SENSITIVITY OF ATAXIA-TELANGIECTASIA FIBROBLAST CULTURES TO THE CHROMOSOME-DAMAGING EFFECT OF HYDROGEN PEROXIDE

by

Ming Yi

B.Sc.(Biochemistry), Beijing University, P.R.China, 1985.

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in the School of Kinesiology

© Ming Yi 1990

Simon Fraser University

November 1990

All rights reserved. This thesis may not be reproduced in whole or in part, by photocopy or other means, without the permission of the author.

APPROVAL

NAME: Ming Yi

DEGREE: M.Sc.

TITLE OF THESIS: Sensitivity of Ataxia-Telangiectasia Fibroblast Cultures to the Chromosome-Damaging Effect of Hydrogen Peroxide

EXAMINIG COMMITTEE:

Chairman: Dr. Glen Tibbits

Dr. Miriam Rosin
Senior Supervisor

Dr. Allan Davison

Dr. Siu Sing Tsang
External Examiner
B.C. Cancer Research Centre

DATE APPROVED: 11 DEC 90

PARTIAL COPYRIGHT LICENSE

I hereby grant to Simon Fraser University the right to lend my thesis, project or extended essay (the title of which is shown below) to users of the Simon Fraser University Library, and to make partial or single copies only for such users or in response to a request from the library of any other university, or other educational institution, on its own behalf or for one of its users. I further agree that permission for multiple copying of this work for scholarly purposes may be granted by me or the Dean of Graduate Studies. It is understood that copying or publication of this work for financial gain shall not be allowed without my written permission.

Title of Thesis/Project/Extended Essay

Sensitivity of Ataxia-Telangiectasia Fibroblast Cultures to the Chromosome-Damaging Effect of Hydrogen Peroxide.

Author:

(signature)

Ming Yi

(name)

Jan 4, 1991

(date)

ABSTRACT

The aim of this thesis was to compare responses of fibroblast cultures from patients with ataxia-telangiectasia (A-T) and normal individuals to hydrogen peroxide (H_2O_2) at the level of chromosomal breakage in order to determine whether A-T cells are more sensitive to external oxidative stress. Chromosomal breakage was assayed with the micronucleus test.

Four cultures from A-T patients and four from normal individuals were exposed to H_2O_2 and the induction of micronucleated cells was examined. The cytokinesis-block method was also employed to determine the frequencies of micronucleated cells among cells dividing after chemical treatment and to provide an index of toxicity of H_2O_2. Spontaneous frequencies of micronuclei formation (in the absence of H_2O_2 treatment) were significantly different for A-T and normal cultures (mean value for percentage of cells with micronuclei $\pm$ S.E.: A-T cultures, $7.73\pm1.25\%$; normal cultures, $0.69\pm0.13\%$, $p<0.01$). A-T cultures responded to H_2O_2 treatment with a greater increase in micronucleus frequencies than that observed in normal cultures. In the micronucleus test without using cytokinesis-block method, the mean values induced by H_2O_2 treatment were $9.27\pm1.47\%$ for A-T cultures and $2.77\pm0.16\%$ for normal cultures ($p<0.01$). In the micronucleus test with using cytokinesis-block method the mean values induced by H_2O_2 were $19.04\pm3.21\%$ for A-T cultures and $3.47\pm0.25\%$ for normal cultures ($p<0.01$). A time course study of H_2O_2 effect showed that an elevation in micronucleus frequencies occurred earlier in A-T cultures (significant increase by 1 hour after treatment) than in normal cultures ($p<0.01$). A significant increase in micronucleus frequencies in normal cultures was not seen until 9 hours after H_2O_2 treatment.

These data suggest a G_2-phase sensitivity of A-T cells to H_2O_2, and indicate that significant differences exist between A-T and normal cultures in the pattern of response to H_2O_2, a chemical produced during normal cellular metabolism. The results support the hypothesis that the exposure of cell cultures to active oxygen species may produce a higher level of chromosomal damage in cells from A-T patients than in normal individuals. Therefore, this study may support the use of the A-T syndrome as a model system for studying the role of DNA damage by active oxygen species in human cancer.

ACKNOWLEDGEMENTS

Many thanks to all the people who helped.

Particularly, I want to thank my supervisor, Dr. M. Rosin for her expert supervision and Dr. A. Davison for his kind guidance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1. Ataxia-telangiectasia (A-T)

1.1.1. Clinical characteristics of A-T

A-T is a syndrome which shows human autosomal recessive inheritance. It can best be clinically and pathologically delineated as a disease featuring a progressively developing cerebellar ataxia from early childhood, and an oculocutaneous telangiectasia, which appears somewhat later. Cerebellar ataxia is the failure of muscular coordination due to a disorder of the cerebellum. Telangiectasia is a skin lesion which is commonly described as a permanent dilation of blood vessels over the eyes and ears. A-T patients possess a proneness to progressive broncho-pulmonary disease and to lymphoreticular neoplasia. A-T is not limited to any particular ethnic group. Because of the variability in some of the clinical and laboratory features of A-T, this syndrome has been thought to be genetically heterogeneous. Death typically occurs during the second decade of life either from overwhelming pulmonary infection or cancer. A-T is also characterized by immunodeficiency, endocrine abnormalities, clinical and cellular radiosensitivity, chromosomal instability and a DNA repair/process defect [reviewed by Sedgwick and Boder, 1972; Bridges and Harnden, 1982; Gatti, et al., 1982; Boder 1985; Gatti and Swift, 1985; Lehmann, et al., 1987; McKinnon, 1987]. The clinical characteristics of A-T are presented in Table 1.

The diagnosis of A-T is based both on the clinical phenotypes of ataxia and telangiectasia (for which there is an absolute requirement) and on a

Table 1 The Clinical Features of A-T

1. Ataxic movements

2. Telangiectasias

3. Neurological degeneration

4. Frequent sinopulmonary infection

5. Immunological defects

6. Cancer proneness

7. Progeric changes of hair and skin

8. Retardation of somatic growth

9. Equable disposition

10 Hypersensitivity to ionizing radiation

References: Boder, 1985; McKinnon, 1987.

number of specific laboratory tests. The most constant marker of A-T is an elevated serum alpha-fetoprotein (AFP) level; another useful laboratory marker of A-T is an elevated plasma level of carcinoembryonic antigen (CEA). The humoral or cellular immunological defects also provide a diagnosis of A-T. It is usually demonstrated as absent or low serum levels of IgA, IgG_2 and/or IgE, diminished responses to skin test antigens, and peripheral lymphopenia. The presence of spontaneous chromosome breaks and rearrangements in short term cultures of T-lymphocytes and/or in fibroblast cultures is also an important laboratory marker of A-T. These laboratory markers may be crucial in the diagnosis of an atypical, uncertain, or early case of A-T. However, their presence is not essential for the diagnosis of A-T, if both ataxia and telangiectasia are present [Boder, 1985].

1.1.2. Frequencies of A-T homozygotes and heterozygotes in general population

A-T is a rare condition. Its incidence and gene frequency were reported in 1986 based on a vigorous casefinding in the United States in 1970-72 and 1980-84 which identified 231 white, 29 black, and 3 oriental A-T cases [Swift, et al., 1986]. White individuals homozygous for A-T were born at the rate of 3.0 per million live births in the U.S. in the years 1965-69. The highest observed incidence was in the state of Michigan for 1965-69 where identified white A-T patients were born at the rate of 11.3 per million births. The minimum frequency of one of the hypothetical A-T genes in the U.S. white population was estimated to be 0.0017.

In contrast, the frequency of A-T carriers in the general population may be quite significant. The A-T heterozygote frequency has been estimated to be between 0.68% and 7.7%, with 2.8% being the most likely estimate [Swift, et al., 1986]. Although several biological tests have been proposed for identifying heterozygotes, none has proven to be totally effective. A-T heterozygotes are not clinically recognizable.

A-T homozygotes can be identified by their clinical symptoms and the diagnosis confirmed by in vitro cellular and chromosomal hypersensitivity to x-rays. However, radio-sensitivity of primary and transformed cells derived from obligate heterozygotes usually overlap or fall within the normal range when assayed by colony-forming ability, inhibition of DNA synthesis, chromosome aberrations, micronucleus production or DNA replication and repair [Chen, et al., 1978; Paterson, et al., 1976; Kinsella, et al., 1982; Natarajan, et al., 1982; Little and Nagasawa, 1985; Nagasawa, et al., 1985]. The asymptomatic A-T heterozygotes are similarly difficult to distinguish in cell culture following exposure to radiomimetic drugs [Kohn, et al., 1982]. Although A-T heterozygote lymphoblastoid cells have been identified on the basis of reduced post-irradiation cell viability, as measured with trypan blue [Chen, et al., 1978], this assay did not control for between- or within-group heterogeneity in cell growth rate, which can be significant. The alternative approaches for identification of A-T heterozygotes are by differential survival of fibroblasts exposed to neocarzinostatin [Shiloh, et al., 1982] and by differential chromosomal radiosensitivity of fibroblasts in the G_2 phase of the cell cycle [Parshad, et al., 1985; Shiloh, et al., 1986]. It has been shown by cumulative incorporation of tritiated thymidine that fibroblasts from A-T heterozygotes, irradiated at confluence and then

immediately subcultured, were more likely to be blocked in G_0/G_1 and delayed in cell-cycle entry than were both normal and A-T homozygote fibroblasts [Little and Nagasawa, 1985; Nagasawa, et al., 1985]. Although it was possible to distinguish heterozygotes from normal controls with this method, some presumed normal cell lines resembled heterozygotes in their response [Nagasawa, et al., 1987]. The identification of A-T heterozygotes by flow cytometic analysis of x-ray damage [Rudolph, et al., 1989] showed that the fibroblasts from A-T heterozygotes were distinguished from normal controls by their significantly lower cumulative labeling index following G_0/G_1 x-irradiation and immediate release from confluence into medium containing 5-bromodeoxyuridine (BrdU, thymidine analogue). However, the misassignment of A-T heterozygotes may also occur in this method.

In summary, it is not possible currently to identify A-T heterozygotes in the general population with any degree of certitude. Efforts to identify the A-T loci [Gatti, et al. 1989] may lead to a genetic probe which will make it possible to obtain a more definitive measurement of the actual number of A-T heterozygotes in the general population.

1.1.3. Cancer in A-T homozygotes, A-T heterozygotes, and blood relatives

A-T patients have an unusually high incidence of cancer, particularly lymphomas and lymphocytic leukemias [Swift, et al., 1976; Spector, et al., 1978, 1982]; excesses of 61-fold and 184-fold for all cancers have been reported among white and black patients, respectively [Morrell, et al., 1986]. Primary carcinomas of the stomach, liver, ovary, salivary glands, oral cavity, breast, and pancreas have also been reported [Spector,

et al., 1982; Morrel, et al., 1986; Narita and Takagi, 1984; Lambert, et al., 1982]. A-T heterozygotes also have an excess cancer risk [Swift, 1982, 1985; Swift, et al., 1987]. The relative risk of cancer has been estimated to be 2.3 for men and 3.1 for women [Swift, et al., 1987]. The relative risk of cancer in blood relatives of A-T patients has been estimated as 1.6 for men and 2.0 for women. Breast cancer in women was the cancer most clearly associated with heterozygosity for A-T; its relative risk in heterozygotes was estimated as 6.8. It is estimated that between 8.8 and 25% of patients with breast cancer in the U.S. white population could be heterozygous for A-T [Swift, et al., 1987]. The different types of cancer and their relative risks in A-T homozygotes, heterozygotes and blood relatives are presented in Table 2.

The reasons for the high cancer incidence in the families with A-T remain unknown, despite considerable speculation and extensive investigation of the various immune defects [Roifman and Gelfand, 1985], random chromosome breaks and stable translocations [Taylor, 1982], and post-irradiation abnormalities demonstrated in patients with A-T and in their cells [Gatti and Swift, 1985; Bridges and Harnden, 1982]. Each of these abnormalities will be described in the following sections.

1.1.4. Chromosomal instability in patients with A-T

A-T is one of the chromosome-breakage syndromes [German, 1969]. Both homozygotes and heterozygotes of A-T have an increased spontaneous chromosomal instability [Hecht, et al., 1966, 1973; Cohen, et al., 1973, 1975; Oxford, et al., 1975; Al Saadi, et al., 1980; Taylor, 1982].

Spontaneous chromosomal instability is defined as an increase in chromosome breakage in untreated cell cultures. Spontaneous

Table 2 The incidence and type of cancer in A-T patients (homozygotes), parents of such patients (obligate heterozygotes), and blood relatives

	Relative Risk for Cancer	Types of Cancers	References
Homo-zygotes	61 for whites 184 for blacks	Lymphomas (60%), Leukemias (27%), Hepatocellular carcinoma, Ovarian carcinoma, Cerebellar astrocytoma, Leiomyosarcoma of the uterus, Carcinoma of the parotid gland.	Morrell, et al. 1986 Kumar, et al. 1979 Amromin, et al. 1979
Hetero-zygotes	2.3 for men 3.1 for women	Breast cancer (6.8 for women), Nonmelanoma, Skin cancers, Cancers of pancreas and bladder.	Swift et al. 1987
Blood Relatives*	1.6 for men 2.0 for women	Breast cancer, Nonmelanoma, Skin cancers (3.7), Lung cancer (2.3), Cancers of pancreas and bladder and prostate, Cancers of hematologic and lymphoid.	Swift et al. 1987

*Blood relatives are defined as persons who are consanguineous to the patient, i.e., who are descended from the same person or the same ancestor [from "Webster's Third New International Dictionary of the English language unabridged", G. & C. Merriam Company, Springfield, Massachusetts, U.S.A.].

chromosomal aberrations ordinarily are readily detectable in both blood T lymphocytes and dermal fibroblasts from A-T patients, with the frequency in fibroblasts generally being greater [Cohen, et al., 1973, 1975, 1979]. Also, T lymphocytes and dermal fibroblasts from A-T heterozygotes have elevated chromosome aberration frequencies [Al Saadi, et al., 1980; Cohen, et al., 1975; Oxford, et al., 1975]. However, it should be noted that a large variation in the levels of chromosomal breakage has been observed among different A-T patients, with several reports of individuals with spontaneous frequencies overlapping control values [Pfeiffer, 1970; Hardnden, 1974; Taylor, et al., 1981; Bochkov, et al., 1974; Rosin, et al., 1989]. Similarly, a wide range in spontaneous chromosomal instability has been observed in cultures from A-T heterozygotes [Al Saadi, et al., 1980; Cohen, et al., 1975; Oxford, et al., 1975; Gropp and Flatz, 1967; Rary, et al., 1974; Levitt, et al., 1978; Lampert, 1969; Harnden, 1974; Schmid and Jerusalem, 1972; Rosin, et al., 1989], with only a portion of such individuals showing an elevation above normal controls.

In vivo evidence for an elevation in spontaneous aberration levels comes from studies on increased frequencies of micronuclei in exfoliated cells from A-T patients [Rosin and Ochs 1986]. These exfoliated cells are collected by smearing the buccal mucosa or by centrifuging urine samples. This latter study provided data which suggested that A-T patients differed widely in the level of "spontaneous" chromosomal breakage in epithelial tissue, with some patients showing levels overlapping normal controls. In addition, a significant portion of A-T obligate heterozygotes displayed elevated levels of exfoliated cells with micronuclei.

In summary, although an elevation in spontaneous chromosomal breakage is often reported for A-T homozygotes, and, less frequently, for obligate heterozygotes, this phenotype may be inconsistently expressed. The reason for this heterogeneity in the expression of chromosomal instability has yet to be determined.

1.1.5. Sensitivity of A-T to radiation

Extensive research has been done on the hypersensitivity of A-T fibroblasts to gamma-irradiation, x-ray and radiomimetic drugs [Paterson, et al., 1979, 1985; Taylor, et al., 1975; Paterson and Smith, 1979; Painter and Young, 1980]. A-T patients are extremely sensitive to the therapeutic ionizing radiation used to treat malignancies [Getoff, et al., 1967; Morgan, et al., 1968; Cunliffe, et al., 1975]. In addition, in vitro studies have shown that cell cultures from A-T fibroblasts exhibit poor survival in vitro following ionizing radiation as measured by a cellular survival test for colony formation efficiency [Taylor, et al , 1975; Gatti and Hall, 1983]. At the cellular level, this increased sensitivity is reflected in increased x-ray-induced frequencies of both chromosome- and chromatid-type aberrations at mitosis [Taylor, et al., 1976; Natarajan and Meyers, 1979]. Increased aberration frequencies are also observed in A-T cells after treatment with chemotherapeutic agents such as bleomycin [Rary, et al., 1974; Taylor, et al., 1975, 1976, 1979; Lehmann and Stevens, 1977, 1979; Cohen, et al., 1981; Kohn, et al., 1982; Shaham, et al., 1983; Zampetti-Bosseler and Scott, 1985], neocarzinostatin [Cohen and Simpson, 1983], and etoposide [Henner and Blazka, 1986]. A-T cells exhibit an enhanced frequency of chromosomal aberrations in both G_0 and G_2 phase of the cell cycle [Taylor, 1978, 1982; Bender, et al., 1985; Parshad, et al., 1985] as well as the unusual occurrence

of chromatid deletions in cells exposed to x-rays in G_0 [Taylor, et al., 1976; Taylor, 1978].

Semiconservative DNA replication in A-T cells following x-irradiation differs from that in irradiated normal cells [De Wit, et al., 1981; Edwards and Taylor, 1980; Houldsworth and Lavin, 1980; Painter and Young, 1980; Lehmann, et al., 1982]. Replication normally is suppressed following x-irradiation. This presumably allows time for damaged DNA segments to be repaired before they replicate and possibly produce a permanent alteration of the base sequence of the damaged DNA segment. A-T cells exhibit diminished inhibition of the initiation of DNA synthesis following x-irradiation [Houldsworth and Lavin, 1980; Painter and Young, 1980; Lehmann, et al., 1982] or exposure to the antitumor antibiotic neocarzinostatin, which is a radiomimetic drug [Hatayama, et al., 1978; Hatayama and Goldberg, 1980; Shiloh, et al., 1982a, b; Shiloh and Becker, 1982; Becker and Tabor, 1989]. In A-T cells, DNA synthesis following x-irradiation or the treatment of neocarzinostatin is suppressed only slightly, so that damaged segments of DNA are permitted to replicate; one of the presumed consequences of this defective response is a dramatically increased incidence of microscopically detectable chromosome abnormalities. Therefore, it was suggested that replication of damaged DNA segments may account also for the elevated incidence of chromosome aberrations that is detectable in untreated A-T cells [Huang and Sheridan, 1981]. The diminished inhibition of DNA synthesis was thought to be due to a defect in the DNA-damage-recognition system in A-T cells. Because x-ray-induced DNA damage is not recognized immediately by A-T cells, the cycling cells will progress unchecked through the S phase of the cell-

division cycle and not be permitted to complete necessary DNA repair, so that DNA replication occurs on damaged templets. Increased frequencies of chromosome aberrations such as chromatid breaks are the direct consequence [Cramer and Painter, 1981; Taylor, et al., 1976; Paterson, et al., 1976; Corforth and Bedford, 1985].

1.1.6. Underlying mechanisms of spontaneous chromosomal instability and radiation sensitivity in A-T

The molecular mechanism by which chromosome instability is enhanced in A-T is unknown. There is speculation that it may be in some way related to the enhanced sensitivity of A-T cells in vitro to radiation and radiomimetic agents. It has been suggested that the diverse features of A-T, including the increased spontaneous chromosome instability, might be explained on the basis of a molecular defect involving some cofactor necessary for the proper functioning of multiple enzymes [Harnden and Brown, 1981]. Metals such as zinc, manganese, and copper that are necessary for the proper functioning of metalloenzymes were thought of as good candidates for being deficient in A-T; however, no data derived from the study of A-T supports the suggestion.

The underlying basis of the increased radiation-sensitivity in A-T cells at the chromosome level is not well understood, although the following possibilities have been raised: (a) these cells are intrinsically more sensitive to the DNA-damaging effects of these agents prior to any repair process [Bender, et al., 1985]; (b) A-T cells are deficient in a repair process [Taylor, et al., 1976]; and (c) A-T cells do not slow their rate of DNA synthesis after treatment and thus allow some unrepaired DNA lesions to be fixed into

chromosome lesions during DNA synthesis [Painter and Young, 1980]. Evidence with some A-T lines suggests that the molecular basis of the enhanced sensitivity of A-T cells to genotoxic agents may be due to a defect in excision-repair of base-damaged lesions in DNA [Paterson, et al., 1976; Inouce, et al., 1977; Smith and Paterson, 1983]. However, it has been difficult to distinguish between these hypotheses, since most studies have determined chromosome aberration frequencies only when the cells attain mitosis, long after the time these processes have taken place. One approach to studying the basis of A-T chromosome breakage sensitivity is to visualize damage and its repair directly in interphase cells using the technique of premature chromosome condensation (PCC) [Johnson, et al., 1982; Hittelman, 1984]. PCC technique was used to determine whether the increased chromosome damage observed after bleomycin treatment is due to increased initial chromosome damage or to a decreased capability of these cells to repair chromosome damage.

Chromosome damage is immediately apparent in the G_1 phase of normal cells after treatment with ionizing radiation [Waldren and Johnson, 1974; Hittelman and Rao, 1974a], bleomycin [Hittelman and Rao, 1974b], and neocarzinostatin [Hittelman and Pollard, 1982]. In normal cells after bleomycin and ionizing radiation (x- and gamma-rays), chromosome damage repair shows a two-component curve, a fast repair component occurring within 30 minutes and a continuing slow repair component [Sen and Hittelman, 1984]. Cornforth and Bedford, [1985] used the PCC technique to investigate the nature of radiosensitivity in two A-T cell lines and found that, after x-irradiation, A-T cells had the same initial frequency of breaks and the same initial rate of rejoining of chromosome breaks as

normal fibroblasts. However, the fraction of chromosome breaks that did not rejoin was 5 to 6 times greater for the A-T cells.

The DNA double-strand break is an important type of radiation-induced damage [Hutchinson, 1985; Thacker, 1986] but is produced without apparent site specificity in DNA. However, a simple type of double-strand break is produced by restriction endonucleases and the choice of DNA molecule allows the induction of one break at a specific restriction site per molecule. Use of a recombinant DNA molecule (vector) with a number of unique restriction sites can allow an analysis of the rejoining or recombinational repair of breaks at different sites with different types of DNA termini. If these restriction sites are placed in a selectable gene the fidelity of the repair process is monitored insofar as this affects gene expression in the host cell (transformant). The use of integrating vectors has served to characterize the various radiation-sensitive cell lines including A-T cells. The A-T cells show a consistent reduction in the fidelity of rejoining double-strand breaks [Thacker, 1989]. The studies using recombinant DNA techniques [Cox, et al., 1986; Debenham, et al., 1987] also indicated that the cellular sensitivity of A-T to x-rays could be caused by a disequilibrium between the rejoining and exonuclease degradation of the ends of DNA double-strand breaks.

Studies on the underlying etiology of the high chromosomal sensitivity of A-T have also focused on an assessment of the yields and kinetics of chromatid aberrations of both deletion and exchange types induced in the G_2 phase of the cell cycle by x-rays and the influence of the DNA synthesis inhibiting nucleoside analogue 9-beta-D-arabinofura nosyladenine (araA) on the x-ray-induced chromatid damage [Mozdarani

and Bryant, 1987]. The number of chromatid deletions in G_2 cells decreased and the number of exchanges increased as a function of time after x-ray irradiation in normal human cells. In the presence of araA the decrease in deletions with time, observed after x-rays alone, was inhibited; but the increase in exchanges was threefold larger in the presence of araA than in its absence. These kinetics were thought to reflect the underlying repair of DNA double-strand breaks since the rate of disappearance (rejoining) of x-ray-induced chromatid deletions paralleled the repair of DNA double-strand breaks as measured in other cell lines by biochemical methods [Bryant and Blocher, 1980; Blocher and Pohlit, 1982] and was also similar to the kinetics of rejoining of chromosome breaks in G_0 human cells as determined by the technique of premature chromosome condensation [Cornforth and Bedford, 1983, 1985].

Moreover araA was shown to inhibit repair of DNA double-strand breaks [Bryant and Blocher, 1982]. The rejoining of chromatid deletions was found to be inhibited by araA, thus the frequency of deletions in the presence of araA should represent the frequency of deletions in the absence of double-strand break repair. The rejoining kinetics for deletions in A-T [Mozdarani and Bryant, 1989] was found to be similar to that found in normal human fibroblasts [Mozdarani and Bryant, 1987]. The number of deletions in x-irradiated A-T cells was found to be higher by a factor of approximately 2 than that found in normals, indicating that in A-T a higher rate of conversion of double-strand breaks into chromatid deletions occurs.

In summary, the aforementioned studies are supportive of a defect existing in A-T cells in a DNA repair system which acts on free radical-

induced damage to sugar/base moieties in the DNA double helix [Paterson, et al., 1976; Shiloh, et al., 1983]. However, other possibilities have been explored including an anomaly in specific DNA cutting and splicing enzymes, such as topoisomerase II [Mohamed, et al., 1987]. In addition, evidence of consistent anomalies in extracellular proteins such as fibronectin [Murname and Painter, 1983; Lavin and Seymour, 1984] and actin [McKinnon and Burgoyne, 1985a], and in other cell surface aberrations (e.g. actin-containing microfilaments [McKinnon and Burgoyne, 1985b]) in A-T cells has supported a hypothesis that a deranged cytoskeletal structure could be the root cause of the many defects observed in this syndrome [McKinnon, 1987].

Several attempts have been made to use the information generated by studies of x-ray-induced effects in A-T cells to generate hypotheses for the etiology of the increased spontaneous chromosomal breakage in A-T cells. One such hypothesis is that cells from A-T patients are subjected to proxidant stress produced either by an increased production of active oxygen species or a defect in the cells capacity to scavenge or inactivate such radicals. Support for this hypothesis comes from reports of a defect in glutathione metabolism in A-T fibroblasts [Meredith and Dodson, 1987] and a decrease in catalase activities in biopsies from A-T patients [Vuillaume, et al., 1989; Vuillaume, 1987]. Both of these observations have been contested by other scientists [Sheridan III and Huang, 1979; Dean, 1987]. Emerit, Cerutti and co-workers have observed the release of clastogenic factors (CF) from individuals belonging to Bloom's syndrome (another chromosome breakage syndrome), individuals with chronic inflammation, patients with autoimmune disease, and from fibroblast cells after

stimulation with TPA [Emerit and Lahoud-Maghani, 1989]. This CF appears to be a lipophilic substance of MW less than 10,000 daltons, possibly resulting from the arachidonic acid of cell membranes released as a consequence of oxidative damage and subsequently degraded to genotoxic aldehydes in an autoxidative process. Whether such a clastogenic factor is present in serum of A-T patients is currently being investigated [Emerit and Rosin personal communication]. Shaham and co-workers have already isolated a clastogenic factor from the serum of A-T patients and have shown it to be a peptide with a 500-1,000 molecular weight. Shaham speculated that this CF could be involved in spontaneous chromosome instability in A-T [Shaham and Becker, 1981; Shaham, et al., 1980, 1982, 1984; Becker, et al., 1983, 1984; Becker, 1986].

It is possible that the spontaneous DNA-damage in A-T patients comes not from a defect in production/detoxification of active oxygen species, but rather from a defect in repair of the damage. Active oxygen species can cause macromolecular damage whose biological effects resemble those of x-rays (see chapter 2.2.). Whether the repair of DNA damage produced by active oxygen species is similar to that by x-rays is yet to be resolved.

1.2. Active oxygen species

Oxygen is indispensable for aerobic life, but it is also a potential threat. The stress of oxygen is mainly due to its tendency to form active oxygen species, which are active intermediates produced during biological metabolism of oxygen. These intermediates are molecules or free radicals which are prone to gaining electron(s). These include superoxide ($O_2^{\bullet -}$),

hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^{\bullet}$). [Numerous reviews on various aspects of oxidative stress have been published: Hochstein and Atallah, 1988; Vuillaume, 1987; Cerutti, 1985; Boveris, 1978; Cadet and Berger, 1985; Cadet, et al., 1986; Chance, et al., 1979; Friedberg, 1985; Jones, 1982; Jouve, 1985; Koch, 1979; Marcel, 1986; Norman, et al., 1986; Oberley, 1983; Pryor, 1976-1986; Sarasin, 1981].

There is an impressive list of degenerative processes that may have their etiologic origin in events mediated by active oxygen species. It comprises Parkinson's disease [Cohen and Heikkila, 1974], ischemic damage [McCord, 1985], emphysema [Dooley and Pryor, 1982], arthritis [McCord, 1974], mutagenesis [Moody and Hassan, 1982], cancer [Ames, 1983; Cerutti, 1985; Borek and Troll, 1983], and senescence [Cutler, 1984]. It has been suggested by many investigators that even at normal steady-state concentration, active oxygen species may be continuously producing cumulative damage in specific targets which may eventually lead to tissue degeneration.

The importance of active oxygen species in both cancer initiation and promotion has become increasingly appreciated in recent years [Ames 1983]. One very important discovery was that certain antioxidants can prevent the promotion process in several cell transformation systems [Solanki, et al., 1984; Goldstein, et al., 1981; Kensler, et al., 1983; Kennedy, et al., 1984; Borek and Troll, 1983; Nakamura, et al., 1985; Armato, et al., 1984]. In addition, free radical-generating systems are promoters of cell transformation [Slaga, et al., 1981; Zimmerman and Cerutti, 1984; Weitzman, et al., 1985]. It has been proposed that the etiology of Bloom's syndrome, a disease whose patients develop malignancies at a much

higher frequency than normal individuals and whose cells exhibit a high frequency of chromosome aberrations, resides in a deficiency in the detoxification of active oxygen species [Emerit and Cerutti, 1981]. The knowledge that active oxygen species are clearly involved in carcinogenesis is of utmost importance. These species are normal metabolites in the cells where their levels can be changed by oxidants and antioxidants present in diet and in the general environment [Ames, 1983].

1.2.1. Source of active oxygen species

1.2.1.1. Endogenous source

Most of the oxygen consumed in biological systems is reduced to water by the transfer of 4 electrons:

$$O_2 + 4e^- + 4H^+ \text{---> } 2H_2O.$$

This reduction is primarily involved in energy coupling, which takes place over highly organized membrane systems, e.g., oxidative phosphorylation over membranes of mitochondria. This constitutes the energetic basis for aerobic life. However, some oxygen in living cells is also reduced by bivalent as well as univalent processes enzymatically or nonenzymatically. These processes result in the formation of hydrogen peroxide (H_2O_2):

$$O_2 + 2e^- + 2H^+ \text{---> } H_2O_2$$

and the superoxide anion radical ($O_2^{\bullet -}$):

$$O_2 + e^- \text{---> } O_2^{\bullet -}.$$

Superoxide anions, which serve as an additional source of hydrogen peroxide, undergo both a spontaneous and a very rapid enzymatic dismutation to hydrogen peroxide. Although hydrogen peroxide may act directly on the biologically important molecules, it also undergoes metal-catalyzed decomposition to yield reactive hydroxyl radical ($HO^{\bullet}$):

$$e^- + Fe^{3+} \text{----> } Fe^{2+} ;$$

$$Fe^{2+} + H_2O_2 \text{---> } Fe^{3+} + HO^- + HO^{\bullet}.$$

The toxicity of hydrogen peroxide seems most related to its capacity to form the hydroxyl radical. The hydroxyl radical is a potent known oxidant, among the most reactive free-radicals formed in biological systems.

Many biological oxidation reactions can generate active oxygen species. Such reactions include redox reactions of catecholamines, hydroquinone, xanthine, and the biosynthesis of prostaglandins and leucotriens in macrophages and monocytes. [Boveris, 1978; Fridovich, 1976; Loschen, et al., 1974a,b; Marklund, et al., 1982; Nohl and Hegner, 1978a,b].

Hydrogen peroxide is also produced by a number of intracellular flavin oxidases. These enzymes are usually localized within peroxysomes which contain high amounts of catalase. However, xanthine oxidase is an extra-peroxysomal enzyme which generates peroxide. The leak of electrons from the mitochondrial electron transport chain can also form superoxide and hydrogen peroxide [Forman and Boveris, 1982]. The autoxidation of heme proteins such as hemoglobin and myoglobin may contribute to superoxide and peroxide formation in vivo, although its extent is difficult to estimate [Hochstein and Atallah, 1988].

The plasma membrane-bound oxidases, such as those associated with phagocytizing cells, may be activated during inflammation and produce active oxygen species [Hamers and Ross, 1985]. This activity provides an important source of oxidants in interstitial space.

1.2.1.2. Exogenous source

A variety of external agents or conditions can induce oxy-radical stress, such as hyperbaric oxygen tension (exceeding approximately 40%), radiation (UV below 290nm, aerobic ionizing radiations, e.g., x-, alpha-, beta-, gamma-rays), certain drugs (e.g., streptonigrin, adriamycin, mitomycin C, bleomycin), xenobiotics (6-hydroxydopamine, and 6-amino-dopamine), and some membrane active agents (such as peptide hormones, growth factors, silica and asbestos). A number of chemical oxidants, such as hydrogen peroxide and potassium permanganate, are obvious exogenous sources of active oxygen species.

The generation of superoxide and hydrogen peroxide from cigarette smoke may be important in the initiation and promotion steps of tobacco carcinogenesis [Nakayama, et al., 1984]. Cigarette smoke generates hydrogen peroxide and superoxide when it is bubbled through neutral buffer solution. Hydrogen peroxide is thought to be formed by autoxidation of polyphenols such as catechol and hydroquinone in cigarette smoke. The autoxidation of phenolics is also implicated in the formation of active oxygen species in betel quid chewers in India, a population at elevated risk for oral cancer [Stich, 1988].

Coffee was shown to be genotoxic in vitro and contain a mutagen, methylglyoxal. Hydrogen peroxide is thought to be present in instant coffee

because the mutagenicity of this kind of coffee is sensitive to catalase. It was concluded that the hydrogen peroxide-generating system is produced by the roasting of coffee beans and is promoted by the increase in water temperature. Mutagenicity increased with coadministration of methylglyoxal and hydrogen peroxide. Therefore, hydrogen peroxide and methylglyoxal may contribute to the mutagenicity of instant coffee [Furihata, et al., 1985; Nagao, et al., 1985; Fujita, et al., 1985].

1.2.2. Type of damage produced by active oxygen species

Active oxygen species can result in a variety of cell damage by reacting with readily oxidizable target molecules either directly or indirectly. The biological consequences are mutations, sister chromatid exchanges, chromosomal aberrations, cytotoxicity, tumor initiation and promotion, and cellular degeneration. DNA damage produced by free radicals is probably the most frequent lesions in living cells. The most reactive species, the hydroxyl radical, produces a large amount of DNA damage, including single or double strand breaks, damage to the deoxyribose moiety, and damage to the purine and pyrimidine bases (e.g., pyrimidine dimers or adducts, depurination or depyrimidination, guanosine derivatives) [for reviews, see Ward, 1975; Hagen, 1986; Hutchinson, 1985; Von Sonntag and Schuchman, 1986; Teoule, 1987].

Hyperbaric oxygen causes mutations in bacteria [Amstad, et al., 1984; Amstad and Cerutti, 1983] and chromosomal aberrations in eukaryotic cells [Sanford, et al., 1979; Winter and Smith, 1972; Sturrock and Nunn, 1978; Yost and Fridovich, 1976; Bruyminckx, et al., 1978]. Superoxide radical and H_2O_2 induce a small number of mutations

[Cunningham and Lokesh, 1983; Levin, et al., 1982] but a lot of DNA breaks and chromosomal aberrations in bacteria [Lesko, et al., 1980; Braun and Fridovich, 1981; Bradley and Erickson, 1981; Emerit, et al., 1982].

Human leukocyte cell cultures release $O_2^{\bullet -}$ after stimulation with 12-O-tetradecanoylphorbol-13-acetate (TPA), and produce strand breakage in intracellular DNA a few minutes later. The mechanism may involve one or more metabolic steps with hydroxyl radical production [Birnboim and Kanabus-Kaminska, 1985].

Hartman, et al.[1984] showed that Salmonella typhimurium strains grown under aerobic conditions exhibited enhanced frequencies of small deletion and frameshift mutations compared to strains grown anaerobically. Farr, et al.[1986] showed that Escherichia coli (E.coli) mutants that lack both the manganese- and iron-containing superoxide dismutases (sodA sodB double mutants) have increased spontaneous-mutation frequencies relative to wild-type strains during aerobic growth. In addition, several E.coli DNA repair mutants, e.g., recA xth double mutants [Imlay and Linn, 1986], certain polA mutants [Boling, et al., 1984], and polA recB double mutants [Morimyo, 1982], form filaments and die aerobically, although they grow normally under anaerobic conditions.

Hoffmann, et al. [1984] compared the cytotoxic effect and the yield of DNA strand breaks by oxy-radical stress in cells of different species. The rate of loss of reproductive capacity caused by hydrogen peroxide was shown to be 6 times faster in human fibroblasts than in Chinese hamster fibroblasts. The response in mouse fibroblasts was between these two. A similar H_2O_2 concentration produced 5-10 times more strand breaks in

human DNA than in hamster DNA and 2-4 times more than in mouse DNA. They proposed that this difference may be due to the different amount of chromatin-bound iron and the level of superoxide ion in these cells. They also found that H_2O_2 does not cause breaks in purified DNA and its effect in the cells is probably mediated by a transition metal (probably ferrous ion) complexed to a macromolecule in the nucleus. Hydrogen peroxide may react with this metal and produce hydroxyl radical, which is thought to be the ultimate species to damage the DNA [Meneghini and Hoffmann, 1980].

8-Hydroxydeoxyguanosine (8-OH-dG) is formed in vivo in cellular DNA on treatment with various oxygen radical-producing agents and it is repairable [Kasai, et al., 1986]. 8-OH-dG was detected in DNA isolated both from HeLa cells after the cells in tissue culture had been irradiated with x-rays and from the liver of mice after the whole animals had been irradiated with gamma-rays. The amounts of 8-OH-dG in DNA after in vivo irradiation were three orders of magnitude lower than those after in vitro irradiation. The 8-OH-dG produced in liver DNA by irradiation of mice decreased with time, suggesting the presence of a repair enzyme(s) acting on 8-OH-dG in mouse liver. Treatment of Salmonella typhimurium cells with hydrogen peroxide also caused increase in the 8-OH-dG content.

It was demonstrated by Schraufstatter, et al. [1988] that H_2O_2 added extracellularly (in micromolar concentrations 10-100uM) induced DNA strand breaks in various human target cells (cultured human fibroblast cells, lymphocytes, PMN). The sensitivity of a specific target cell was inversely correlated to its catalase content and the rate of removal of H_2O_2 by the target cell. Oxidant species produced by xanthine oxidase/purine or phobol myristate acetate-stimulated monocytes induced DNA breakage of

target cells in proportion to the amount of H_2O_2 generated. These DNA strand breaks were prevented by extracellular catalase, but not by superoxide dismutase. It was also shown that H_2O_2 formed hydroxyl radical intracellularly, which appeared to be the most likely free radical responsible for DNA damage for three reasons: $HO^{\bullet}$ was detected in cells exposed to H_2O_2; the DNA base, deoxyguanosine, was hydroxylated in cells exposed to H_2O_2; and intracellular iron was essential for induction of DNA strand breaks.

1.3. The involvement of chromosomal change in cancer development

Chromosome aberrations have been implicated in several aspects thought to be involved in development of cancer. Chromosomal instability is characteristic of dysplasias and many premalignant conditions. Moreover, specific chromosomal aberrations appear to be associated with many types of cancer [Mitelman and Heim, 1988; Berne, et al., 1989] (Table 3). A role for chromosomal breakage, translocation, or loss is implicated in the sequence of events leading to development of neoplasia [Cooper, 1984; Flier, et al., 1988; Knudson, 1985]: (a) Such changes can activate oncongenes. For example, the translocation involved in chronic myelogenous leukemia (CML) removes abl oncogene from chromosomes 9 to 22 and sis oncogene from chromosomes 22 to 9. A new abl protein is synthesized, which increases tyrosin phosphorylation. The translocation involved in Burkitt's lymphoma results in movement of myc oncogene from chromosome 8 to the other chromosomes near to immunoglobin gene sites. (b) Chromosomal changes can also result in the loss of tumor "antioncogenes" or "suppressor" genes, as in Retinoblastoma(RB) and Wilm's tumor, which

Table 3 Association between karyotypes and specific cancers

Cancers	Chromosome aberrations
Chronic myelogenous leukemia (CML)	translocation between 22 and 9
Acute promyelocytic leukemia	translocation between 15 and 17
Acute myelogenous leukemia	abnormalities of 8 (trisomy of 8, or translocation of 8 and 21)
Burkitt's tumor	translocation of 8 and 2, 14, 22
Ovarian tumor	translocation of 6 and 14
Retinoblastoma	deletion on chromosome 13
Wilm's tumor	deletion on chromosome 11

References: Mitelman and Heim, 1988; Berne, et al., 1989.

are heritable human cancers. RB is a tumor of the eye found exclusively in young children. Wilm's tumor is a kidney tumor of children.

One of the strongest and oldest pieces of evidence supporting the involvement of chromosome change in cancer development comes from studies with individuals belonging to genetic syndromes in which chromosomal breakage rates are elevated, such as Bloom's syndrome, ataxia-telangiectasia, Fanconi anemia, and xeroderma pigmentosum. These syndromes are all characterized by an increase in risk for cancer [German, 1983; Ray and German, 1983].

1.4. Micronuclei

Because it is technically difficult to obtain metaphase cells from tissues, most chromosomal data obtained for humans has been restricted to blood cells and to *in vitro* cultured fibroblast cells; very little is from *in vivo* study. The micronucleus assay provides a relatively easy way to examine chromosomal breakage *in vivo* as well as *in vitro*.

Micronuclei are formed from chromosomal fragments (and, less frequently, from entire chromosomes) which lack connection to the spindle apparatus and lag behind when the cell divides. Such fragments are excluded from the main nucleus when it reforms and are visible as Feulgen-positive extranuclear bodies in the daughter cells. The use of micronucleus frequencies as a quantifiable indicator of chromosomal breakage has been validated on more than 100 genotoxic and/or carcinogenic chemicals using either cultured fibroblasts, hepatocytes, human lymphocytes, myelo- and erythroblasts, or animals and people

[Heddle, et al., 1978; Schmid, 1976; Stich and Rosin, 1983]. In vivo, the micronucleus assay serves as an endogenous dosimeter of DNA damage directly in the specific tissue which is the target for the carcinogens. The elevation in micronucleus frequency is dependent on the dose of carcinogen exposure. For example, a dose-dependent increase in micronucleus frequency has been observed throughout the course of radiotherapy in samples from the irradiated buccal cavity of head and neck cancer patients [Rosin and Stich, 1983; Stich and Rosin, 1983; Stich, et al., 1983].

Micronuclei require one cell division to be expressed. Therefore the frequency of micronuclei scored in a given number of cells depends on (a) the proportion of cells that have responded to the exposure of genotoxicants; (b) the proportion of the responding cells that have divided; (c) and the fate of micronuclei in cells which have divided more than once. These factors may vary greatly between different cell cultures, between different repeat experiments using same cultures, and between different doses of genotoxicant exposure. To overcome these problems, the cytokinesis-block method has been proposed [Fenech and Morley, 1985], in which cytochalasin B is added to cell cultures after genotoxicant exposure so that cytokinesis is inhibited and binucleate cells are accumulated in their first division cycle. The enumeration of micronuclei is restricted to the cytokinesis-blocked cells. Since cytochalasin B does not induce micronuclei or chromosomal damage, the cytokinesis-block method can be used both to measure the base-line level of micronuclei, which reflects spontaneous chromosomal damage, and to allow comparison of genotoxicant-induced micronuclei in different cell cultures (e.g., normal fibroblast cultures and A-T fibroblast cultures).

In recent years, a variety of experiments have been carried out using the cytokinesis-block method for analyzing micronuclei. These studies have included both in vitro and in vivo/in vitro studies in rodent and human cells [Yager and Sorsa, 1987; Wakata and Sasaki, 1987; Erexson, et al., 1987; Maki-Paakkanen, 1987; Ramalho, et al., 1988; Prosser, et al., 1988; Krishna, et al., 1989].

1.5. Relevance

Studies on the significance of active oxygen species in the development of cancer would benefit from the identification of human populations with a defect in response to active oxygen species, either at the level of production/detoxification of these species or with respect to the repair of damage produced by their activity. This study will explore the possibility that A-T may represent a group of people with a clinically identifiable syndrome which can be used as such a model.

Data supporting an increased sensitivity to active oxygen species for A-T patients would be significant for several reasons. First, although the frequency of individuals homozygous for this locus is low (0.0017), it has been suggested that heterozygous carriers of the gene may represent from 1-8% of the general population and be at elevated risk for development of a variety of cancers (see section 1.1.3.). A portion of these carriers may also be defective in the metabolism of active oxygen species and/or repair of the damage they produce. Fibroblast cultures from several A-T heterozygotes have been shown to have an elevated sensitivity to x-ray and an increased level of spontaneous chromosomal breakage (see section 1.1.4.). Secondly, a significant number of xenobiotics produce active oxygen species during

their metabolism. These xenobiotics could have an impact on individuals with an increased sensitivity to such agents, perhaps leading to an elevation in the level of "spontaneous" chromosomal breakage observed in vivo. Compounds which generate active oxygen species during their metabolism are ubiquitously present in our environment: in lifestyle habits (cigarette smoking, tobacco chewing), in the diet (plant phenolics from coffee, tea, wine), and in occupational exposures (asbestos workers), as well as being produced by some medicines (chemotherapeutic agents such as bleomycin). Finally, an understanding of the mechanism by which active oxygen species (and xenobiotics generating such agents) alter DNA is crucial to a development of preventive measures that could act to suppress cancer development in exposed humans.

1.6. Hypothesis

The hypothesis to be tested in this thesis is that A-T patients have an elevated sensitivity to oxidative stress when compared with normal individuals and that this elevated sensitivity is responsible for the increased level of spontaneous chromosomal breakage observed in vivo and in vitro in such patients.

The proposed study will involve a characterization of the response of A-T and normal cultures to hydrogen peroxide. The endpoint chosen for study will be the induction of chromosomal breakage, measured by the frequency of micronucleated cells produced. The following questions will be answered:

1. Does hydrogen peroxide produce a higher level of chromosomal breakage in A-T cells than in normal cells? This question will require

establishing harvest time- and dose- response curves of effects of hydrogen peroxide in fibroblast cultures.

2. Is there any correlation between the frequency of micronucleated cells observed spontaneously in a culture and the level of such cells induced by treatment with hydrogen peroxide?

3. Is there a cell cycle-dependence in the response of A-T cells to hydrogen peroxide treatment?

1.7. Objectives and approaches

1. To determine whether A-T cultures are more sensitive than cultures from "normal" individuals to the chromosome-damaging activity of hydrogen peroxide.

Four A-T homozygote and four normal fibroblast cultures will be chosen for study. The four A-T cultures which will be used will represent a wide range of spontaneous mutation frequencies (Fig.1). The cultures will be exposed to a dose range of H_2O_2, and micronucleus frequencies will be determined.

2. To determine whether there is a correlation between the spontaneous frequency of micronucleus formation and the maximum level of micronucleated cells induced by hydrogen peroxide.

A comparison will be made between spontaneous and H_2O_2-induced levels of micronucleus formation in the cultures used in the above section 1 to determine whether there is any correlation between these two events.

3. To characterize the pattern of chromsomal sensitivity to hydrogen peroxide with respect to dose- and cell cycle-dependence.

This portion of the thesis will require the use of cytochalasin B treatment, which allows us to study kinetics of culture growth, and G_2 phase sensitivity. Cytochalasin B treatment will be employed to determine the frequencies of micronucleated cells among cells dividing after chemical treatment and to provide an index of toxicity of H_2O_2. First, the response of these two variables with increasing doses of H_2O_2 will be determined. Second, cytochalasin B will be added to cultures at different times after H_2O_2 exposure and the time-dependence of H_2O_2-induced micronuclei formation will be determined. The cytochalasin B technique will also be used to determine the frequencies of micronuclei in cells exposed in G_2 phase by using the drug to collect cells from 0.5 to 3.5 hours after H_2O_2 treatment. Cells collected during this time have been previously shown to be those which had received their H_2O_2 exposure during G_2 phase.

CHAPTER 2

METHODS AND MATERIALS

2.1. Chemicals and media

Dulbecco's modified minimal essential (DME) medium is obtained from Flow Laboratories, McLean, Va. Fetal calf serum (FCS) is obtained from Gibco Laboratories, Grand Island, NY. Cytochalasin B is purchased from Sigma, St. Louis, Mo. Cytochalasin B stock solution is prepared as 1ug/ul in dimethyl sulfoxide (Sigma, St. Louis, Mo.), which is stored at -20°C. Cornoy's solution is composed of methanol/glacial acetic acid (3:1), prepared just before use. Fast Green (Fisher Scientific, Chemical Manufacturing Division, Fairlawn, NJ) is dissolved in 95% ethanol. H_2O_2 is obtained from BDH Chemicals, Toronto.

2.2. Fibroblast cultures

Human fibroblast cultures are obtained from the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). The fibroblast lines used, along with the characteristics of the donor from which each line was obtained, are listed in Table 4.

2.3. Tissue culture maintainence

Stock cultures are grown in plastic flasks (80 cm^2 surface area, 260ml, Gibco Canada Inc., Burlington, Ontario) in DME medium supplemented with 10% heat-inactivated FCS, penicillin (100 units/ml), and streptomycin (100 ug/ml). Cultures are routinely passaged when the cell density reaches confluency. Cultures are maintained in a water-jacketed

Table 4 Characteristics of fibroblast cultures

Diagnosis	Line No.	Age (at biopsy)	Sex	Race	Passage No. (shipped)
Normal	5757	7yr	M	BL	3
	3440A	20yr	M	WH	6
	4390	23yr	F	WH	4
	3651C	25yr	F	WH	6
A-T	647A	17yr	M	WH	9
homo-	1588		M	WH	14
zygous	1829	7yr	M	WH	11
	2052A	15yr	F	WH	7

incubator at 37°C in an atmosphere of 5% CO_2/95% air. All experiments are performed on the cultures between passages 7 and 20.

2.4. Micronucleus assay

2.4.1. Cell seeding

Logarithmically-growing cells were trypsinized off of stock culture flasks, suspended in 10% DME and seeded on sterile glass coverslips (Baxter, Canlab, 20*20mm) in 30*10mm petri dishes (Nunc, Denmark). The cell concentration at seeding (2 -- $4*10^4$ cells/dish) was adjusted for each cell line so that cell cultures grew to cover approximately 50% of the coverslip within 2 days. Each petri dish contained a total volume of 2 ml 10% DME. Coverslip cultures were incubated in 5% CO_2 in air at 37°C.

2.4.2. Treatment -- Hydrogen peroxide exposure

When the cultures reached 50% coverage of the coverslip, culture media was removed by suction from the petri dishes and replaced with three washes of DME (without FCS, 2 ml/dish). This wash media was removed by suction, followed by the addition of hydrogen peroxide solution, prepared in phosphate-buffered saline (PBS). The dishes were returned to the incubator for the duration of the treatment. As controls, cultures were treated with PBS without hydrogen peroxide. After treatment, the hydrogen peroxide solution was removed by suction and coverslips were rinsed 3 times with DME (without FCS). Fresh DME with 10% FCS was added to each dish and the dishes were returned to the incubator until harvest.

Two approaches were used for determining micronucleus frequencies: 1) direct measurement, in which cell cultures were left in the incubator for designated times (generally 48 hours) and then harvested; and 2) harvest after blocking cell cytokinesis by addition of cytochalasin B at a final concentration of 2ug/ml to each petri dish. This procedure involves adding 4ul cytochalasin B stock solution to the 2ml DME solution in each petri dish.

2.4.3. Harvesting, staining and slide preparation

The coverslip cultures were removed with forceps from the petri dishes, placed in dishes containing PBS for 20 seconds, and then allowed to air dry. The coverslips were then placed into dishes containing Cornoy's solution for 20 minutes and allowed to air dry.

The coverslips were stained with the Feulgen reaction as follows: 1N HCl, 10 seconds at room temperature; 1N HCl, 10 minutes at 63°C; 1N HCl, 10 seconds at room temperature; rinse in distilled water; Schiff's reagent, 120 minutes at room temperature; rinse with tap water, 15 minutes; followed by Fast Green, 1 minute; 70% ethanol, 1 minute; 95% ethanol, 1 minute; butanol, twice, 1 minute each; 1:1(v/v) butanol/xylol, twice, 1 minute each; xylol, twice, 1 minute each. The coverslips were mounted directly from xylol onto slides (76*26*1mm, plain, W.-Germany) using Permount.

2.4.4. Scoring

The slides were randomized and coded prior to scoring under a microscope (Olympus) 20*100-magnification oil lens. The criteria followed

for estimating the frequency of micronucleated cells were those already established (Stich and Rosin 1983a,b, 1984). For determinations made directly (without cytochalasin B), a total of 1000 intact cells were scored for the presence of micronuclei. For cultures treated with cytochalasin B, a minimum of 500 cytochalasin B-blocked cells were assayed for the presence of micronuclei. In the latter case, the fraction of cells which had undergone karyokinesis subsequent to the addition of the cytochalasin B was estimated.

2.5. Statistical analysis

Each experiment was repeated three times and the data analyzed using the computer software StatView or Minitab. The mean values for percentage of cells with micronuclei and the standard errors were determined and the t-TEST used for comparing the differences between effects in A-T cultures and normal cultures. The correlation analyses were performed with Pearson correlation and Spearman's rank correlation.

CHAPTER 3

RESULTS

3.1. Spontaneous levels of chromosomal breakage in A-T and normal cell cultures

Logarithmically growing cultures from four A-T patients and from four normal individuals were seeded onto sterile coverslips and incubated for 48 hours before harvest. The level of chromosomal breakage in each culture was determined by scoring the frequency of cells with micronuclei (Figure 1). The spontaneous levels of micronuclei production were 11-fold higher for A-T cultures than for normal cultures (average spontaneous frequency for A-T cultures, $7.73 \pm 1.25\%$; for normal cultures, $0.69 \pm 0.13\%$; $P<0.01$).

3.2. Effect of H_2O_2 exposure on A-T and normal cell cultures

The aforementioned A-T and normal cultures were treated for 1 hour with H_2O_2 at concentrations of 0.2 to 1.2 μg/ml. The cultures were harvested at 48 hours after the end of the treatment and the cells were scored for the presence of micronuclei. The level of micronucleated cells which was induced by the peroxide treatment was determined by subtracting spontaneous frequencies in untreated controls from the frequencies observed in the cultures after treatment with H_2O_2. Figure 2 shows the micronucleus frequencies which were induced in each cell culture by H_2O_2 treatment.

A-T cultures responded to the H_2O_2 treatment with a greater increase in micronucleus frequencies than that observed in normal

Figure 1

Spontaneous micronucleus frequencies in A-T and normal cultures

(Direct method)

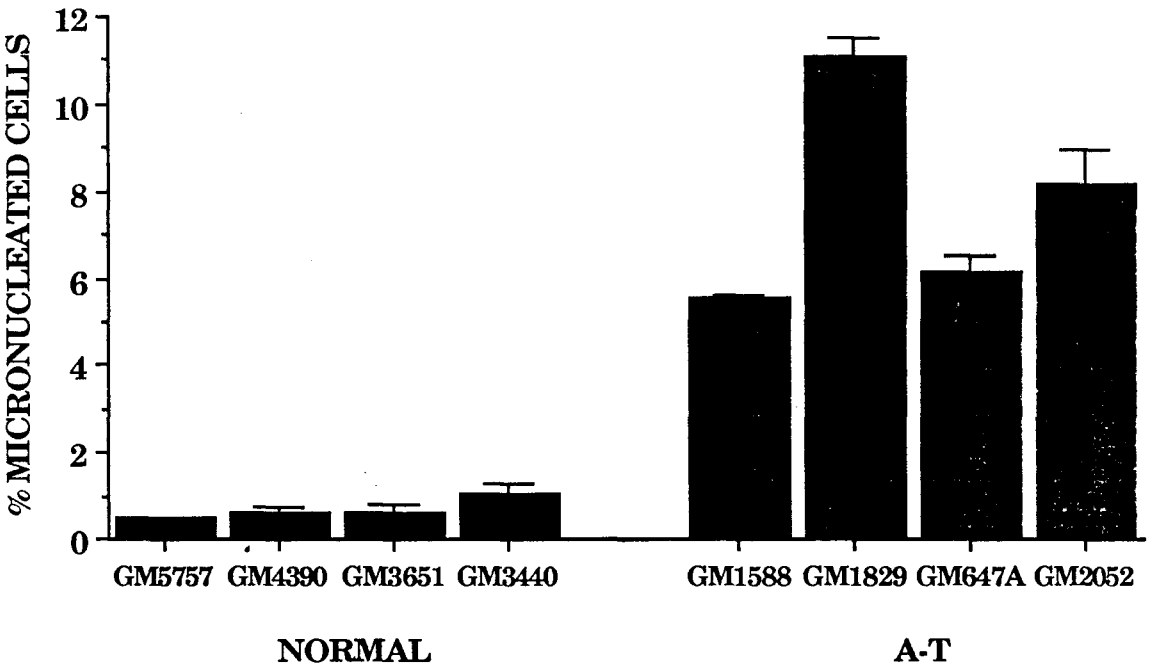

Fig.1. Frequency of micronucleated cells in untreated cultures from normal individuals and from A-T patients. Data shown are the mean ± S.E. of 3 replicate experiments for each culture.

Figure 2

Induction of micronucleated cells by H_2O_2 treatment of A-T and normal cultures (Direct method)

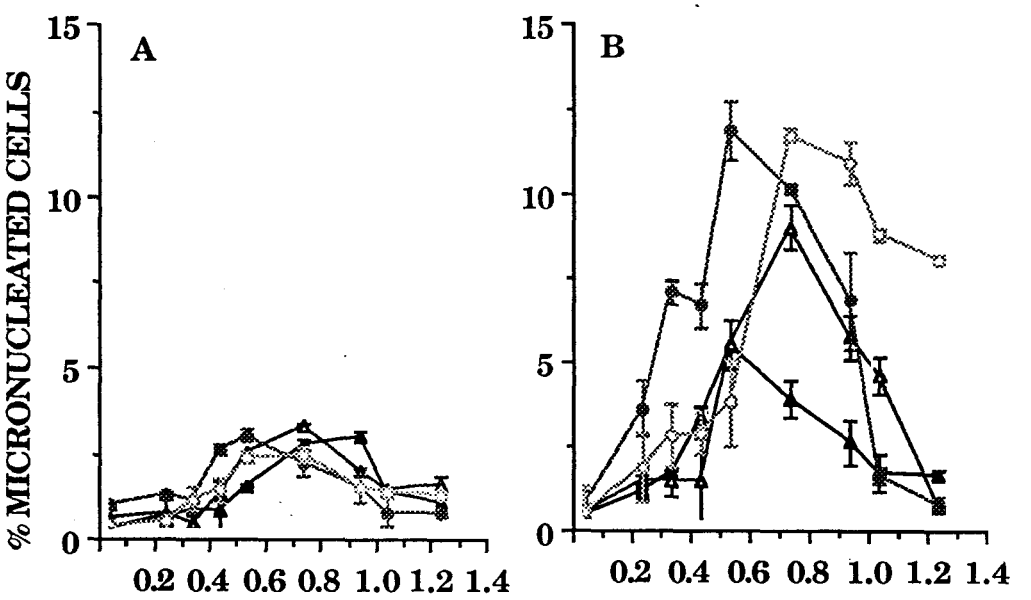

Fig.2. Frequency of micronucleated cells induced by H_2O_2 treatment of cultures from normal individuals (A) and from A-T patients (B). Each point represents the results from three experiments (mean ± S.E.). Normal cultures: GM4390 (△), GM3651 (○), GM5757 (▲), and GM3440 (●). A-T cultures: GM1588 (○), GM1829 (△), GM647A (▲), and GM2052 (●). Spontaneous frequencies (observed in untreated cultures) have been subtracted from each plotted value: 0.45% for GM4390, 0.60% for GM3651, 0.45% for GM5757, 1.05% for GM3440, 5.53% for GM1588, 11.10% for GM1829, 6.17% for GM647A, and 8.13% for GM2052.

cultures. The highest micronucleus frequencies for normal and A-T cultures were induced at concentrations of 0.5 to 0.9 μg/ml. At these concentrations, induced frequencies were 3.3-fold higher for A-T cultures than for normal cultures (average induced frequency for A-T cultures, 9.27±1.47%; for normal cultures, 2.77±0.16%; $P<0.01$). At higher peroxide concentrations, there was a decrease in the induction of micronucleated cells. This effect was presumably due to toxicity and/or growth inhibition since cell division after treatment is required for micronucleus formation [see chapter 1.4.].

In order to control for variation among cultures in the toxicity or the growth-inhibiting action of H_2O_2, a series of studies were done in which micronucleus frequencies were determined in cells identified as having completed one nuclear division following H_2O_2 treatment. These cells were collected by adding cytochalasin B to the cultures after treatment. This drug blocks cytokinesis without affecting karyokinesis. Cytokinesis-blocked (CB) cells appear binucleated.

3.3. Pattern of chromosomal sensitivity to hydrogen peroxide with respect to stage in cell cycle

Figure 3 shows the results obtained when an A-T and a normal culture were treated with H_2O_2 (0.7μg/ml) for 1 hour and CB cells collected by the addition of cytochalasin B at a series of time intervals after the treatment. In each case, the cytochalasin B was present in the culture for 4 hours prior to harvest. Control cultures receiving PBS only were harvested at each time interval. The frequency of CB cells with micronuclei in these PBS controls remained unchanged for both A-T and normal cultures,

Figure 3

A time course study of H_2O_2 effect on A-T and normal cultures

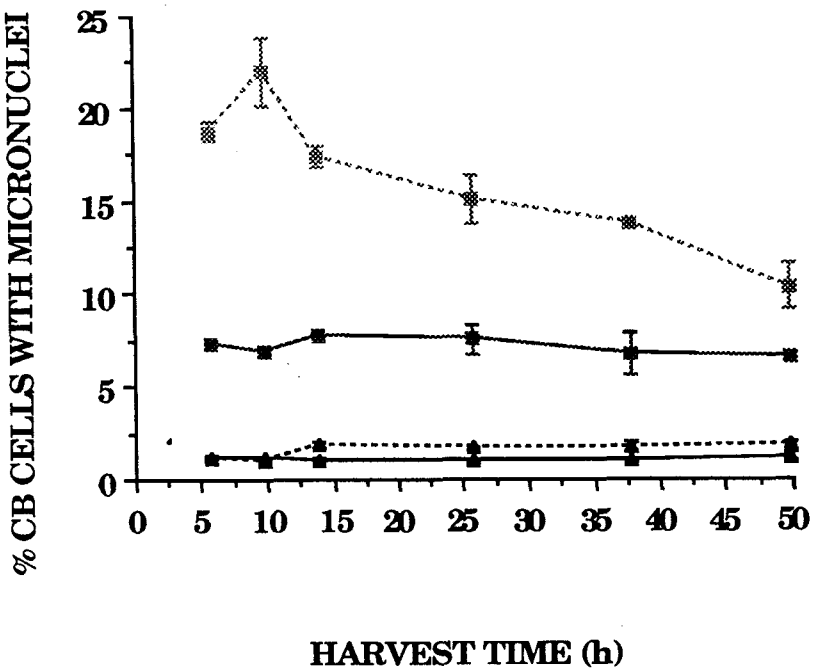

Fig.3. Frequency of cytokinesis-blocked (CB) cells with micronuclei (mean ± S.E., n=3) in cultures harvested at different time intervals after exposure to H_2O_2 (0.7μg/ml, 1 h). For each time point, cytochalasin B was added 4 h before harvest. Normal culture, GM5757 (▲), A-T culture, GM1588 (■). Solid lines, cultures receiving a PBS treatment; dash lines, H_2O_2 treatment.

showing no significant variation between harvest times. In contrast, peroxide-treated cultures of both A-T and normal cells showed a significant elevation in micronucleated CB cells above PBS controls. In A-T cells, this increase was first apparent among cells collected from 1 to 5 hours after H_2O_2 treatment ($P<0.01$). In contrast, the first significant induction of micronucleated cells in the normal culture occurred among cells collected from 9 to 13 hours after H_2O_2 treatment (% CB cells with micronuclei: PBS treatment, 0.49 ± 0.09; H_2O_2 treatment, 1.25 ± 0.13, $P<0.01$). The A-T and normal cultures also differed in the extent of induction of micronucleated CB cells by the H_2O_2 treatment, with the A-T culture displaying a greater induction at all harvest times ($P<0.01$). The frequency of CB cells with micronuclei declined in A-T cells harvested at later time intervals. Since cell cycle times for A-T cultures range between 24 to 36 hours [Tatsuka, et al., 1989], this decline in frequencies could reflect the collection of cells in a second division cycle after treatment.

The variation in temporal response to peroxide treatment for A-T and normal cultures was confirmed in a subsequent study in which micronuclei frequencies were determined in CB cells collected at even earlier intervals after treatment: from 0.5 to 1.5 hours for harvest 1 and from 1.5 to 2.5 hours for harvest 2 (Table 5). Micronucleus frequencies in peroxide-treated A-T cultures were significantly elevated above PBS controls at both harvests ($P<0.01$). There was no significant difference between micronucleus frequencies in PBS and H_2O_2-treated cultures of normal cells. These data support a G_2-phase sensitivity for the A-T culture to peroxide treatment since cells collected during these time intervals would

Table 5 G_2-phase sensitivity of A-T cells to H_2O_2[a]

Cultures	% of CB cells with micronuclei (Mean±S.E.)			
	Harvest 1		Harvest 2	
	PBS	H_2O_2	PBS	H_2O_2
Normal (GM5757)	0.44 ± 0.04	0.51 ± 0.09	0.38 ± 0.01	0.42 ± 0.04
A-T (GM1588)	6.67 ± 0.20	15.98 ± 0.45[b]	6.57 ± 0.32	17.93 ± 0.41[b]

[a] Cultures were exposed to either PBS (control) or H_2O_2 (0.7μg/ml) for 1 hour, washed, and the treatment media replaced by DMEM with 10% serum. Cytochalasin B was used to collect cells undergoing karyokinesis from 0.5 to 1.5 hours after the end of the treatment for harvest 1. For harvest 2, cytochalasin B was added 1.5 hours after the end of H_2O_2 or PBS treatment and cells fixed 1 hour thereafter.

[b] Statistically significant as compared to control (PBS) treatment ($P<0.01$).

presumably be those in G_2-phase at the time of treatment [Mozdarani and Bryant, 1989; Parshad, et al., 1983; Sanford, et al., 1989].

3.4. Cell cycle kinetics and micronucleus frequencies in untreated cultures

A comparison was made of the percentage of cells which are passing through the cell cycle in A-T and normal cultures. These data are important for two reasons: 1) cells which are actively cycling are generally more sensitive to DNA damage than noncycling cells and 2) nuclear division is required before chromosomal damage can be observed by assaying for micronuclei. Cytochalasin B was used to collect all cells undergoing nuclear division in a 43 hour collection period (Figure 4). This percentage was significantly greater for normal cultures than for A-T cultures (mean $\pm$ S.E.: normal cultures, 61.95 $\pm$ 8.11%; A-T cultures, 25.76 $\pm$ 4.55%; $P<0.01$).

Micronucleus frequencies were determined in these cycling cells (Figure 5). Spontaneous frequencies of CB cells with micronuclei differed significantly for A-T and normal cultures (average spontaneous frequency for A-T cultures, 11.384$\pm$2.013%; for normal cultures, 1.189$\pm$0.059%; $P<0.01$). These results show that although there are fewer cells cycling in A-T cultures, chromosomal damage and micronuclei frequencies are higher.

3.5. The effect of H_2O_2 treatment on the fraction of cells which are cytokinesis-blocked (CB)

The effect of H_2O_2 treatment on the percentage of cells undergoing karyokinesis in treated A-T and normal cultures is shown in Figure 6.

Figure 4

Frequencies of cytokinesis-blocked cells in untreated A-T and normal cultures

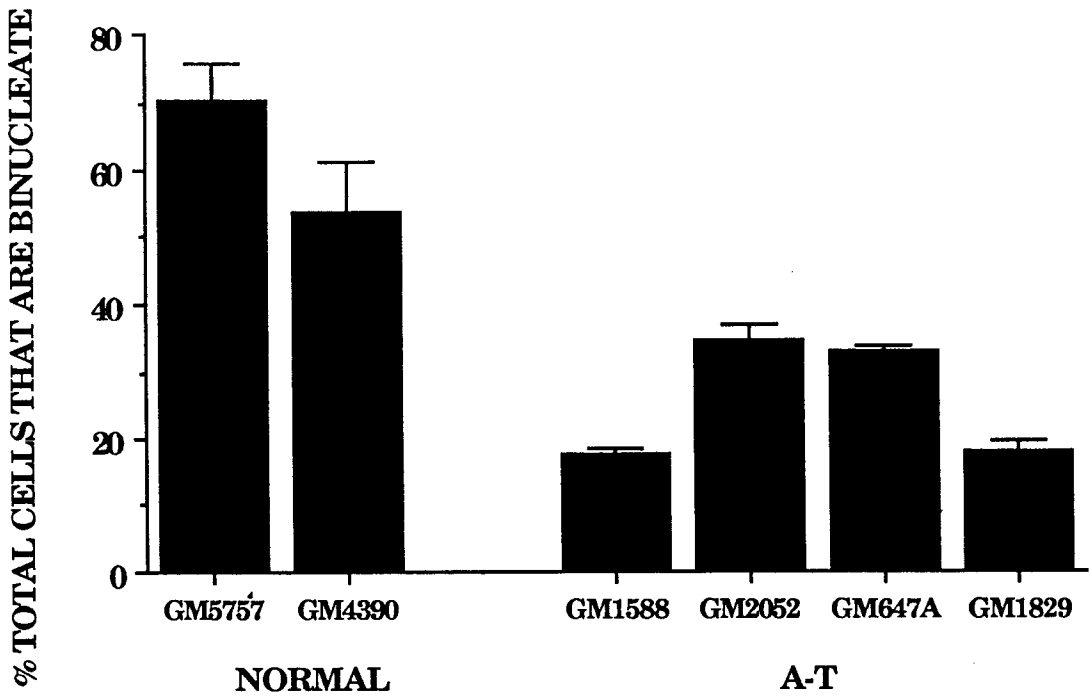

Fig.4. Frequency of cytokinesis-blocked (CB) cells occurring in untreated cultures from normal individuals and from A-T patients. Each point represents the results from three experiments (mean ± S.E.). Cytochalasin B was added 43 hours before harvest.

Figure 5

Spontaneous micronucleus frequencies in A-T and normal cultures (CB Technique)

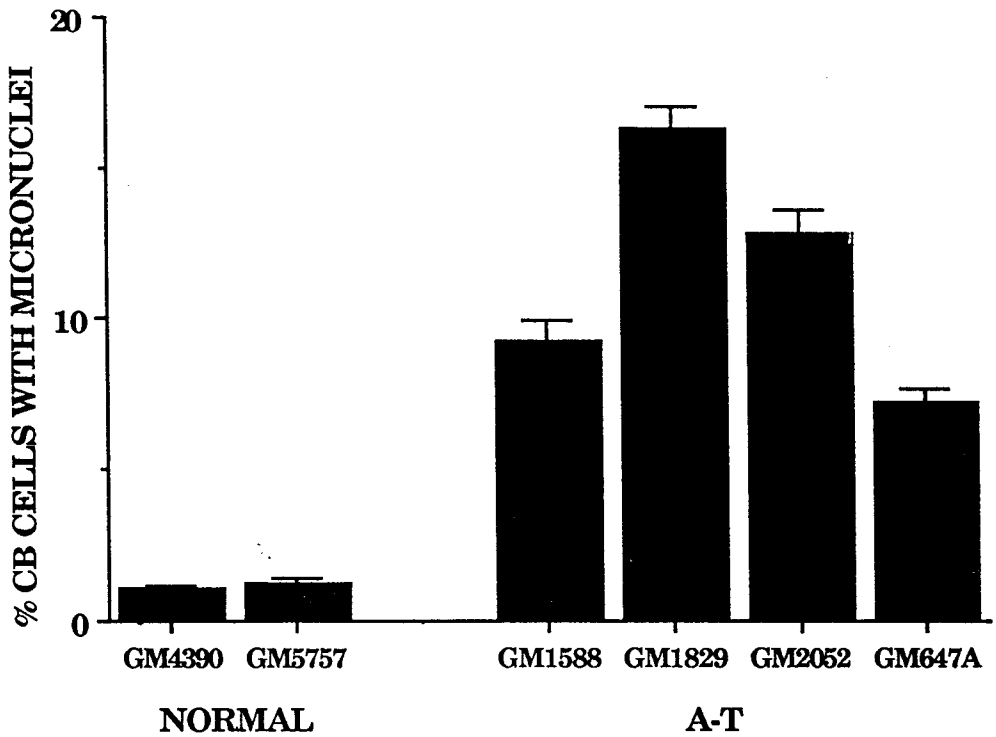

Fig.5. Frequency of cytokinesis-blocked (CB) cells with micronuclei in untreated cultures from normal individuals and from A-T patients. The cultures were incubated for 48 hours before harvest. Cytochalasin B was added 43 hours before harvest.

Figure 6

Effect of H_2O_2 treatment on cell cycle kinetics in A-T and normal cultures

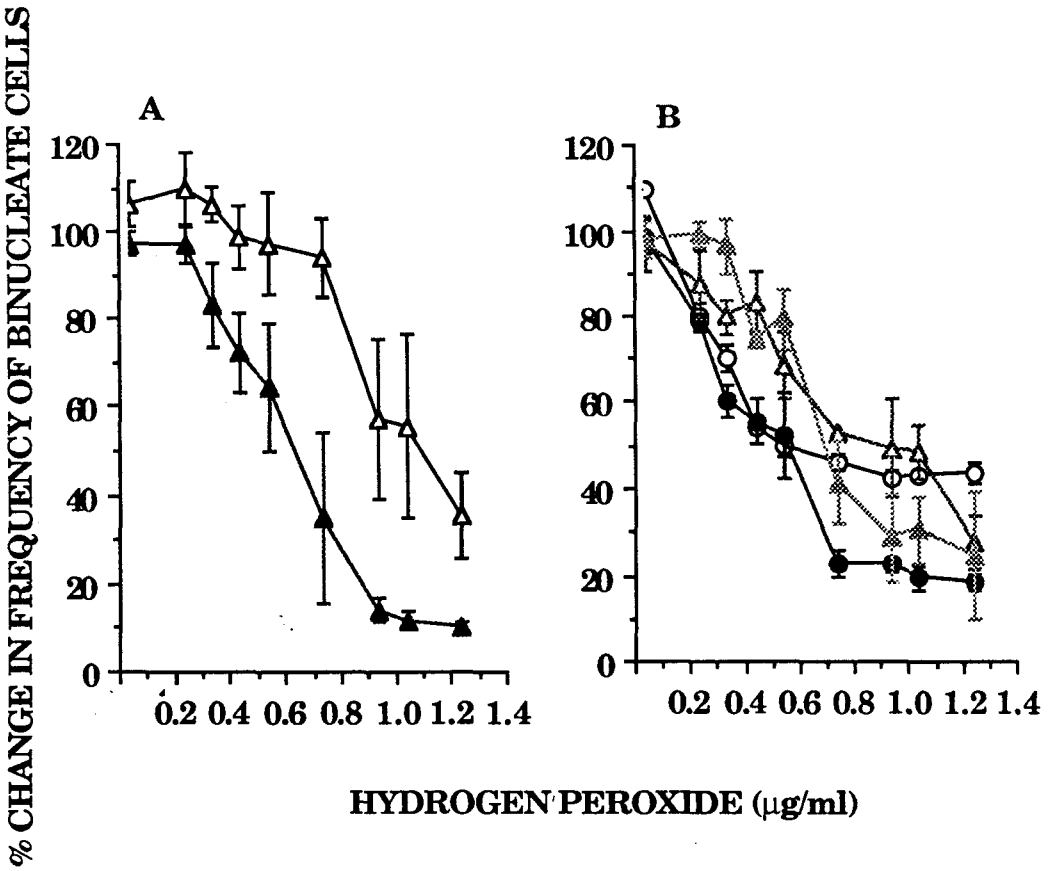

Fig.6. Decrease in proportion of cytokinesis-blocked (CB) cells in H_2O_2-treated cultures from 2 normal individuals (A) and from 4 A-T patients (B). The cultures were exposed to H_2O_2 for 1 hour and then incubated for 48 hours before harvest. Cytochalasin B was added 43 hours before harvest. Values for each culture represent the mean of 3 replicate experiments ± S.E.. Normal cultures: GM 4390 (△), GM5757 (▲). A-T cultures: GM1588 (○), GM1829 (△), GM647A (▲), and GM2052 (●).

These data were obtained by exposing cells to a dose range of H_2O_2 for 1 hour. The cells were then washed and incubated for 48 hours in fresh medium prior to harvest. Cytochalasin B was added 43 hours before harvest. By using this procedure it is possible to obtain a rough estimate of toxicity and/or cell cycle inhibition in a culture resulting from exposure to H_2O_2.

The dose-dependent decrease in percentage of CB cells after peroxide treatment was calculated for 4 A-T and 2 normal cultures (Figure 6). Values were normalized by setting the %CB cell frequency (i.e. percentage of cells which are binucleated) in untreated cultures to 100 and calculating the relative decrease in binucleated cells compared with this value for each culture. There was no significant difference between normal and A-T cultures in the reduction of the number of CB cells after H_2O_2 treatment.

3.6. Pattern of chromosomal sensitivity to hydrogen peroxide with respect to dose-dependence

Micronucleus frequencies were determined specifically in cells undergoing karyokinesis after H_2O_2 treatment by using the CB technique. Figure 7 shows the dose-dependent increase in frequencies of micronucleated cells in A-T and normal cultures. Micronucleus frequencies induced in A-T cultures were significantly greater than those observed in normal cultures (average induced frequency for A-T cultures for H_2O_2 exposure of 1.2μg/ml, $19.04 \pm 3.21\%$; for normal cultures, $3.47 \pm 0.25\%$; $P<0.01$). This increase in micronucleus frequencies in A-T cultures occurred without a significant change in cell cycle kinetics compared with normal cultures. A-T and normal cultures displayed

Figure 7

Induction of micronucleated cells by H_2O_2 in A-T and normal cultures

(CB Technique)

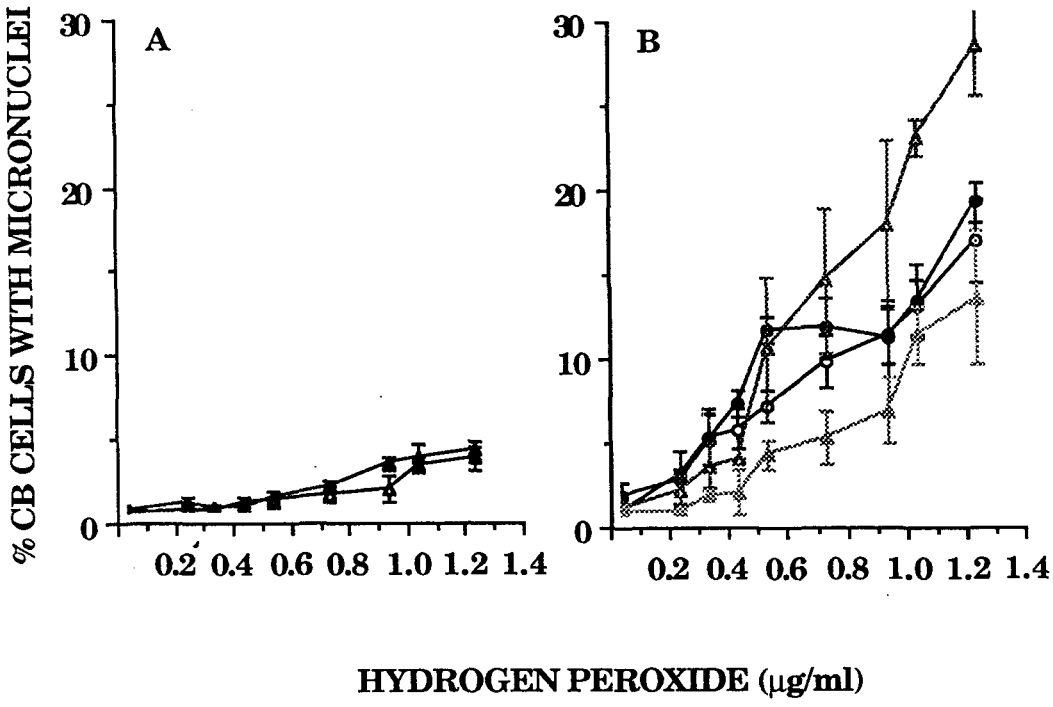

Fig.7. Frequency of cytokinesis-blocked (CB) cells with micronuclei induced by H_2O_2 treatment of cultures from normal individuals (A) and from A-T patients (B). Each point represents the results from three experiments (mean ± S.E.). The cultures were exposed to H_2O_2 for 1 hour and then incubated for 48 hours before harvest. Cytochalasin B was added 43 hours before harvest. Normal cultures: GM4390 (△), GM5757 (▲). A-T cultures: GM1588 (○), GM1829 (△), GM647A (▲), and GM2052 (●). Plotted values have been corrected for frequencies observed in untreated cultures.

similar reductions in the percentage of CB cells after H_2O_2 treatment (Figure 8).

Table 6 and Figure 9 present the frequencies of cytokinesis-blocked (CB) cells with 1, 2, 3, or >3 micronuclei per cell. The type of micronuclei formed gives an indication of the frequency of multiple chromosomal breakage and spindle dysfunction events. A-T cultures displayed increased frequencies of CB cells with one or two micronuclei compared with values observed in normal cultures at $P<0.05$. However, the frequencies of CB cells with three micronuclei and greater did not differ significantly at $P=0.05$. This effect was observed for both spontaneous and induced micronucleus frequencies.

3.7. Correlation between spontaneous frequency and H_2O_2-induced frequency of micronucleated cells

A comparison was made between spontaneous frequencies of micronucleus production and frequencies induced by H_2O_2 treatment of 4 A-T cultures. Two approaches were used: 1) direct method (no cytochalasin B) and 2) CB technique.

When the micronucleus assay was used directly, spontaneous and H_2O_2-induced micronucleus frequencies were poorly correlated. This result was observed at H_2O_2 doses of 0.7μg/ml and 1.2μg/ml (Fig.10: $r=0.1$ for 0.7μg/ml, $r=-0.65$ for 1.2μg/ml). A comparison was made of the spontaneous frequency of micronucleated cells in these cultures as determined by the two techniques. A significant correlation was obtained (Fig.11: $r=0.94$, $P<0.01$). However, when induced values obtained with these

Figure 8

Effect of H_2O_2 on cell cycle and production of micronuclei

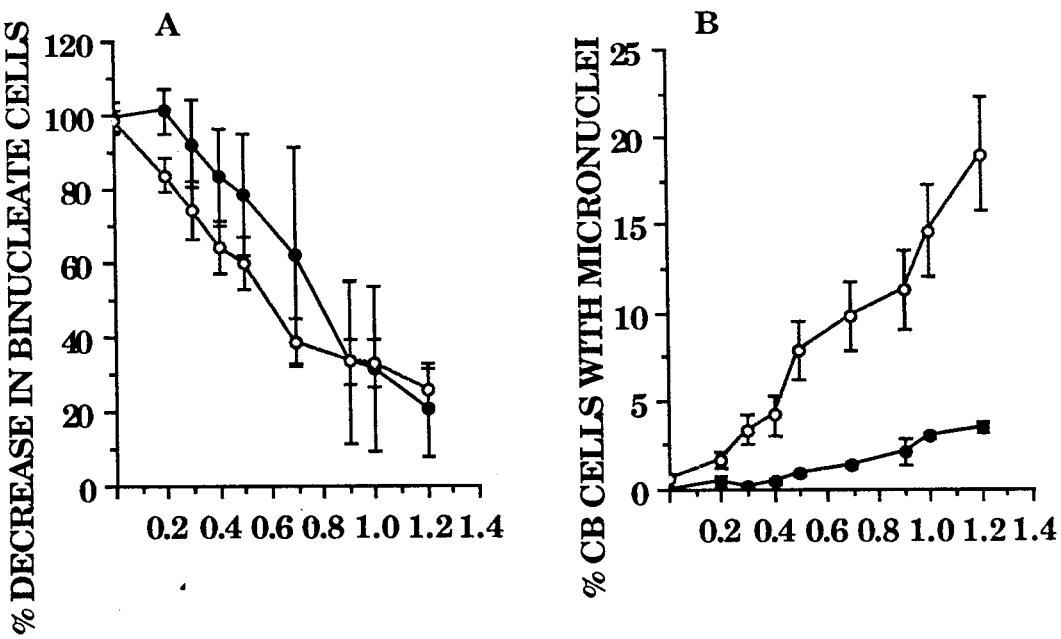

Fig.8. Effect of H_2O_2 treatment on cell cycle kinetics (A) and on induction of CB cells with micronuclei (B). Values are mean ± S.E. for 2 normal cultures (●) and 4 A-T cultures (○). Test for significance: no difference for (A), P<0.01 for (B).

Table 6 Pattern of micronuclei production

Spontaneous [a]	% of CB cells with indicated number of MN[b]			
	1	2	3	>3
A-T cultures				
1588	6.72±0.61	2.28±0.39	0.22±0.22	ND[b]
647A	6.60±0.53	0.60±0.31	ND	ND
2052	11.38±0.92	0.91±0.27	0.49±0.25	ND
1829	15.50±0.50	0.38±0.38	0.46±0.21	ND
$\bar{x}$±S.E.	10.05±2.13	1.04±0.43	0.39±0.09	ND
Normal cultures				
5757	1.10±0.10	0.15±0.03	ND	ND
4390	0.99±0.23	0.08±0.08	0.06±0.06	ND
$\bar{x}$±S.E.	1.05±0.06	0.12±0.04	0.06±0.06	ND
Induced [c]	% of CB cells with indicated number of MN[b]			
	1	2	3	>3
A-T cultures				
1588	9.28±3.23	4.72±0.61	2.44±0.29	ND
647A	10.47±2.27	2.04±1.24	0.50±0.50	ND
2052	15.65±0.75	1.56±0.51	1.06±0.81	0.42±0.42
1829	24.00±1.00	3.13±1.88	0.38±0.38	0.84±0.84
$\bar{x}$±S.E.	14.85±3.35	2.86±0.70	1.10±0.47	0.63±0.21
Normal cultures				
5757	2.69±1.12	1.07±0.67	ND	ND
4390	2.22±0.86	0.52±0.29	0.49±0.39	ND
$\bar{x}$±S.E.	2.46±0.24	0.80±0.28	0.49±0.39	ND

[a] Cultures were incubated for 48 hours before harvest, cytochalasin B was added 43 hours before harvest.

[b] MN, micronuclei; ND, none detected.

[c] Cultures were exposed to H_2O_2 (1.2 μg/ml, 1 hour) and then incubated for 48 hours before harvest, cytochalasin B was added 43 hours before harvest. Spontaneous frequencies have been subtracted.

Both spontaneous and induced frequencies of CB cells with one micronucleus differ significantly between A-T and normal cultures ($P<0.01$). The frequencies of CB cells with micronuclei differ significantly at $P<0.05$ for 1 or 2 micronuclei per cell. The percent of CB cells with 3 micronuclei and greater were not significantly different at $P=0.05$ level.

Figure 9

Pattern of micronuclei production

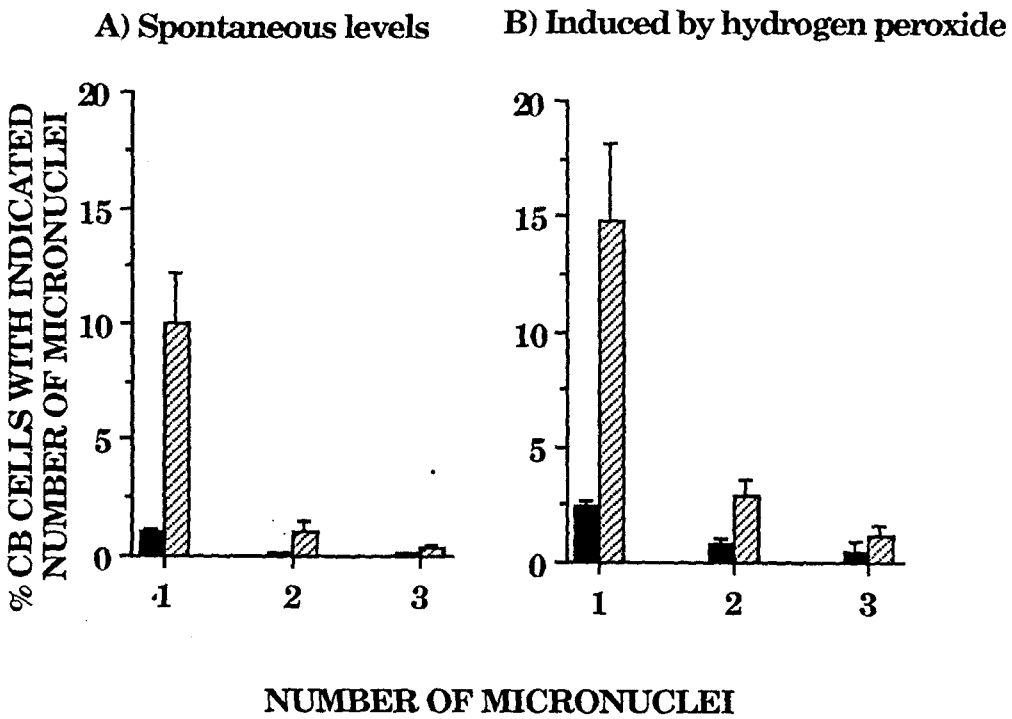

Fig.9. Pattern of micronuclei production in A-T (stippled bars) and normal (solid bars) cultures. The frequency of CB cells with 1, 2, or 3 micronuclei are indicated for A) untreated cultures and B) cultures treated with H_2O_2 (1.2 μg/ml, 1 hour). Induced values in group B have been corrected for spontaneous frequencies. Values shown are mean ± S.E. for 4 A-T and 2 normal cultures. Test for significance: P<0.01 for % with 1 micronucleus, no differences for % with 2 or 3 micronuclei at P=0.01 level.

Figure 10

Correlation between spontaneous and H_2O_2-induced micronucleus frequencies in A-T cultures (Direct method)

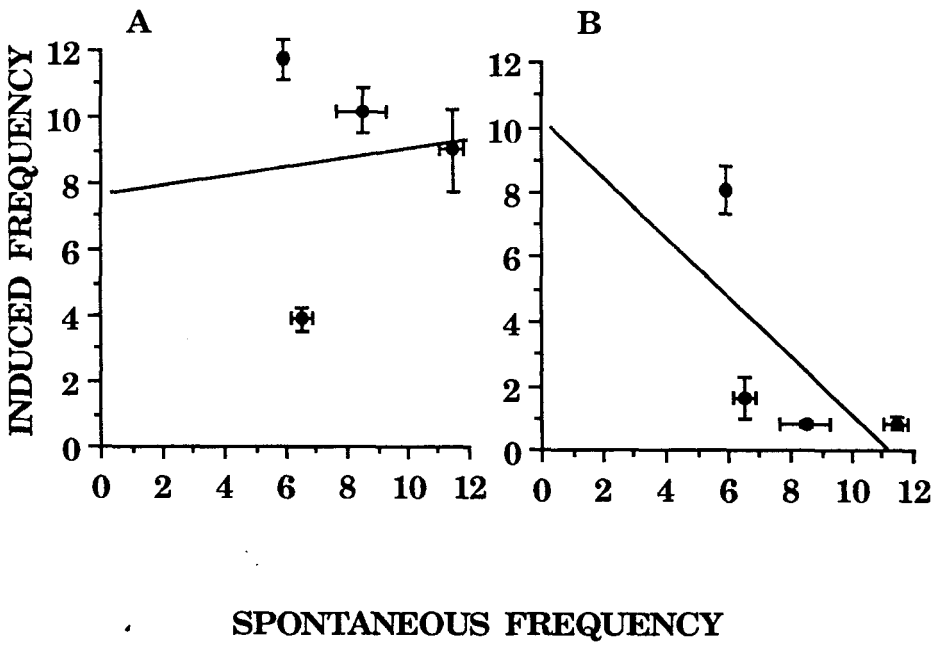

Fig.10. Correlation between spontaneous frequency of micronucleated cells and frequency of micronucleated cells induced by H_2O_2 treatment of 4 A-T cultures. Treatment was A) 0.7 μg/ml or B) 1.2 μg/ml H_2O_2 for 1 hour. The linear equation which best fits this relationship is $y = 7.3400 + 0.13558\,x$ for 0.7μg/ml, r=0.1, P>0.01, and $y = 9.6018 - 0.91673\,x$ for 1.2μg/ml, r= -0.653, P>0.01, where y = induced frequency, and x = spontaneous frequency.

Figure 11

Comparison of spontaneous micronucleus frequencies in A-T cultures determined directly or with CB technique

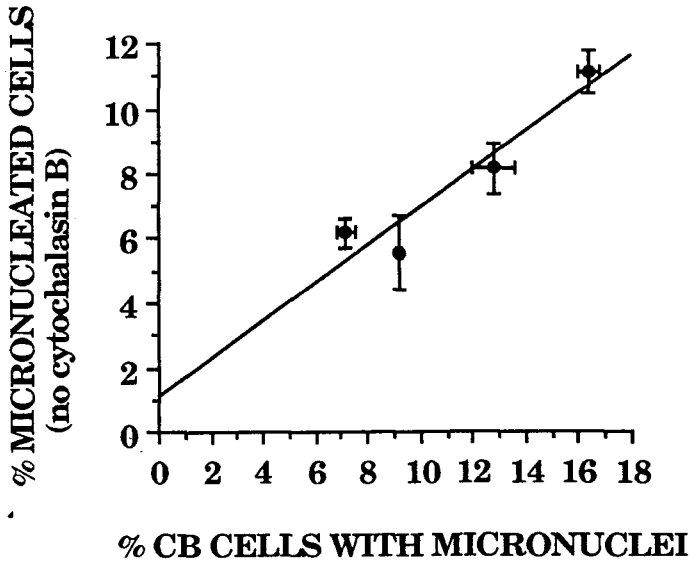

Fig.11. Correlation between spontaneous frequency of micronucleated cells detected directly in 4 A-T cultures with frequencies determined in cytokinesis-blocked (CB) cells. Values are mean ± S.E. (n=3). The equation which best descibes this relationship is $y = 1.0537 + 0.58676\,x$, where y = % micronucleated cells (no cytochalasin B), and x = % CB cells with micronuclei, $r=0.944$, $P< 0.01$.

two procedures were compared no significant correlation was observed (Fig.12: r=0.66 for 0.7μg/ml, r=-0.35 for 1.2μg/ml).

Figure 13 shows a plot of induced vs spontaneous values of micronuclei production when the CB technique was used. A significant correlation was observed between these values ($r=0.96$, $P<0.01$) using Pearson correlation assay. This significant correlation is shown for H_2O_2 doses of 0.7μg/ml and 1.2μg/ml. In addition, a second test using Spearman's rank correlation was performed between spontaneous micronucleus frequencies and observed micronucleus frequencies in the presence of H_2O_2 treatment in A-T cultures. A significant correlation was observed for H_2O_2 doses of 0.7μg/ml ($P<0.02$) and 1.2μg/ml ($P<0.01$). A-T cultures with higher spontaneous levels of micronucleus production displayed a larger increase in micronucleated cells after H_2O_2 treatment. These data support the possibility that the mechanism by which elevated chromosomal breakage is produced "spontaneously" in A-T patients may be the same as the mechanism by which oxidative agents induce elevated chromosomal breakage.

Figure 12

Comparison of H_2O_2-induced micronucleus frequencies in A-T cultures determined directly or with CB technique

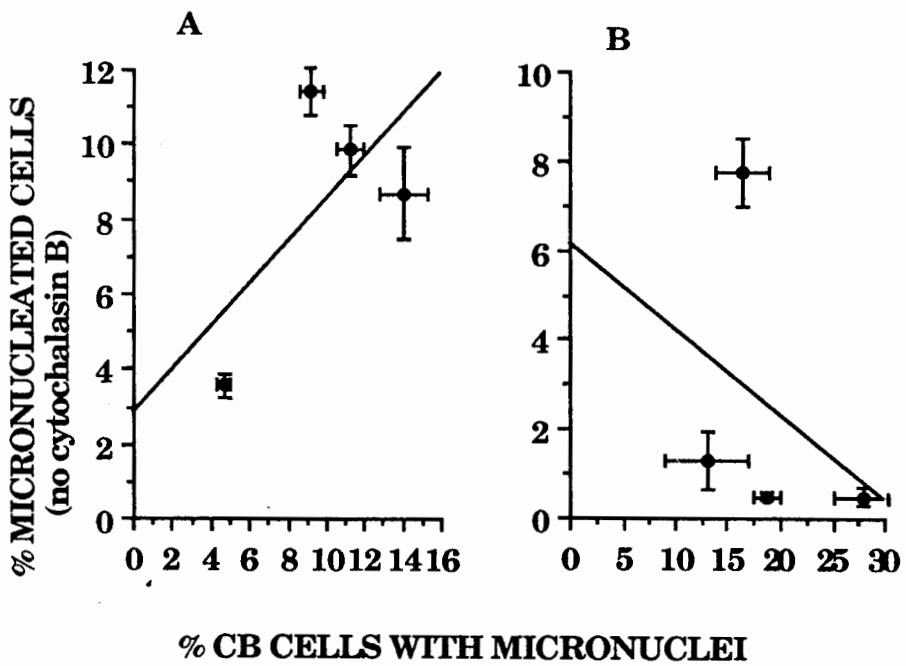

Fig.12. Correlation between H_2O_2-induced frequencies of micronucleated cells detected directly in 4 A-T cultures with frequencies determined in cytokinesis-blocked (CB) cells. H_2O_2 treatment was A) 0.7μg/ml or B) 1.2μg/ml for 1 hour. Values are mean ± S.E. (n=3). The linear equation which best fits this relationship is: $y = 2.8016 + 0.57108\,x$ for 0.7μg/ml, $r=0.66$, $P>0.01$, and $y = 6.1581 - 0.19152\,x$ for 1.2μg/ml, $r= -0.35$, $P>0.01$, where y = % micronucleated cells (no cytochalasin B), and x = % CB cells with micronuclei.

Figure 13

Correlation between spontaneous and H_2O_2-induced micronucleus frequencies in A-T cultures (CB Technique)

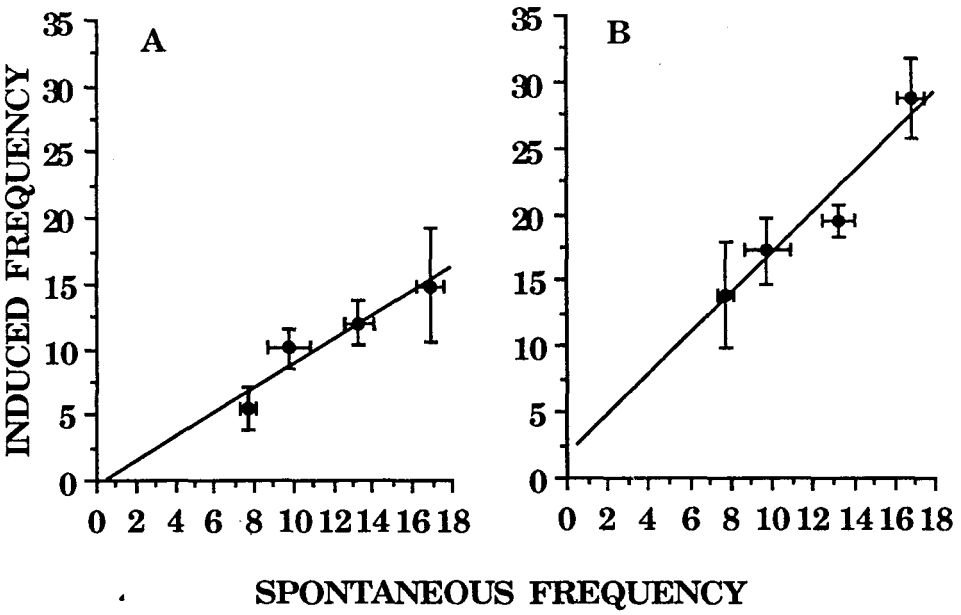

Fig.13. Correlation between spontaneous frequency of CB cells with micronuclei and frequency of micronucleated cells induced by H_2O_2 treatment of 4 A-T cultures. Treatment was A) 0.7 μg/ml or B) 1.2 μg/ml H_2O_2 for 1 hour. The equation which best fits this relationship is $y = -0.84280+0.93342\,x$ for 0.7μg/ml, $r=0.959$, $P<0.01$, and $y = 1.5325+1.5375\,x$ for 1.2μg/ml, $r=0.964$, $P<0.01$, where y = induced frequency, and x = spontaneous frequency.

CHAPTER 4

DISCUSSION

The purpose of this study was to explore the cause of "spontaneous" chromosomal instability in the A-T syndrome by studying the sensitivity of A-T fibroblast cultures from different patients to H_2O_2 exposure. The hypothesis was that DNA damage was produced in A-T patients from endogenous sources of oxidative stress, more specifically, via the generation of active oxygen species such as H_2O_2 or superoxide (which dismutates into H_2O_2) during normal cellular metabolism. It has been speculated that such processes could play a role in spontaneous cancers [Trotter, 1980]. An alternative source of oxidative stress could be exogenous components producing active oxygen species, including H_2O_2, during metabolism. Such "natural" xenobiotics come from diet (e.g., plant phenolics) and from various lifestyle habits, such as betel quid chewing.

The data presented in this thesis show that A-T cells have an elevated level of spontaneous chromosomal breakage and a greater induction of chromosomal damage by H_2O_2 compared with normal cells. The level of spontaneous and H_2O_2-induced micronucleus formation varied among A-T cultures. However, a significant correlation was observed between spontaneous and H_2O_2-induced micronucleus frequencies in A-T cells from different patients. A-T cells also display a greater sensitivity to oxidative stress in the G_2-phase of cell cycle. The relevance of these results and the possible mechanisms underlying them are discussed below.

4.1. Significance of the obtained results

There is a growing list of chemicals which produce active oxygen species (H_2O_2, superoxide, and hydroxyl-free radical) during cell metabolism and have been shown to induce DNA damage in vitro [Ames, 1983; Cerutti, 1985; Vuillaume, 1987]. We are daily exposed to many of these chemicals. For example, simple phenolics, such as caffeic acid, sesamol, and catechol, are ingested daily with food products. These phenolics have been shown to cause single- and double-strand breaks to DNA in cell cultures and to induce cell proliferation, hyperplasia, papillomas, and tumor formation in laboratory animals [reviewed by Stich, 1990].

An unresolved issue is to what extent such agents can cause DNA damage and induce tumor formation in humans. Under normal conditions, protective enzymes (catalase, peroxidases, and superoxide dismutase) and cellular antioxidants prevent DNA damage by these species. When DNA damage is induced, it can still be effectively repaired before it becomes a permanent lesion. However, it may be possible to overwhelm these protective mechanisms [Cerutti, 1985]. For example, that may be the case in chewers of betel quid, where transition metals and alkaline conditions act to accelerate the oxidation of areca nut tannins. The oral cavity of the chewer may be exposed to levels of active oxygen species which exceed the capacity of protective mechanisms [Stich and Anders, 1989; Stich and Tsang, 1989]. Individuals may also be more susceptible to damage by such agents because of defective detoxification and/or repair mechanisms. A-T patients may represent such a group of individuals. In this study, A-T cells did exhibit a hypersensitivity to H_2O_2 (one member of the active oxygen species). The possible mechanisms are discussed in the following sections.

4.2. Mechanism of elevated sensitivity to hydrogen peroxide

Among the possible explanations for the hypersensitivity of A-T cultures to active oxygen species, two hypotheses are most probable: a) There may be lower levels of anti-oxidants or anti-oxidant enzymes in A-T cells than in normal cells, so that greater DNA damage is produced in A-T cells by active oxygen species; and/or b) The DNA repair system in A-T cells may be defective and unable to cope with active oxygen species-induced DNA damage.

4.2.1. Defects in defence against active oxygen species

Previous reports have suggested a reduced catalase activity [Vuillaume, 1987] or a defect in glutathione metabolism [Meredith and Dodson, 1987] in A-T cells. Vuillaume and his colleagues have studied several A -T fibroblast cell lines and have found that A-T cell extracts have a lower catalase activity than normal cells, with the possible exception of one A-T cell line which showed normal catalase activity. Glutathione (GSH) biosynthesis in A-T fibroblast and lymphoblast cultures was assessed by Meredith and Dodson. They concluded that cells from A-T homozygotes are deficient in cysteine (a substrate for GSH synthesis) transport, thus limiting GSH resynthesis after a depleting challenge such as ionizing radiation, which produces H_2O_2, or GSH-depleting xenobiotic compounds. However, these results of defects in antioxidant defences in A-T cells have not been uniformily observed by all investigators [Dean, 1986; Dean and Jaspers, 1988; Sheridan III and Huang, 1979]. Levels of glutathione (GSH) and two enzymes involved in GSH metabolism, glutathione reductase(GR) and glutathione-S-transferase(s)(GST), were measured in A-T fibroblasts in a

separate study by Dean [1987]. The basal GSH levels were similar in A-T and normal cells in this report. Although there was some variation in GST and GR activities, the small deficiency in both enzymes was thought unlikely to be responsible for the radiosensitivity in A-T. With respect to superoxide dismutase and catalase levels in A-T cultures, Sheridan III and Huang [1979] reported that activities of these two enzymes were identical in normal and A-T cultures.

4.2.2. Defects in damage-repair capacity in A-T cells

An alternative hypothesis which can be used to explain the elevated sensitivity to H_2O_2 in A-T cells is that this characteristic results from a defect in the repair of DNA damage, similar to that observed after x-ray damage [Paterson, et al., 1976; Thacker, 1989]. Thacker suggested that there was a consistent reduction in the fidelity of rejoining of DNA double-strand breaks in A-T cells. Shiloh and co-workers [1983] have previously noted decreased colony-forming efficiency in A-T fibroblast cultures, compared with normal cultures, after exposure to H_2O_2. In addition, normal cultures showed a transient inhibition of DNA synthesis after the H_2O_2 treatment, an effect which was significantly reduced in A-T cultures. A reduced inhibition of DNA synthesis compared with normal cultures has also been observed immediately after treatment of A-T cultures with x-ray or bleomycin and may indicate a defect in the capacity of A-T cells to recognize and/or repair damage.

4.3. G_2-phase sensitivity to x-ray and H_2O_2 in A-T cells

A-T cells may be defective in their capacity to monitor and repair DNA damage during the G_2-phase of the cell cycle. An elevation in

chromosomal breakage during the G_2-phase is characteristic of A-T cells after x-ray treatment [Bender, et al., 1985; Mozdarani and Bryant, 1989; Parshad, et al., 1983; Sanford, et al., 1989; Taylor, 1978]. Our results with induction of micronucleated cells by H_2O_2 treatment are consistent with such a G_2-phase sensitivity. Mozdarani and Bryant [1989] suggest that although normal and A-T cells display equal levels of double strand breaks immediately after x-ray treatment, A-T cells subsequently convert more of these double strand breaks into chromatid deletions. A-T heterozygote fibroblasts clearly show increased susceptibility to the lethal effect of x-irradiation. However, the difference in post-irradiation survival between A-T and normal controls is not always large enough to allow the use of x-ray sensitivity as a laboratory assay for carrier detection in A-T. Shiloh, et al. [1989] have shown that the extent of chromatid damage induced in the G_2-phase by x-ray is markedly higher in A-T heterozygous cells than in normal cells. They have successfully applied this test of G_2-phase sensitivity to detect cases of A-T heterozygotes.

4.4. Correlation between spontaneous and H_2O_2-induced micronucleus frequencies in A-T cells

A question which arises is whether there exists a common mechanism for spontaneous and oxidative stress-induced chromosomal breakage in A-T cells. This study showed that cells from different A-T patients displayed a variation in spontaneous micronucleus formation. Furthermore, a significant linear correlation existed between spontaneous and H_2O_2-induced micronucleus frequencies in A-T cells from these different patients. A-T cell lines with higher levels of spontaneous frequencies exhibited higher levels of H_2O_2-induced micronucleus

frequencies. The correlation between spontaneous and H_2O_2 -induced mironucleus frequencies in A-T cells was observed only when the CB technique was used. This technique permitted us to differentiate dividing cells and to correct for variations in toxicity and the fraction of cycling cells in different cell cultures. Although this correlation supports a common mechanism for spontaneous and induced chromosomal breakage in A-T, it does not prove this association. Future studies are required to obtain such evidence.

4.5. Future studies

It has been suggested that hydroxyl radicals may be involved in the formation of damage induced by both hydrogen peroxide and x-ray. Therefore, an analysis of the effect of hydroxyl radical scavenging agents such as desferrioxamine, mannitol, or phenanthroline on spontaneous and H_2O_2-induced chromosomal breakage in A-T cells could further elucidate the mechanism(s) underlying these phenomena. It also might be enlightening to analyze the levels of antioxidant enzymes in A-T cells from different patients and to determine whether there is a correlation between these levels and between spontaneous micronucleus formation or H_2O_2-induced micronucleus production. Alternatively, catalase or other radical scavengers could be added to the A-T cultures with or without H_2O_2 exposure and the preventive effects of these antioxidants on both spontaneous and H_2O_2-induced micronucleus production could be compared. Finally, it might be possible to use microcell-mediated chromosome transfer to study the mechanism of elevated active oxygen species-induced damage in A-T patients. Komatsu, et al. [1990] have reported that radiation resistance is restored in A-T cells by the

introduction of normal human chromosome 11. The hybrid cells showed a level of cytotoxicity similar to normal cells after x-irradiation. The same technique could be used to examine whether the damage induced by H_2O_2 can be reduced to the normal level by the introduction of normal human chromosome 11. In addition, the level of spontaneous micronucleus formation in hybrids could be determined.

4.6. Conclusion

In summary, the results of this thesis suggest a greater sensitivity of A-T patients to chromosomal damage by H_2O_2. These *in vitro* observations may possibly be used to explain the "spontaneous" chromosomal instability observed *in vivo* in A-T patients. Since A-T patients represent a group of human population which have increased risk of cancer development, A-T could be a good model for studying the active oxygen species in carcinogenesis. Finally, an understanding of the mechanism by which active oxygen species alter DNA is crucial to a development of preventive measures that could act to suppress cancer development in exposed humans.

LIST OF REFERENCES

Al Saadi, A., Palutke, M., and Kumar, G. K. (1980) Evolution of chromosomal abnormalities in sequential cytogenetic studies of telangiectasia. Hum. Genet. 55:23-29.

Ames, B. N. (1983) Dietary carcinogens and anticarcinogens -- oxygen radicals and degenerative diseases. Science 221:1256-1264.

Amromin, G. D., Boder, E., and Teplitz, R. (1979) Ataxia-telangiectasia with a 32 year survival. A clinicopathological report. J. Neuropathol. Exp. Neurol. 38:621-643.

Amstad, P., and Cerutti, P.A. (1983) Biochem. Biophys. Res. Commun. 112:1034.

Amstad, P., Levy, A., Emerit, I., and Cerutti, P.A. (1984) Carcinogenesis 5:719.

Armato, U., Andreis, P. G., and Romanano, F. (1984) Exogenous Cu-Zn superoxide dismutase suppresses the stimulation of neo natal rat hepatocytes by tumor promoter. Carcinogenesis 5:1547-1555.

Becker, Y. (1986) Cancer in ataxia-telangiectasia patients: analysis of factors leading to radiation-induced and spontaneous tumors. Anticancer Res. 6:1021-1032.

Becker, Y., and Tabor, E. (1989) Skin fibroblasts form patients with the genetic disorder hereditary hemorrhagic telangiectasia compared with ataxia-telangiectasia fibroblasts in their response to the radiomimetic drug neocarzinostatin. Isr. J. Med. Sci. 25:81-86.

Becker, Y., Shaham, M., Shiloh, Y., and Voss, R. (1983) Chromosome breakage and sensitivity to DNA breaking agents in ataxia-telangiectasia and their possible association with predisposition to cancer, In: Chandra, P. (ed.) Biochemical and Biological Markers of Neoplastic Transformation, Proceedings of the NATO Advanced Study Institute, Corfu Island, Greece, 1981, New York: Plenum Press, pp.171-184.

Becker, Y., Shaham, M., Tabor, E., and Shiloh, Y. (1984) Ataxia-telangiectasia -- a human autosomal recessive disorder predisposing to cancer, In: Giraldo, G., and Beth, G. (eds.) The Role of Viruses in Human Cancer. Vol.11 Amsterdam: Elsevier Science Publishers, pp.397-406.

Bender, M. A., Rary, J. M., and Kale, R. P. (1985) G_2 chromosomal radiosensitivity in ataxia-telangiectasia lymphocytes. Mutat. Res. 152:39-47.

Berne, J., McColl, I., and Adinolfi, M. (1989) Recessive mutations and chromosome deletions leading to cancer. Br. J. Surg. 76:327-330.

Biruboim, H.C., and Kanabus-Kaminska, M. (1985) Proc. Natl. Acad. Sci. (USA) 82:6820-6824.

Blocher, D., and Pohlit, W. (1982) DNA double strand breaks in Ehrlich ascites tumour cells at low doses of X-rays, II: Can cell death be attributed to double strand breaks? International Journal of Radiation Biology 42:329-338.

Bochkov, N. P., Lopukhin, Y. M., Kuleshov, N. P., and Kovalchuk, L. V. (1974) Cytogenetic study of patients with ataxia-telangiectasia. Humangenetik 24:115-128.

Boder, E. (1985)Ataxia-telangiectasia:an overview, In: Gatti, R. A., and Swift, M. (eds.) Ataxia-Telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood, Kroc Foundation Series, Vol.19, Alan R. Liss, Inc., N.Y. pp:1-63.

Boling, M., Adler, H., and Masker, W. (1984) J. Bacteriol. 160:706-710.

Borek, C., and Troll, W. (1983) Modifiers of free radicals inhibit in vitro the oncogenic action of x-rays, bleomycin and the tumor promoter 12-o-tetradecanoyl-phorbol 13-acetate. Proc. Natl. Acad. Sci. (U.S.A.) 80:1304-1307.

Bosi, A., and Olivieri, G. (1989) Variability of the adaptive response to ionizing radiations in humans. Mutation Res. 211:13-17.

Boveris, A. (1978) In: Biochemistry and Genetics of Yeast. Academic Press, New York, pp.65-81.

Bradley, M. O , and Erickson, L. C. (1981) Comparison of the effects of hydrogen peroxide and x-ray irradiation on toxicity, mutation , and DNA damage/repair in mammalian cells (V-79). Biochim. Biophys. Acta. 654:135-141.

Brawn, K. K., and Fridovich, I. (1981) Arch. Biochem. Biophys. 206:414

Bridges, B. A., and Harnden, D. G. (eds.) (1982) Ataxia-Telangiectasia:A Cellular and Molecular Link Between Cancer, Neuropathology and Immune Deficiency, Chichester, England: John Wiley.

Bryant, P. E., and Blocher, D. (1980) Measurement of the kinetics of DNA double strand break repair in Ehrlich ascites tumor cells using unwinding method. International Journal of Radiation Biology 38:335-347.

Bryant, P. E., and Blocher, D. (1982) The effects of 9-beta-D-arabinofuranosyladenine on the repair of DNA strand breaks in X-

irradiated Ehrlich ascites tumor cells. International Journal of Radiation Biology 42:385-394.

Cadet, J., and Berger, M. (1985) Int. J. Radiat. Biol. 47:127-143.

Cadet, J., Berger, M., and Shaw, A. (1986) In: Simic, M.G., Grossman, L., and Upton, A.D. (eds.) Mechanisms of DNA Damage and Repair, Implications for Carcinogenesis and Risk Assessment. Plenum, New York, pp.69-74.

Carter, S.B. (1967) Nature (London) 213:261.

Carter, S.B. (1972) Endeavor 31:77-82.

Cerutti, P.A. (1985) Prooxidant states and tumor promotion. Science 227:375-381.

Chen, P. C., Lavin, M. F., and Kidson, C. (1978) Identification of ataxia-telangiectasia heterozygotes, a cancer prone population. Nature (London) 274:484-486.

Christman, M.F., Morgan, R.W., Jacobson, F.S., and Ames, B.N. (1985) Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in Salmonella typhimurium. Cell 41:753-762.

Cohen, G., and Heikkila, R. E. (1974) The generation of hydrogen peroxide, superoxide radiacal and hydroxyl radical by 6-hydroxydopamine, dialuric acid and related cytotoxic agents. J. Biol. Chem. 249:2447-2452.

Cohen, M. M., and Simpson, S. J. (1983) Increased clastogenicity and decreased inhibition of DNA synthesis by neocarzinostatin and tallyomycin in ataxia-telangiectasia lymphoid cells. Mutat. Res. 112:119-128.

Cohen, M. M., Kohn, G., and Dagan, J. (1973) Chromosomes in ataxia-telangiectasia. Lancet II:1500.

Cohen, M. M., Sagi, M., Ben-Zur, Z., Schaap, T., Voss, R., Kohn, G., and Ben-Bassat, H. (1979) Ataxia-telangiectasia: chromosomal stability in continuous lymphoblastoid cell lines. Cytogenet. Cell. Genet. 23:44-52.

Cohen, M. M., Shaham, M., Dagan, J., Shnuell, E., and Kohn, G. (1975) Cytogenetic investigations in families with ataxia-telangiectasia. Cytogenet. Cell. Genet. 15:338-356.

Cohen, M. M., Simpson, S. J. , and Pazos, L. (1981) Specificity of bleomycin-induced cytotoxic effects on ataxia-telangiectasia lymphoid cell lines. Cancer Res. 41:1817-1823.

Copper, G. M. (1984) Activation of transforming genes in neoplasms. Br. J. Cancer 50:137-142.

Cornforth, M.N., and Bedford, J.S. (1985) On the nature of the defect in cells from individuals with ataxia-telangiectasia. Science (Wash.DC) 227:1589-1591.

Cornforth, M. N., and Bedford, J. S. (1983) X-ray induced breakage and rejoining of human interphase chromosomes. Science 222:1141-1143.

Cox, R., Debenham, R.G., Masson, W.K., and Webb, M.B.T. (1986) Ataxia-telangiectasia: a human mutation giving high-frequency misrepair of DNA double-strand scissions. Molecular Biology and Medicine 3:229-244.

Cramer, P., and Painter, R.B. (1981) Bleomycin-resistant DNA synthesis in ataxia-telangiectasia cells. Nature 291:671-672.

Cunliffe, P.N., Mann, J.R., Cameron, A.H., Roberts, K.D., and Ward, H.W.C. (1975) Radiosensitivity in ataxia-telangiectasia. Br. J. Radiol. 48:374-376.

Cunningham, M., and Lokesh, B. (1983) Mutat. Res. 121:299

Cutler, R. (1984) Antioxidants, aging and longevity, In: Pryor, W. (ed.) Free Radicals in Biology. vol. 6, Academic Press, New York, pp. 371-428.

De Wit, J., Jaspers, N. G. J., and Bootsma, D. (1981) The rate of DNA synthesis in normal human and ataxia-telangiectasia cells after exposure to x-irradiation. Mutat. Res. 80:221-226.

Dean, S. W. (1987) Some aspects of glutathione metabolism in ataxia-telangiectasia fibroblasts. Int. J. Radiat. Biol. 52(1):43-48.

Dean, S. W., and Jaspers, N. G. J. (1988) Correspondence re: Michael J. Meredith and Marion L. Dodson. Impaired glutathione biosynthesis in cultured ataxia-telangiectasia cells. Cancer Res. 48:5374-5375.

Debenham, P. G., Webb, M. B. T., Jones, N. J., and Cox, R. (1987) Molecular studies on the nature of the repair defect in ataxia-telangiectasia and their implications for cellular radiology. Journal of Cell Science (Supplement) 6:177-189.

Demple, B., and Halbrook, J. (1983) Inducible repair of oxidative DNA damage in Escherichia coli. Nature 304:466-468.

Dooley, M. M., and Pryor, W. A. (1982) Free radical pathology: inactivation of human alpha-proteinase inhibitor by products from the reaction of nitrogen dioxide with hydrogen peroxide and the etiology of emphysema. Biochem. Biophys. Res. Commun. 106:981-987.

Edwards, M. J., and Taylor, A. M. R. (1980) Unusual levels of (ADP-ribose) and DNA synthesis in ataxia-telangiectasia cells following gamma-ray irradiation. Nature 287:745-747.

Edwards, N. W., Taylor, A. M. R., and Duckworth, G. (1980) Biochem. J. 188:677-683.

Emerit, I., Keck, M., Levy, A., Feingold, J., and Michelson, A. M. (1982) Mutat. Res. 103:165

Emerit, I., and Cerutti, P. (1981) Clastogenic activity from Bloom syndrome fibroblasts cultures. Proc. Natl. Acad. Sci. (U.S.A.) 78:1868-1872.

Erexson, G., Kligerman, A., and Allen, J. (1987) Diaziquone-induced micronuclei in cytochalasin B-blocked mouse perippheral blood lymphocytes. Mutation Res. 178:117-122.

Farr, S. B., D'Ari, R., and Touati, D. (1986) Proc. Natl. Acad. Sci. USA 83:8268-8272.

Fenech , M., and Morley, A. (1985) Measurement of micronuclei in lymphocytes. Mutation Res. 147:29-36.

Flier, J. S., Underhill, L. H., Friend, S. H., Dryja, T. P., and Weinberg, R. H. (1988) Oncogenes and tumor-suppressing genes. New. Engl. J. Med. 318(10):618-622.

Ford, M. D., and Lavin, M. F. (1981) Nucleic Acids Res. 9:1395-1401.

Forman, H. J., and Boveris, A. (1982) In: Pryor, W. A. (ed.) Free Radicals in Biology. Academic Press, New York, pp.65-90.

Fridovich, I. (1976) In: Pryor, W. A. (ed.) Free Radicals in Biology. Vol.1 Academic Press, New York, pp.239-277.

Friedberg, E. C. (1985) In: Friedberg, E. C. (ed.) DNA Repair. Freeman, New York.

Fujita, Y., Wakabayashi, K., Nagao, M., and Sugimura, T. (1985) Mutation Res. 144:227-230.

Furihata, C., Sata, Y., Matsushima, T., and Tatematsu, M. (1985) Carcinogenesis 6:91-94.

Gatti, R. A., and Swift, M. (eds.) (1985) Ataxia-Telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood. Kroc Foundation Series, Vol.19, Allan R. Liss, N.Y.

Gatti, R. A., and Hall, K. (1983) In: German, J. (ed.) Chromosome Mutation and Neoplasia. Alan R Liss, New York. pp.23-41.

Gatti, R. A., Bick, M., Tam, C. F., Medici, M. A., Oxelius, V. A., Holland, M., Goldstein, A. L., and Boder, E. (1982) Ataxia-telangiectasia: a multiparameter analysis of eight families. Clin. Immunol. Immunopathol. 23:501-516.

German, J. (1969) Chromosomal breakage syndromes. Birth Defects 5(5):117-131.

German, J. (1983) Patterns of neoplasia associated with the chromosome-breakage syndromes, In: German, J. (ed.) Chromosome Mutation and Neoplasia. Liss, New York, pp. 97-134.

Goldstein, B. D., Witz, G., Amoruso, M., Stone, D. S., and Troll, W. (1981) Polymorphonuclear leukocyte superoxide anion radical production by tumor promoters. Cancer Lett. 11:257-262.

Gotoff, S. P., Amirmokri, E., and Liebner, E. J. (1967) Am. J. Dis. Child. 114:617-625.

Gropp, A., and Flatz, G. (1967) Chromosome breakage and blastic transformation of lymphocytes in ataxia-telangiectasia. Humangenetic 5:77-79.

Hagen, U. (1986) Current aspects on the radiation induced base damage in DNA. Radiat. Environ. Biophys. 25:261-271.

Hamers, M., and Roos, D. (1985) In: Sies, H. (ed.) Oxidative Stress. Academic Press, New York, pp.351-381.

Hanham, A.F., Dunn, B. P., and Stich, H. F. (1983) Clastogenic activity of caffeic acid and its relationship to hydrogen peroxide generated during autooxidation. Mutation Res. 116:333-339.

Harnden, D. G. (1974) Ataxia-telangiectasia syndrome: cytogenetic and cancer aspects, In: German, J. (ed.) Chromosomes and Cancer. New York: John Wiley, pp. 619-636.

Hartman, Z., Hartman, P. E., Barnes, W. M., and Tuley, E. (1984) Environ. Mutagen. 6:633-650.

Hatayama, T., and Goldberg, I. H. (1980) Deoxyribonucleic acid sugar damage in the action of neocarzinostatin. Biochemistry 19:5890-5898.

Hatayama, T., Goldberg, I. H., Takeshita, M., and Grollman, A. P. (1978) Nucleotide specificity in DNA scission by neocarzinostatin. Proc. Natl. Acad. Sci. USA 75:3603-3607.

Hecht, F., Koler, R. D., Rigas, D. A., Dahnke, G. S., Case, M. P., Tisdale, V., and Miller, R. W. (1966) Leukaemia and lymphocytes in ataxia-telangiectasia. Lancet II: 1193.

Hecht, F., McCaw, B. K. , and Koler, R. D. (1973) Ataxia-telangiectasia-clonal growth of translocation lymphocytes. N. Eng. J. Med. 289:286-291.

Heddle, J. A., Benz, R. D., and Countryman, P. I. (1978) Measurement of chromosomal breakage in cultured cells by the micronucleus technique, In: Evans, H. J., and Lloyd, D. C. (eds.) Mutagen-Induced Chromosome Damage in Man. Edinburgh University Press, Edinburgh, pp. 191.

Henner, W. D., and Blazka, M. E. (1986) Hypersensitivity of cultured ataxia-telangiectasia cells to etoposide. J. Natl. Cancer. Inst. 76:1007-1011.

Hirose, M., Kurata, Y., Tsuda, H., Fukushima, S., Ito, N. (1987) Catechol strongly enhances rat stomach carcinogenesis: a possible new environmental stomach carcinogen. Jap. J. Cancer Res. (Gann) 78:1144-1149.

Hittelman, W. N. (1984) Prematurely condensed chromosomes: a model system for visualizing effecs of DNA damage, repair, and inhibition at the level of chromosome structure, In: Collins, A., Downes, S., and Johnson, R. T. (eds.) DNA Repair and Its Inhibition. Oxford: IRL Press, pp.317-341.

Hittelman, W. N., and Pollard, M. (1982) Induction and repair of DNA and chromosome damage by neocarzinostatin in quiescent normal human fibroblasts. Cancer Res. 42:4584-4590.

Hittelman, W. N., and Rao, P. N. (1974a) Premature chromosome condensation. I.Visualization of X-ray induced chromosome damage in interphase cells. Mutat. Res. 23:251-258.

Hittelman, W. N. , and Rao, P. N. (1974b) Bleomycin induced damage in prematurely condensed chromosomes and its relationship to cell cycle progression in CHO cells. Cancer Res. 34:3433-3439.

Hochstein, P., and Atallah, A. S. (1988) The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. Mutation Res. 202:363-375.

Hoffmann, M. E., Mello-Filho, A. C., and Meneghini, R. (1984) Correlation between cytotoxic effect of hydrogen peroxide and the yield of DNA strand breaks in cells of different species. Biochimica et Biophysica Acta 781:234-238.

Houldsworth, J., and Lavin, M. F. (1980) Effects of ionizing radiation on DNA synthesis in ataxia-telangiectasia. Nucleic Acids Research 8:3709-3720.

Huang, P. C., and Sheriadan, R. B. (1981) Genetic and biochemical studies with ataxia-telangiectasia. A review. Hum. Genet. 59:1-9.

Hutchinson, F. (1985) Chemical changes induced in DNA by ionizing radiation. Prog. Nucleic Acid Res. Mol. Biol. 32:115-154.

Inoue, T., Hirano, K., Yokoiyama, A., Kada, T., and Kato, H. (1977) DNA repair enzymes in ataxia-telangiectasia and Bloom's syndrome frbroblasts. Biochim. Biophys. Acta 479:497-500.

Inoue, T., Yokoiyama, A., and Kada, T. (1981) Biochim. Biophys. Acta 655:49-53.

Jaspers, N. G. J., et al. (1982) Cancer Res. 42:335.

Johnson, R. T., Collins, A. R. S., and Waldren, C. A. (1982) Prematurely condensed chromosomes and the analysis of DNA and chromosome lesions, In: Rao, P. N., Johnson, R. T., and Sperling, K. (eds.) Premature Chromosome Condensation: Application in Basic, Clinical, and Mutation Research. New York: Academic Press, pp.253-308.

Jones, P. (1982) In: Dunford, H. B. (ed.) The Biological Chemistry of Iron. Reidel, Dordrecht, pp.427-438.

Jouve, H. M. (1985) Etude de la catalase chez proteus mirabilis. These de Doctorat en Sciences. Grenoble.

Kasai, H., Crain, P. F., Kuchino, H. (1986) Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 7:1849-1851.

Kennedy, A. R., Troll, W., and Little, J. B. (1984) Role of free radicals in the initiation and promotion of radiation transformation in vitro. Carcinogenesis 5:1213-1218.

Kensler, T. W., Bush, D. M., Kosumbo, W. J. (1983) Inhibition of tumor promotion by a biomimetic superoxide dismutase. Science 221:75-77.

Kinsellla, T. J., Mitchell, J. B., McPherson, S., Russo, A., and Tietze, F. (1982) In vitro x-ray sensitivity in ataxia-telangiectasia homozygote and heterozygote skin fibroblasts under oxic and hypoxic conditions. Caner Res. 42:3950-3956.

Knudson, A. G. (1985) Hereditary cancer, oncogenes, and antioncogenes. Cancer Res. 45:1437-1443.

Koch, C. J. (1979) Advances in Radiation Biology. 8:274-315.

Kohn, P. H., Kraemer, K. H., and Buchanan, J. K. (1982) Influence of ataxia-telangiectasia gene dosage on bleomycin induced chromosome breakage and inhibition of replication in human lymphoblastoid cell lines. Exp. Cell. Res. 137:387-395.

Komatsu, K., Kodama, S., Okumura, Y., Koi, M., and Oshimura, M. (1990) Restoration of radiation resistance in ataxia telangiectasia cells by the introduction of normal human chromosome 11. Mutation Research 235:59-63.

Krishan, A. (1971) J. Ultrastruct. Res. 36:191.

Krishna, G., Kropko, M. L., and Theiss, J. C. (1989) Use of the cytokinesis-block method for the analysis of micronuclei in V79 Chinese hamster lung cells: results with mitomycin C and cyclophosphamide. Mutation Res. 222:63-69.

Kumar, G. K., Al Saadi, A., Yang, S. S., et al. (1979) Ataxia-telangiectasia and hepatocellular carcinoma. Am. J. Med. Sci. 278:157-160.

Lambert, W. C., Kolber, L. R., Gorelli, L. A., Muglia, J. J., and Kardashian, J. F. (1982) Epidermoid carcinoma of the oral cavity following diagnostic roentgnographic examination in a patient with ataxia-telangiectasia. Clin. Prev. Dent. 4:3-8.

Lampert, F. (1969) Akute lympoblastische leukamie bei Geschwistern mit progressiven kleinhirnataxia (Louis-Bar-Syndrom). Dtsch Med Wochenschr 94:217-220.

Laval, F. (1988) Pretreatment with oxygen species increases the resistance of mammalian cells to hydrogen peroxide and gamma-rays. Mutation Res. 201:73-79.

Lavin, M. F., and Seymour, G. J. (1984) Int. J. Cancer 33:359.

Lehmann, A. R., and Stevens, S. (1977) The production and repair of double-strand DNA breaks in cells from normal humans and from patients with ataxia-telangiectasia. Biochimica et Biophysica Acta 474:49-60.

Lehmann, A. R., and Stevens, S. (1979) The response of ataxia-telangiectasia cells to bleomycin. Nucleic Acids Research 6:1953-1960.

Lehmann, A. R., James, M. R., and Stevens, S. (1982) Miscellaneous observations on DNA repair in ataxia-telangiectasia, In: Bridges, B. A., and Harnden, D. G. (eds.) Ataxia-Telangiectasia: A Cellular and Molecular Link Between Cancer, Neurophathology, and Immune Deficiency. Chichester: John Wiley and Sons, pp.347-353.

Lehmann, A. R., Jaspers, N. G. J., and Gatti, R. A. (1987) Meeting report: workshop on ataxia-telangiectasia. Cancer Res. 47:4750-4751.

Lesko, S., Lorentzen, R., and Ts'o, P. (1980) Biochemistry 19:3023

Levin, D., Hollstein, M., Christman, M., Schwiers, E., and Ames, B. N. (1982) Proc. Natl. Acad. Sci. USA 79:7445

Levitt, R., Pierre, R. V., White, W. L., Siekert, R. G. (1978) Atypical lymphoid leukemia in ataxia-telangiectasia. Blood 52:1003-1011.

Little, J. B., and Nagasawa, H. (1985) Effect of confluent holding on potentially lethal damage repair, cell cycle progression and

chromosomal aberrations in human normal and atacia-telangiectasia cells. Radiat. Res. 101:81-93.

Loschen, G. A., Azzi, A., and Flohe, L. (1974a) In: Thruman, R. G., Yonetani, T., Williamson, J. R., and Chance, B. (eds.) Alcohol and Aldehyde Metabolizing Systems. Academic Press, New York, pp.215-229.

Loschen, G. A., Azzi, A., Richter, C., and Flohe, L. (1974b) FEBS Lett. 42:68-72.

MacMahon, B., Yen, S., Trichopoulos, D., Warren, K., and Nardi, G. (1981) Coffee and cancer of the pancreas. N. Engl. J. Med. 304:630-633.

Maki-Paakknen, J. (1987) Chromosome aberrations, micronuclei and sister-chromatid exchanges in blood lymphocytes after occupational exposure to low levels of styrene. Mutation Res. 189:399-406.

Marcel, G. A. (1986) Biofutur 46:58-61.

Marklund, S. L., Holme, E., and Hellemr, L. (1982) Clin. Chim. Acta 126:41-51.

McCord, J. M. (1974) Free radicals and inflammation: Protection of synovial fluid by superoxide dismutase. Science 185:529-531.

McCord, J.M. (1985) Oxygen-derived free radicals in post ischemic tissue injury. N. Engl. J. Med. 312:159-163.

McKinnon, P. J. (1987) Ataxia-telangiectasia: an inherited disorder of ionizing-radiation sensitivity in man . Hum. Genet. 75:197-208.

McKinnon, P. J., and Burgoyne, L. A. (1985a) Exp. Cell. Res. 158:413.

McKinnon, P. J., and Burgoyne, L. A. (1985b) Eur. J. Cell. Biol. 39:161.

Meneghini, R., and Hoffmann, M. E. (1980) Biochim. Biophys. Acta 608:167-173.

Meneghini, R. (1988) Genotoxicity of active oxygen species in mammalian cells. Mutation Research 195:215-230.

Meredith, M. J., and Dodson, J. L. (1987) Impaired glutathione biosynthesis in cultured human ataxia-telangiectasia cells. Cancer Res. 47:4576-4581.

Mirzayans, R., Smith, B. P., and Paterson, M. C. (1989) Hypersensitivity to cell killing and faulty repair of 1-beta-D-arabinofuranosyl cytosine-detectable sites in human (ataxia-telangiectasia) fibroblasts treated with 4-nitroquinoline 1-oxide. pp. 5523-5529.

Mitelman, F. (ed.) (1985) Catalog of Chromosome Aberrations in Cancer, 2nd Ed. Alan R.Liss, Inc., N.Y.

Mitelman, F., and Heim, S. (1988) Consistent involvement of ony 71 of the 329 chromosomal bands of the human genome in primary neoplasia-associated rearrangements. Cancer Res. 48:7115-7119.

Mohamed, R., et al. (1987) Biochem. Biophys. Res. 149:233.

Moody, C. J., and Hassan, H. M. (1982) Mutagenicity of oxygen free radicals. Proc. Natl. Acad. Sci. (U.S.A.) 79:2855-2859.

Morgan, J. L., Holcomb, R. M., and Morrissey, R. W. (1968) Am. J. Dis. Child. 116:557-558.

Morgan, R. W., Christman, M. F., Jacobson, F. S., Storz, G., and Ames, B. N. (1986) Hydrogen peroxide-inducible protein in Salmonella typhimurium overlap with heat shock and other stress proteins. Proc. Natl. Acad. Sci. USA 83:8059-8063.

Morimyo, M. (1982) J. Bacteriol. 152:208-214.

Morrell, D., Cromartie, E., and Swift, M. (1986) Mortality and cancer incidence in 263 patients with ataxia-telangiectasia. J. N. C. I. 77:89-92.

Mozdarani, H., and Bryant, P. E. (1987) The effect of 9-beta-D-arabinofuranosyladenine on the formation of X-ray induced chromatid aberrations in x-irradiated G_2 human cells. Mutagenesis 2:371-374.

Mozdarani, H., and Bryant, P. E. (1989) Kinetics of chromatid aberrations in G_2 ataxia-telangiectasia cells exposed to X-rays and ara A. Int. J. Radiat. Biol. 55(1):71-84.

Murname, J. P., and Painter, R. B. (1983) Biochemistry 22:1217.

Nagao, M., Fujita, Y., Sugimura, T., and Kosuge, T. (1985) In: Singer, B., and Bartsch, H. (eds.) Role of Cyclic Adducts in Carcinogenesis, Mutagenesis, IARC, Lyon.

Nagasawa, H., Kraemer, K. H., Shiloh, Y., and Little, J. B. (1987) Detection of ataxia-telangiectasia heterozygous cell lines by postirradiation cumulative labelling index: measurements with coded samples. Cancer Res. 47:398-402.

Nagasawa, H., Latt, S. A., Lalaude, M. E., and Little, J. B. (1985) Effects of x-irradiation on cell-cycle progression, induction of chromosomal aberrations and cell killing in ataxia-telangiectasia (A-T) fibroblasts. Mutation Res. 148:71-82.

Nakamura, Y., Colburn, N. H., and Gindhart, T. (1985) Role of reactive oxygen in tumor promotion: implication of superoxide anion in promotion of neoplastic transformation in JB6 cells by TPA. Carcinogenesis 6:229-235.

Nakayama, T., Kodama, M., and Nagata, C. (1984) Gann 75:95-98.

Narita, T. and Takagi, K. (1984) Ataxia-telangiectasia with dysgerminoma of right ovary, papillary carcinoma of thyroid, and adenocarcinoma of pancreas. Cancer 54:1113-1116.

Natarajan, A. T. and Meyers, M. (1979) Chromosomal radiosensitivity of ataxia-telangiectasia cells at different cell cycle stages. Hum. Genet. 52:127-132.

Natarajan, A. T., Meijers, M., van Zeeland, A. A., and Simons, J. W. I. M. (1982) Attempts to detect ataxia-telangiectasia (A-T) heterozygotes by cytogenetical techniques. Cytogenet. Cell. Genet. 33:145-151.

Nohl, H. and Hegner, D. (1978a) FEBS Lett. 89:126-130.

Nohl, H. and Hegner, D. (1978b) Eur. J. Biochem. 82:563-567.

Norman, R. O. C., Hill, F. R. S., and Hill, H. A. O. (1986) In: Hill, H. A. O. (ed.) Inorganic and Organic Radicals: Their Biological and Clinical Relevance. Royal Society, London.

Oberley, L. W. (1983) In: Oberley, L. W. (ed.) Oncology Overview: Selected Abstract on Modulation of Cancer by Superoxide Dismutase. U.S.Department of Health and National Institute of Health. pp.1-47.

Oxford, J. M., Harnden, D. G., Parrington, J. M., and Delhanty, J. D. A. (1975) Specific chromosome aberrations in ataxia-telangiectasia. J. Med. Genet. 12:251-262.

Painter, R. B., and Young, B. R. (1980) Radiosensitivity in ataxia-telangiectasia:a new explanation. Proc. Natl. Acad. Sci. USA 77:7315-7317.

Parshad, R., Sanford, K., and Jones, G. M. (1983) Chromatid damage after G_2 phase x-irradiation of cells from cancer-prone individuals implicates deficiency in DNA repair. Proc. Natl. Acad. Sci. USA 80:5612-5616.

Parshad, R., Sanford, K. K., Jones, G. M., and Tarone, R. E. (1985) G_2 chromosomal radiosensitivity of ataxia-telangiectasia heterozygotes. Cancer. Genet. Cytogenet. 14:163-168.

Paterson, M. C., and Smith, P. J. (1979) Ataxia-telangiectasia: an inherited human disorder involving hypersensitivity to ionizing radiation and related DNA damaging chemicals. Ann. Rev. Genet. 13:291-318.

Paterson, M. C., Anderson, A. K., Smith, B. P., and Smith, P. J. (1979) Enhanced radiosensitivity of cultured fibroblasts from ataxia-telangiectasia heterozygotes manifested by defective colony forming ability and reduced DNA repair replication after hypoxic gamma-irradiation. Cancer Res. 39:3725-3734.

Paterson, M. C., MacFarlane, S. J., Gentner, N. E., and Smith, B. P. (1985) Cellular hypersensitivity to chronic gamma-irradiation in cultured fibroblasts from ataxia-telangiectasia heterozygotes, In: Gatti, R. A., and Swift, M. (eds.) Ataxia-Telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood. New York: Alan R.Liss. pp.73-83.

Paterson, M. C., Smith, B. P. , Lohman, P. H. M., Anderson, A. K., and Fishman, L. (1976) Defective excision repair of gamma-ray damaged DNA in human (ataxia-telangiectasia) fibroblasts. Nature (Lond.) 260:444-447.

Paterson, M. C., Anbin, R. A., Fourney, R.M., and Mirzayans, R. (1989) 19 Survey of post-gamma-ray colony-forming ability, DNA metabolism and oncogene status in non-malignant fibroblast strains from cancer-prone families and individual cancer patients. pp. 227-239.

Paterson, M. C., Smith, B. P., Knight, P. A., and Anderson, A. K. (1977) In: Castellani, A. (ed.) Research in Photobiology. Plenum, New York.

Pfeiffer, R. A. (1970) Chromosomal abnormalities in ataxia-telangiectasia (Louis-Bar syndrome). Humangenetik 8:302-306.

Prosser, J.S., Moquet, J.E., Lloyd, D.C., and Edqards, A.A. (1988) Radiation induction of micronuclei in human lymphocytes. Mutation Research 199:37-45.

Pryor, W. A. (1976-1986) (ed.) In: Free Radicals in Biology. Vols.1-5. Academic Press, New York.

Ramalho, A, Sunjevaric, I, and Natarajan, A.T. (1988) Use of the frequencies of micronuclei as quantitative indicators of X-ray-induced chromosomal aberrations in human peripheral blood lymphocytes: Comparison of two methods. Mutation Research 207:141-146.

Rary, J. M., Bender, M. A., and Kelly, T. E. (1974) Cytogenetic studies of ataxia-telangiectasia. American Journal of Human Genetics 26:70A.

Ray, J. H., and German, J. (1983) The cytogenetics of the "chromosomal breakage syndromes", In: German, J. (ed.) Chromosome Mutation and Neoplasia. Liss, New York, pp. 135-167.

Roifman, C. M. and Gelfand, E. W. (1985) Heterogeneity of the immunological deficiency in ataxia-telangiectasia: absence of a clinical-pathological correlation. In: Gatti, R. A. and Swift, M. (eds.) Ataxia-Telangiectasia:Genetics, Neuropathology, and Immunology of a Degenerative Disease of Childhood. Alan R. Liss, N.Y. pp:273-285.

Rosin, M. P., and Ochs, H. D. (1986) In vivo chromosomal instability in ataxia-telangiectasia homozygotes and heterozygotes. Hum. Genet. 74:340-345.

Rosin, M. P., and Stich, H. F. (1983) The identification of antigenotoxic/anticarcinogenic agents in food, In: Roe, D. A. (ed.) Diet and Cancer: From Basic Research to Policy Implications. Alan R. Liss, New York, pp. 141-154.

Rosin, M. P., Ochs, H. D., Gatti, R. A., and Boder, E. (1989) Heterogeneity of chromosomal breakage levels in epithelial tissue of ataxia-telangiectasia homozygotes and heterozygotes. Human Genetics 83:133-138.

Rudolph, N. S., Nagasawa, H., Little, J. B., and Latt, S. A. (1989) Identification of ataxia-telangiectasia heterozygotes by flow cytometric analysis of x-ray damage. Mutation Research 211:19-29.

Sanford, K. K., Parshad, R., Gantt, R., Tarone, R. E., Jones, G.M., and Price, F. M. (1989) Factors affecting and significance of G_2 chromatin radiosensitivity in predisposition to cancer. Int. J. Radiat. Biol. 55:963-981.

Sankaranarayanan, K., Duyn, A. V., Loos, M. J., and Natarajan, A. T. (1989) Adaptive response of human lymphocytes to low-level radiation from radioiosotopes or X-rays. Mutation Res. 211:7-12.

Sarasin, A. (1981) La Recherche 124:824-838.

Schmid, W. (1976) The micronucleus test for cytogenetic analysis, In: Hollaender, A. (ed.) Chemical Mutagens: Principles and Methods for Their Detection. Plenum Press, New York, vol.4, pp. 31.

Schmid, W., and Jerusalem, F. (1972) Cytogenetic findings in two brothers with ataxia-telangiectasia (Louis Bar's syndrome). Arch. Genet. (Zurich) 45:49-52.

Schraufstatter, I., Hyslop, P. A., Jackson, J. H., and Cochrane, C. G. (1988) Oxidant-induced DNA damage of target cells. J. Clin. Invest. 82:1040-1050.

Sedgwick, R. P. and Boder, E. (1972) Ataxia-telangiectasia. In: Vinken, P. V. and Bruyn, G. W. (eds.): Handbook of Clinical Neurology. Vol.14, pp:267--339. North-Holland, Amsterdam.

Sen, P., and Hittelman, W. N. (1984) Kinetics and extent of repair of bleomycin-induced chromosome damage in quiescent normal human fibroblasts and human mononuclear blood cells. Cancer Res. 44:591-596.

Shaham, M., and Becker, Y. (1981) The ataxia-telangiectasia clastogenic factor is a low molecular weight peptide. Hum. Genet. 58:422-424.

Shaham, M., Becker, Y., and Cohen, M. M. (1980) A diffusable clastogenic factor in ataxia-telangiectasia. Cytogenet. Cell. Genet. 27:155-161.

Shaham, M., Becker, Y., Lerer, I., and Voss, R. (1983) Increased level of bleomycin-induced chromosome breakage in ataxia-telangiectasia skin fibroblasts. Cancer Res. 43:4244-4247.

Shaham, M., Choreb, M., and Becker, Y. (1984) Further characterization of the ataxia-telangiectasia clastogenic factor by reversed-phase liquid chromatography. J. Biotechnol. 1:351-354.

Shaham, M., Voss, R., Becker, Y., Yarkoni, S., Ornoy, A., and Kohn, G. (1982) Prenatal diagnosis of ataxia-telangiectasia. J. Pediatr. 100:134-137.

Sheridan III, R. B., and Huang, P. C. (1979) Superoxide dismutase and catalase activities in ataxia-telangiectasia and normal fibroblast cell extracts. Mutation Res. 61:381-386.

Shiloh, Y., Tabor, E., and Becker, Y. (1982a) Cellular hypersensitivity to neocarzinostatin in ataxia-telangiectasia skin fibroblasts. Cancer Res. 42:2247-2249.

Shiloh, Y., Tabor, E., and Becker, Y. (1982b) The response of ataxia-telangiectasia homozygous and heterozygous skin fibroblasts to neocarzinostatin. Carcinogenesis 3:815-820.

Shiloh, Y., Tabor, E., and Becker, Y. (1983) Abnormal response of ataxia-telangiectasia cells to agents that break the deoxyribose moiety of DNA via a targeted free radical mechanism. Carcinogenesis 4:1317-1322.

Shiloh, Y., Parshad, R., Sanford, K. K., and Jones, G. M. (1986) Carrier detection in ataxia-telangiectasia. Lancet ii:689-690.

Shilooh, Y., and Becker, Y. (1982) Reduced inhibition of replicon initiation and chain elongation by neocarzinostatin in skin fibroblasts from patients with ataxia-telangiectasia. Biochim. Biophys. Acta 721:485-488.

Shiloh, Y., Parshad, R., Frydman, M., Sanford, K. K., Portnoi, S., Ziv, Y., and Jones, G. M. (1989) G_2 chromosomal radiosensitivity in families with ataxia-telangiectasia. Hum. Genet. 84:15-18.

Slaga, T. J., Klein-Szanto, A.J. P., Triplet, I. L., Yotti, L. P., and Trosko, J. E. (1981) Skin tumor promoting activity of benzoyl peroxide, a widely used free radical generating compound. Science 213:1023-1025.

Smith, P. J., and Paterson, M. C. (1983) Effects of aphidicolin on de novo DNA synthesis, DNA repair and cytotoxicity in gamma-irradiated human fibroblasts: implications for the enhanced radiosensitivity in ataxia-telangiectasia. Biochimica et Biophysica Acta 739:17-26.

Smith, P. J. (1984) Carcinogenesis 5:1345.

Solanki, V., Yotti, L., Logani, M. K., and Slaga, T. J. (1984) The reduction of tumor initiating activity and cell mediated mutagenicity of dimethylbenz [a] anthracene by a copper coordination compound. Carcinogenesis 5:129-131.

Spector, B., Perry, G. S., and Kersey, J. H. (1978) Genetically determined immunodeficiency diseases (GDID) and malignancy. Report from the Immunodeficiency Cancer Registry. Clin. Immunol. Immunopathol. 11:12-29.

Spector, B. D., Filipovich, A. H., Perry, G. S. III, and Kersey, J. H. (1982) Epidemiology of cancer in ataxia-telangiectasia. In: Bridges, B. A. and Harnden, D. G. (eds.) Ataxia-Telangiectasia: A Cellular and Molecular Link between Cancer, Neuropathology and Immune Deficiency. Chichester, England:John Wiley. pp:103-138.

Spitz, D. R., and Li, G. C. (1988) Stable H2O2-resistant variants of Chinese hamster fibroblasts demonstrate increases in catalase activity. Radiation Research 114:114-124.

Stich, H. F., and Rosin, M. P. (1983a) Quantitating the synergistic effect of smoking and alcohol consumption with the micronucleus test on human buccal mucosa cells. Int. J. Cancer 31:305-308.

Stich, H. F., and Rosin, M. P. (1983b) Micronuclei in exfoliated human cells as an internal dosimeter for exposures to carcinogens. In: Stich, H. F. (ed.) Carcinogens and mutagens in the environment. Vol.II: Naturally occuring compounds: endogenous formation and modulation. CRC Press, Boca Raton, Fla, pp:17-25.

Stich, H. F., and Rosin, M. P. (1984) Micronuclei in exfoliated human cells as a tool for studies in cancer risk and cancer intervention. Cancer Lett. 22:241-253.

Stich, H. F., and Anders, F. (1989) The involvement of reactive oxygen species in oral cancers of betel quid/tobacco chewers. Mutat. Res. 214:47-61.

Stich, H. F., and Tsang, S. S. (1989) Promoting activity of betel quid ingredients and their inhibition by retinol. Cancer Letters 45:71-77.

Stich, H. F., Rosin, M. P., Wu, C. H., and Powrie, W. D. (1981a) A comparative genotoxicity study of chlorogenic acid (3-O-caffeoylquinic acid). Mutation Res. 90:201-212.

Stich, H. F., Rosin, M. P., Wu, C. H., and Powrie, W. D. (1981b) The action of transition metals on the genotoxicity of simple phenols, phenolic acids and cinnamic acids. Cancer Lett. 14:251-260.

Stich, H. F., San, R. H. C., and Rosin, M. P. (1983) Adaptation of the DNA repair and micronuleus tests to human cell suspensions and exfoliated cells. Ann. N. Y. Acad. Sci. 407:93-105.

Stich, H. F. (1990) Genotoxic and antigenotoxic effects, carcinogenic and anticarcinogenic actions, and promoting and actipromoting effects of simple phenolic compounds. Mutat. Res. (in press).

Storz, G., Michael, F., Christman, M. F., Sies, H., and Ames, B. N. (1987) Spontaneous mutagenesis and oxidative damage to DNA in Salmonella typhimurium. Proc. Natl. Acad. Sci. USA 84:8917-8921.

Swift, M. (1982) Disease predisposition of ataxia-telangiectasia heterozygotes. In: Bridges, B. A. and Harnden, D. G. (eds.) Ataxia-Telangiectasia: A Cellular and Molecular Link between Cancer, Neuropathology and Immune Deficiency. Wiley, London, pp:355-361.

Swift, M. (1985) Genetics and epidemiology of ataxia-telangiectasia. In: Gatti, R. A. and Swift, M. (eds.) Ataxia-Telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood. Alan R.Liss, N.Y. pp:133-146.

Swift, M., Morrell, D., Cromartie, E., Chamberlin, A. R., Skolnick, M. H. and Bishop, D. T. (1986) The incidence of gene frequency of ataxia-telangiectasia in the United States. Hum. Genet. 39:573-583.

Swift, M., Reitnauer, P. J., Morrell, D. and Chase, C. L. (1987) Breast and other cancers in families with ataxia-telangiectasia. N. Engl. J. Med. 316:1289-1294.

Swift, M., Sholman, L., Perry, M. and Chase, C. (1976) Malignant neoplasms in the families of patients with ataxia-telangiectasia. Cancer Res. 36:209-215.

Tannock, I. F. and Hill, R. P. (eds.) (1987) The Basic Science of Oncology. Pergamon Press, Inc.

Tates, A. D., Neuteboom, I., Hofker, M., and Den Enbelsen, L. (1980) A micronucleus technique for detecting clastogenic effects of mutagens/carcinogens (DEN, DMN) in hepatocytes of rat liver in vivo. Mutat. Res. 74:11.

Tatsuka, M., Nikaido, O., Tatsumi, K., and Takebe, H. (1989) X-ray-induced G_2 arrest in ataxia telangiectasia lymphoblastoid cells. Mutat. Res. 214:321-328.

Taylor, A. M. R. (1978) Unrepaired DNA strand breaks in irradiated ataxia-telangiectasia lymphocytes suggested from cytogenetic observations. Mutation Research 50:407-418.

Taylor, A. M. R. (1982) Cytogenetics of ataxia-telangiectasia, In:Bridges, B. A. and Harnden, D. G. (eds.) Ataxia-Telangiectasia: A Cellular and Molecular Link Between Cancer, Neuropathology, and Immune Deficiency. Chichester, England: John Wiley. pp:53-81.

Taylor, A. M. R. (1983) The effect of radiation on the chromosomes of patients with an unusual cancer susceptibility. In: Takaaki, I., and Sasaki, M. S. (eds.) Radiation-Induced Chromosome Damage in Man. New York: A.R Liss, pp.167-199.

Taylor, A. M. R., Harnden, D. G., Arlett, C. F., and et al. (1975) Ataxia-telangiectasia: a human mutation with abnormal radiation sensitivity. Nature 258:427-429.

Taylor, A. M. R., Metcalfe, J. A., Oxford, J. M., and Harnden, D. G. (1976) Is chromatid type damage in ataxia-telangiectasia after irradiation at Go a consequence of defective repair? Nature 260:441-443.

Taylor, A. M. R., Rosney, C. M., and Campbell, J. B. (1979) Unusual sensitivity of ataxia-telangiectasia cells to bleomycin. Cancer Res. 39:1046-1050.

Taylor, A. M. R., Oxford, J. M., Metcafe, J. A. (1981) Spontaneous cytogenetic abnormalities in lymphocytes from thirteen patients with ataxia-telangiectasia. Int. J. Cancer 27:311-319.

Teoule, R. (1987) Radiation-induced DNA damage and its repair. Int. J. Radiat. Biol. 51:573-589.

Thacker, J. (1986) The use of recombinant DNA techniques to study radiation-induced damage, repair and genetic change in mammalian cells. Int. J. Radiat. Biol. 50:1-30.

Thacker, J. (1989) The use of integrating DNA vectors to analyse the molecular defects in ionising radiation-sensitive mutants of mammalian cells including ataxia-telangiectasia. Mutation Research 220:187-204.

Trotter, J. R. (1980) Spontaneous cancer and its possible relationship to oxygen metabolism. Proc. Natl. Acad. Sci. USA 77:1763-1767.

Von Sonntag, C., and Schuchmann, H. P. (1986) The radiolysis of pyrimidines in aqueous solutions: an updating review. Int. J. Radiat. Biol. 49:1-34.

Vuillaume, M. (1987) Reduced oxygen species, mutation, induction and cancer initiation. Mutation Res. 186:43-72.

Wakata, A., and Sasaki, M. (1987) Measurement of micronuclei by cytokinesis-block method in cultured Chinese hamster cells: comparison with types and rates of chromosome aberrations. Mutation Res. 190:51-57.

Waldren, C. A., and Johnson, R. T. (1974) Analysis of interphase chromosome damage by means of premature chromosome condensation after X-ray and ultraviolet irradiation. Proc. Natl. Acad. Sci. USA 71:1137-1141.

Ward, J. F. (1975) Molecular mechanism of radiation-induced damage to nucleic acids. Adv. Radiat. Biol. 5:181-239.

Weitzman, S. A., Weitberg, A. B., Clark, E. P., and Stones, T. P. (1985) Phagocytes as carcinogens: malignant transformation produced by neutrophils. Science 227: 1231-1233.

Winquist, L., Rannug, U., Rannug, A., and Ramel, C. (1984) Protection from toxic and mutagenic effects of H_2O_2 by catalase induction in 59. Salmonella typhimurium. Mutat. Res. 141:145-147.

Yager, J., and Sorsa, M. (1987) Evaluation of the cytokinesis block modification of the peripheral lymphocyte micronucleus method. Environ. Mutagen. 9:116 (abstr.).

Zampetti-Bosseler, F., and Scott, D. (1981) Cell death, chromosome damage and mitotic delay in normal human, ataxia-telangiectasia and retinoblastoma fibroblasts after X-irradiation. International Journal of Radiation Biology 39:547-558.

Zampetti-Bosseler, F., and Scott, D. (1985) The response of normal and ataxia-telangiectasia cells to bleomycin: relationships between chromosome damage, cell cycle delay and cell killing. Mutation Research 151:89-94.

Zimmerman,, R., and Cerutti, P. (1984) Active oxygen acts a promoter of transformation in mouse embryo C3H /10T1/2/C18 fibroblasts. Proc. Natl. Acad. Sci. (U.S.A.) 51:2085-2087.