving rise to the greatly increased number of translocations and T-cell tumors.

Carbonari et al proposed that the recombination defect in A-T cells affected both Ig isotype switching and TCR rearrangement in B and T lymphocytes. These investigators suggested that the decreased ratio of \( \alpha/\beta \) to \( \gamma/\delta \) expressing T cells and the impaired Ig switch process are consequences of the same recombinational defect in A-T patients. Although this may be true for some, not all A-T patients have a measurable defect in humoral immunity, although the defect in cellular immunity is more consistent. It is possible, therefore, that the defect in immune system gene recombination in some patients does not affect both B and T cells. This heterogeneity between patients may be the consequence of different combinations of A-T gene mutations in an individual.

If the excess production of translocations contributes to the increased likelihood of leukemia, what is the basic defect in A-T causing this excess? Is it the result of a defect in V(DJ) recombination in A-T cells? All the evidence suggests that the specific V(DJ) recombination process in A-T cells is normal. At present, the role of the A-T gene product in recombination is unknown. There is also no evidence that the excess of A-T translocations arises from a deficiency to select against their production compared with normal. However, A-T cells show increased intrachromosomal recombination in constructs introduced into the host genome compared with normal cells, although no difference was observed between A-T and normal cells if constructs were maintained episomally. In addition, other translocations not involving the sites of TCR genes occur in A-T lymphocytes, although more rarely. These observation suggests a wider abnormality of recombination that may be important in the development of nonlymphoid tumors in A-T patients.

A-T cells also show additional defects, including retention of a higher level of radiation-induced chromosome breakage, failure to inhibit DNA synthesis after exposure to ionizing radiation, and abnormal telomeric associations under some circumstances. One attractive idea is that the A-T gene product allows access of enzymes for different aspects of DNA processing. The substrates for such DNA processing would include any form of DNA strand breakage, whether it occurred during normal cellular processes such as immune system gene recombination or after exposure to DNA breaking agents. The DNA processing itself would include recombination, repair, telomeric replication, and recognition of damaged DNA before inhibition of DNA synthesis, etc. In this way, the A-T gene product is likely to influence a regulatory control over several different cellular processes. This may involve regulation of chromatin structure. The pleiotropic effect of the A-T gene mutation may result from the action of a protein that is a recognizable mosaic showing homologies to other known proteins and with an additional capacity for the production of several proteins formed by alternative splicing.

A deficiency in A-T cells in a component common to different recombination processes, including V(DJ) rejoining, might explain the predisposition to all tumor types in A-T, with the largest proportion arising in lymphoid tissue presumably because of their obligation to undergo immune system gene rearrangement. It is not known whether an inability to repair DNA damage resulting from exposure to background radiation might contribute to cancer development in A-T, although the major predisposition to lymphoid leukemias makes this unlikely.

What are the genes that, when translocated to the TCR region, can eventually give rise to a preleukemic clone? There are many genes involved in the primary translocations giving rise to T-ALL in non-A-T patients, and it is assumed that these will also be present in A-T T-ALL. In contrast, T-PLL development is associated with expression of either TCL1 at 14q32 or a homologous gene, c6.1B/MTCP1 at Xq28, in both A-T and non-A-T patients. It is known that the primary translocation by itself in A-T is not sufficient for the development of the tumor. This means that expression of the TCL1 or c6.1B/MTCP1 alone, although probably necessary for the tumorigenic process, is unlikely to be sufficient to give the tumor phenotype. Other, as yet unrecognized, genes will also be important in this process. It is not known whether the order of appearance of these is important for tumor development or whether the unordered accumulation of particular mutations is sufficient. It has been suggested that not only the number of mutations but also the order in which they occur can be critical for the development of human colon carcinoma.

Because the same translocations involving TCL1 and c6.1B/MTCP1 genes are seen in sporadic T-PLL patients, we assume that these patients also develop large premalignant translocation clones in the same way as do A-T patients.
important difference between A-T and non–A-T patients is the age at which these tumors develop. According to Matutes et al., 24 half of all sporadic T-PLL cases are in individuals more than 69 years of age; therefore, it is unclear when the primary translocation might have occurred. It is also not known whether the A-T gene is involved in the formation of the translocation chromosome in these sporadic T-PLL cases.

In addition to explaining cellular features such as increased V(D)J recombination in A-T cells, the molecular defect must also account for the various degenerative clinical features such as the cerebellar degeneration, the hypogonadism, the abnormally high levels of serum AFP, the presence of thymic hypoplasia, and growth retardation. These may be the clinically observable consequences of easier accessibility to chromatin of apoptotic endonucleases leading to higher than normal levels of cell death. 10

The cloning of the A-T gene will now allow an analysis of the mutations that occur in the gene and their relation to the clinical phenotype, including the types of tumor that occur in families. It is to be hoped that an understanding of the relationship of all the cellular and clinical features of A-T to the gene defect will rapidly follow.

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