

Meeting Report

Fifth International Workshop on Ataxia-Telangiectasia¹

A wide spectrum of investigators are interested in A-T² because of the pleiotropic effects of the gene. Patients show a progressive cerebellar degeneration, first evident in infancy, resulting in severe ataxia, dysarthria, and oculomotor dyspraxia. All patients show a defect of cell mediated immunity and many show a significant deficiency in humoral immunity. There is a greatly increased predisposition to both lymphoma and lymphoid leukemia. T-cell leukemia appears to be much more common in A-T patients than in the general population. In addition there is an excess of particular epithelial cell tumors including carcinoma of the stomach, liver, and ovaries. A-T patients are unusually sensitive to the effects of ionizing radiation. This increased radiosensitivity can be observed at the cellular level as a reduced colony forming ability of cultured fibroblasts, compared with normal, following exposure to X- or γ -rays. Another intriguing response to radiation can be measured as the effect on DNA synthesis. Following exposure of normal cells to γ -rays DNA synthesis is inhibited in a biphasic manner with an initial steep component. In irradiated A-T cells there is not the same reduction in the rate of DNA synthesis and levels remain high. This is termed radioresistant DNA synthesis. The responses of A-T cells to ionizing radiation have provided a means for assaying the cellular phenotype and in analyzing genetic heterogeneity (see below).

The Fifth International Workshop was organized by Dr. R. A. Gatti (University of California, Los Angeles, Los Angeles, CA). The major aim of the Workshop was to discuss progress toward mapping and cloning A-T gene(s). Other important sessions included discussion on the possibility that the A-T mutation produces a defect in recombination, the role of the A-T gene in the heterozygous state in cancer predisposition, the status of heterozygote detection, and the range of clinical heterogeneity in patients.

Cloning of A-T Genes

A major aim of current work on A-T is to isolate the gene and characterize its product. An important finding of recent years, in this respect, was the localization of the A-T gene to chromosome 11q22-23 by conventional linkage analysis (1). This observation was subsequently confirmed by others (2, 3).

There was hope that further progress had been made in cloning the gene for A-T but while the genetic map of the 11q22-23 region had shown considerable improvement it became clear that compared with the situation at the time of the previous Workshop (4) the mapping of the A-T locus was proving to be more complex. One complicating factor is genetic heterogeneity in A-T. This was largely established, some years ago, by the tedious analysis of rates of DNA synthesis in γ -irradiated heterodikaryons obtained after fusion of different A-T fibroblast cell strains. By producing different combinations of fusion four different complementation groups were defined. These are des-

ignated A (55% of patients), C (28% of patients), D (14% of patients), and E (3% of patients) (5, 6). One approach to isolating a possible A-T gene involves functional complementation. L. Kapp (University of California, San Francisco, San Francisco, CA) described his mammoth study involving the transfection of the SV40 virus transformed cell line AT5BIVA (from complementation group D) with a human cosmid library and the subsequent isolation of a cell line 1B3 showing partial restoration to the radiosensitivity associated with normal cells. The radioresistant DNA synthesis associated with A-T cells was, however, not corrected. He rescued integrated cosmid sequences from this line and showed by *in situ* hybridization that they mapped to 11q22-23 in normal cells. J. Murnane, working in the same laboratory, showed that the *ATDC* (A-T D complementing) gene was present as a single copy in the human genome and that there was no cross hybridization with other possible A-T genes. Northern filter analysis revealed mRNA species of several sizes. Transfection of the AT5BIVA cell line with the 3.0-kilobase cDNA in an expression vector produced cell lines with a small increase in survival. No rearrangement was detected in the *ATDC* gene and thus far no point mutations have been found (7). One very interesting feature is that the *ATDC* gene isolated is very closely linked to the *THY1* gene as shown by radiation hybrids (M. James, CEPH, Paris, France) and thus is outside the region flanked by *STMY/D11S385* and *NCAM/DRD2/D11S132* believed to contain the A-T genes for complementation groups A and C.

S. Meyn (Yale University School of Medicine, New Haven, CT) and Y. Shiloh (Tel Aviv University, Tel Aviv, Israel) both described attempts at functional complementation of A-T cells. S. Meyn described cDNAs rescued from A-T group D fibroblasts that had acquired streptomycin resistance after transfection with a human cDNA library constructed using an Epstein-Barr virus-based episomal expression vector. Different cDNAs were isolated and when transfected into A-T fibroblasts corrected one or more of the A-T cellular phenotypes (*i.e.*, sensitivity to streptomycin, sensitivity to ionizing radiation, and high spontaneous recombination rate). Mapping is under way to identify candidate A-T genes at position 11q22-23. Y. Shiloh used complementation group A cells and also rescued cDNA clones one of which represented an extensive gene family with members localized to chromosomes 1, 2, 6, 8, 10, and 15 as well as 11. The chromosome 11 clone did not hybridize, however, to a radiation hybrid containing the A-T gene region.

Repair deficient rodent cell lines have shown to be essential in the isolation of xeroderma pigmentosum genes. Another approach to cloning A-T genes therefore was illustrated by M. Zdzienicka (University of Leiden, Leiden, the Netherlands). She reported on further genetic analysis of Chinese hamster mutant cells with a phenotype closely resembling A-T cells. Using microcell hybrids and supporting marker analysis she was able to exclude the human 11q22-23 band as the region involved in these mutants. Perhaps, another A-T gene (*e.g.*, from group F, or from the groups V1 or V2 from the closely related Nijmegen breakage syndrome) is affected here.

Fine Mapping of A-T Genes

Using microcell hybrids containing chromosomes 11 or t(X;11) translocations, Y. Ejima (University of Kyoto, Kyoto, Japan) showed

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² The abbreviations used are: A-T, ataxia-telangiectasia; cDNA, complementary DNA; NBS, Nijmegen breakage syndrome.

that hybrids with 11pter→q23 or 11p12→qter conferred radioresistance on A-T cells, but in one hybrid a deletion of 11q23.1→q23.3 did not confer radioresistance suggesting the A-T gene is at this position. Genetic complementation in A-T cells had been established previously by measuring DNA synthesis in heterodikaryons, formed between different A-T fibroblast cell strains, following γ -irradiation. An alternative method for analyzing the more numerous A-T lymphoblastoid cell lines was given by P. Chen (Queensland Institute of Medical Research, Brisbane, Australia) involving analysis of the levels of radiation induced chromosome damage in heterodikaryons. M. Paterson (Cross Cancer Institute, Edmonton, Alberta, Canada) described yet another approach to complementation testing based on the transfer of soluble factors during cocultivation of pairs of fibroblast strains.

R. A. Gatti described the results of his genetic linkage analysis on an international consortium of over 100 A-T families and reported linkage evidence for genetic heterogeneity suggesting that the *ATA* and *ATC* genes may both be between STMY/D11S385 and NCAM/DRD2/D11S132 and that *ATD* may be 30 cM distal, between D11S147 and D11S133 but still within 11q23. C. M. McConville (Birmingham University, Birmingham, England) showed good evidence from recombination events in A-T families for a common A-T gene proximal to D11S535 which is itself proximal to both NCAM/DRD2 and D11S424 (see Fig. 1). Another approach therefore to locating the A-T gene is far more precise mapping initially to identify candidate regions for subsequent gene transfer studies. This approach, however, requires the isolation of additional polymorphic markers across the region of the A-T gene(s). M. James described the isolation

of six new microsatellite markers from a region between STMY/D11S385 and D11S351 derived from (a) a cosmid library from a radiation hybrid, (b) previously localized cosmids (8) and (c) J. Weissenbach (Genethon, Paris, France). He also mapped the *CRYA2* gene to this region. C. M. McConville described two new markers, D11S535 (J12.8) and D11S611 (J12/IC2), flanking the *A-TA* gene. P. Byrd *et al.* (Birmingham University, Birmingham, England) showed the construction of YAC contigs around STMY/D11S385, D11S611 and D11S535 with a total of 2 mb covered. P. Charnley (Virginia Mason Research Center, Seattle, WA) also discussed in a poster the use of CA repeats in detecting linkage disequilibrium with disease alleles.

Defective Recombination in A-T?

The presence of spontaneously occurring chromosome translocations involving break points in T-cell receptor genes in A-T lymphocytes suggests a defect in some form of recombination. Meyn described an increased intrachromosomal recombination rate in A-T fibroblast lines underlining this increased propensity for abnormal recombination. R. J. Albertini (Vermont Cancer Center, Burlington, VT) gave an *hprt* mutant frequency in T-cells in young adults of $5-10 \times 10^{-6}$ and in the fetus of about 0.6×10^{-6} . Most *hprt* mutations in the foetus involved precise break points in introns 1 and 3 within a typical heptamer recognition sequence. C. F. Arlett (University of Sussex, Brighton, England) showed that the *in vivo* mutation frequency at the *hprt* locus in A-T T-lymphocytes was 5-10-fold higher than in age matched normals. The frequency of *hprt* mutants in A-T heterozygotes was the same as in normals.

The high rate of interlocus recombination seen in A-T lymphocytes may occur because controls for preventing inappropriate rearrangement have broken down. M. R. Lieber (Stanford University, Stanford, CA) described ways in which under normal circumstances simultaneous gene rearrangements are prevented including, for example, differential locus accessibility to recombinase. He showed using minichromosome substrates that CpG methylation decreases the V(D)J recombination of these substrates by more than 100-fold. Studies with A-T cell extracts have yet to be undertaken. L. R. D. Kingsbury (University of California, Berkeley, Berkeley, CA) reported her evidence that somatic DNA rearrangements can occur in the brains of transgenic mice (9). This recombination appears to differ in site specificity from V(D)J recombination in lymphocytes. These results are intriguing in the light of the combination of features in A-T patients, including cerebellar degeneration together with evidence of immunodeficiency and translocations at sites of V(D)J recombination. The defect in SCID mice involves V(D)J joining which does not appear abnormal in A-T patients. K. Komatsu (Nagasaki University School of Medicine, Nagasaki, Japan) suggested from observations on SCID/human cell fusions that human chromosome 8 or 16 complemented the mouse SCID mutation. [The *RAG1* and *RAG2* genes which influence an earlier stage of V(D)J joining are on chromosome 11p (10)].

Cancer and the A-T Gene

One of the most important consequences in somatic cells of such a possible recombination defect in A-T patients is the development of lymphoma/leukemia. P. Sherrington (M.R.C. Laboratory of Molecular Biology, Cambridge, United Kingdom) and C. Croce (Jefferson Cancer Institute, Philadelphia, PA) both described the importance of a region proximal to IgH in t(14;14) and inv(14) inversions in the development of T-cell tumors in both A-T and non-A-T individuals. J. Thick (Birmingham University, Birmingham, England) also described an A-T patient with t(X;14)(q28;q11) who developed a T-cell leukemia.

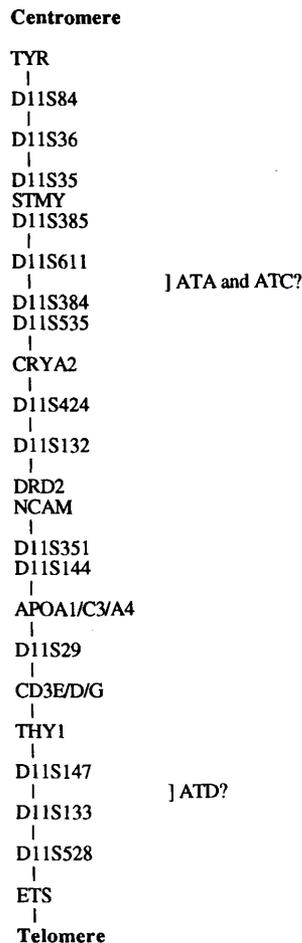


Fig. 1. Map of the chromosome 11q22-23 region showing the relative order of genes and RFLPs. The likely positions of the *ATA*, *ATC*, and *ATD* genes are indicated.

F. Li (Dana-Farber Cancer Institute, Boston, MA) reviewed recent work on familial cancer syndromes and the importance of particular genes in cancer causation. He stressed work on *p53* in Li-Fraumeni syndrome. If carriers of the A-T gene can be shown to have a clearly increased risk of some cancers then the A-T gene will assume an importance comparable to those seen in some familial cancer syndromes. M. Swift (University of North Carolina, Chapel Hill, NC) reiterated his recent results suggesting not only that female carriers of the A-T gene were at increased risk of breast cancer but also that exposure of these carriers to ionizing radiation may increase the risk. R. Wooster (Institute of Cancer Research, London, England) could find no evidence for linkage of breast cancer to loci on chromosome 11q after allowing for possible linkage to 17q. He even reported obligate recombination between the A-T locus and breast cancer. The relative risk for breast cancer in A-T heterozygotes may not be high enough to generate the large breast cancer families necessary for linkage analysis. R. Haile (University of California, Los Angeles, Los Angeles, CA) is currently undertaking a similar study. Despite reports about breast cancer in A-T heterozygotes, R. Withers (University of California, Los Angeles, Los Angeles, CA) suggested that mammography in heterozygous A-T patients should be carried out in a manner comparable to that for the general population.

Heterozygote Detection

Identification of known heterozygotes was reported by M. Lavin (Queensland Institute of Medical Research, Brisbane, Australia) by the longer G_2 phase delay seen in fluorescence-activated cell sorter analysis following γ -irradiation of lymphoblastoid cells. Minimal overlap occurred with controls. D. Scott [Paterson Institute of Cancer Research, Manchester, England] measured chromosomal radiosensitivity of cells irradiated in G_2 [assay of Sanford *et al.* (11)] or low dose rate G_0 irradiation and found considerable overlap of A-T heterozygotes and normals. This was also true for low dose rate G_0 irradiation using cell survival as the end point. Interestingly some breast cancer patients who showed excessive skin reactions after radiotherapy showed significantly increased radiosensitivity by these criteria. This work is continuing. It seems likely that the A-T gene(s) will be cloned before too long so that heterozygotes will be identifiable in the general population and the role of the A-T gene in breast cancer can be tested.

Clinical Heterogeneity in A-T

A-T is regarded as a clinically homogeneous condition. L. Chessa (University "La Sapienza," Rome, Italy) described the establishment of a register of 56 families in Italy and emphasized their clinical homogeneity. She also suggested that A-T patients might have an increased incidence of cardiac anomalies. O. Porras (Hospital Nacional de Neros, San Jose, Costa Rica) described a group of 27 Costa Rican families, noting a high incidence of clubbing in his patients and gastric cancer in their families. There is growing evidence, however, of variations in the age of onset of A-T in *bona fide* patients as well as the rates of progress of the cerebellar degeneration. C. G. Woods (Oxford, England) presented data from 60 British A-T families where a minority showed unusually severe or mild or atypical clinical features. Two patients in a family described by D. Hernandez (Birmingham, England) showed milder than usual cerebellar degeneration but very typical severe cellular radiosensitivity. There was no evidence for linkage of the A-T gene, in this family, to chromosome 11. At the cellular level it is clear from some studies that 10–15% of A-T patients have a radiosensitivity which is less than that normally seen in other patients. L. Chessa also described 3 patients with an intermediate level of radiosensitivity. The most fascinating clinical relationship, however, is between A-T and the NBS. Although these are known to be in

different complementation groups NBS patients show the increased cellular radiosensitivity seen in A-T patients as well as the increased frequency of chromosome translocations involving chromosomes 7 and 14 in T-lymphocytes. C. M. A. Weemaes (University Hospital, Nijmegen, Nijmegen, the Netherlands) described the NBS syndrome as well as a new variant without increased radiosensitivity. It is interesting that although NBS patients, like A-T patients, also develop lymphoid tumors it is not clear whether they preferentially develop T-cell tumors.

Conclusion

The meeting began with a clinical overview of A-T by R. Sedgwick who with E. Boder (12) gave the disorder its name in 1957 and closed with an appropriate mixture of clinical and scientific overviews. S. Perlmann (University of California, Los Angeles, Los Angeles, CA) gave a summary of the approaches to the treatment of ataxia-telangiectasia. While there is no curative or even stabilizing treatment for the disorder, numerous medications are available for treatment of individual neurological symptoms. Screening for development of neoplastic conditions is clearly important and treatment for nonneurological symptoms such as recurrent sinopulmonary infections is obviously available. R. Painter (University of California, San Francisco, San Francisco, CA) gave an overview of the radiobiology of A-T emphasizing, in particular, radioresistant DNA synthesis, a topic that will always be associated with his laboratory. Finally M. Lavin gave an overview of the biochemical defects that have been reported in A-T over the years. These are many and disparate and it is difficult to synthesize a scheme including them all. M. Paterson also proposed a new explanation for the increased radiosensitivity observed in A-T cells by invoking a defect in signal transduction in the cell. Essentially the fundamental defect in A-T remains unknown. The most certain way to understanding A-T is to first clone what now appear to be several different A-T genes. The period to the next A-T Workshop will be exciting and promises revelations that will continue to be of great interest across the disciplines.

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