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Transformation and Repair Replication in Lymphocytes from Ataxia Telangiectasia[1] (41598)

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Abstract. Peripheral blood leukocytes (PBL) isolated from five patients with ataxia telangiectasia (AT) proved more difficult to transform following addition of exogenous Epstein–Barr virus than PBL isolated from AT heterozygotes or normal adults. PBL isolated from one AT patient transformed within the range expected for normal PBL. Once established in culture, the resulting lymphoblastoid cell lines (LCLs) were immortal and, though they grew slower than normal control LCLs, provided useful material for studying cellular phenotypes associated with AT lymphoid cell lines. All the resulting LCLs established from ataxia were more sensitive to X-irradiation than were LCLs established from controls as measured by colony formation in microtiter plates. Furthermore, X-ray-induced inhibition of semiconservative DNA synthesis in ataxia LCLs was less than that seen in normal LCLs. These results are in agreement with those obtained using cultured AT fibroblasts, indicating that *in vitro* transformation by exogenously added Epstein–Barr virus does not alter the phenotype of the ataxia cell as measured by these two parameters. However, no deficiency in X-ray-induced excision repair of DNA was demonstrable in LCLs established from four AT patients. Nor was there a deficiency in AT LCL host cell reactivation of herpes simplex virus X-irradiated under anoxic conditions. Taken together, these data point toward a defect in ataxia lymphoblasts other than repair enzyme(s) per se, one possibly associated with chromosomal structure, function, or modification.

Ataxia telangiectasia (AT) is an autosomal recessive disease in humans characterized *in vitro* at the cellular level by an increased sensitivity to ionizing radiation as measured by cell survival (1–3). It has also been reported that colony formation by AT cells is more sensitive to the radiomimetic compounds methylmethanesulfonate (4), *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (5), bleomycin (6, 7), and neocarcinostatin (8), each of which can cause DNA strand breaks and DNA base modifications. At the level of DNA repair, although all AT cells tested have been shown to be capable of normal rejoining of radiation-induced single- (1, 9) and double-strand (10) breaks, some, but not all, AT cell lines have been shown to be deficient in ionizing radiation-induced excision repair as measured by either unscheduled DNA synthesis (11) or [^{3}H]thymidine:5-bromo-2-deoxyuridine incorporation into unreplicated cellular DNA (12). More recently, the interesting observation has been made that DNA synthesis as measured by [^{3}H]thymidine incorporation is not as sensitive in AT cell lines to the inhibitory effects of ionizing radiation as DNA synthesis in normal cells (13–15). A similar resistance in AT cells has been reported to be seen after treatment with bleomycin but not *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (16). In addition, it has been reported that the levels of poly (ADP-ribose)$_n$ formed from NAD$^+$ was lower in permeabilized AT cells than in normal cells following radiation treatment (13). Since it is believed that poly (ADP-ribose) polymerase is involved in the modifications of chromosomal or other proteins preceding or concomitant with DNA repair (17–19), these observations give rise to the possibility that the molecular defect in AT does not reside in DNA repair enzymes per se but in chromosomal structure or metabolism. It should be noted, however, that uracil glycosylase (20, 21) as well as nucleotide excision and base excision repair processes (22) have been reported to be cell cycle dependent in cultured human cells, and that AT cells in

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culture have been shown by Elmore and Swift (23) to have an abnormally long doubling time, with the longer cell cycle apparently being associated with an increased length of S phase (24). Since sensitivity to certain types of DNA lesions, such as O^6-methylguanine induced by certain alkylating agents, may be increased during the S phase of the cell cycle (20, 25–27), the possibility exists that alterations in the cell cycle in AT cells could also influence measurements of DNA repair and associated phenomena.

In this report, we have quantitated the Epstein–Barr virus (EBV)-induced transformation efficiencies of peripheral blood leukocytes (PBL) from six ataxia homozygotes and the DNA repair capabilities of the resulting lymphoblastoid cell lines (LCLs) as measured by (i) assaying recovery of DNA synthetic capabilities by [^{3}H]thymidine incorporation; (ii) cell survival in microtiter plates following X-irradiation; and (iii) classical excision repair of DNA as measured by isopyknic sedimentation of DNA density labeled with 5-bromo-2-deoxyuridine (BrdUrd). From these combined studies we conclude that the difficulty in establishing AT LCLs can be overcome by using exogenous EBV of high transforming titer and that the resulting LCLs once established retain their phenotype following *in vitro* transformation by the B95-8 strain of EBV.

Materials and Methods. *Cell culture.* PBL were purified by the ficoll-hypaque method of Böyum (28) from 5–10 ml of heparinized whole blood drawn from volunteers. PBL (between 1×10^6 and 5×10^6 cells total) from individuals homozygous for the AT gene, and from normal adults, were transformed by use of the B95-8 transforming strain of EBV. Transformation efficiencies of various cell populations were determined in microtiter plates using the limiting dilution method (29). The resulting LCLs that were established were then maintained in RPMI-1640 supplemented with 20% heat-inactivated fetal bovine serum (Flow Laboratories) and the antibiotics penicillin and streptomycin. Under these culture conditions the AT LCLs did not produce detectable biologically active transforming virus. Second- or third-passage cells at 1×10^7 cells/ml were slowly ($\leq$1°C/min) frozen in prescored glass vials with 10% dimethylsulfoxide under liquid nitrogen, and are available upon request.

Assessment of cell survival. Measurements of cell survival were accomplished by the method of cell plating in microtiter plates. Briefly, exponentially growing LCLs adjusted to 1×10^6 cells/ml were treated at 4°C with X-irradiation and then immediately serially diluted into individual flat-bottom wells of 96-well microtiter plates (identical to the type of plates used to originally initiate the LCLs from quiescent PBL) previously inoculated with complete media. The plates were then incubated in a humidified 5% CO_2 in air atmosphere. Colonies which developed within 3 weeks were scored with an inverted tissue culture microscope and the microculture-initiating efficiencies were calculated as determined by Kraemer *et al.* (30). Mean microculture-initiating efficiencies of treated cultures were then compared to nontreated cultures and expressed as a percentage of this nontreated control. Filtered (0.50 mm Cu and 1.04 mm Al) X-radiation was emitted from a General Electric Maxitron 300 therapeutic X-ray machine. The dose rate for cellular inactivation was determined to be 125 rad/min at 50 cm and 300 kV, 20 MA by a Victoreen Model 570 rate meter, calibrated and generously lent by Dr. Alan Conger, Fels Research Institute. To alter the dose rate for DNA repair experiments the distance of the target from the source was changed.

DNA metabolism. Effects of X-irradiation on cellular DNA synthetic capabilities were determined by measuring the incorporation of [^{3}H]thymidine into cold trichloroacetic acid-precipitable material. Due to the small numbers of PBL obtainable from AT patient blood, these experiments were carried out only on LCLs. Briefly, the cells cultured in plastic tissue culture flasks were exposed to various doses of X-irradiation and then distributed at 2.5×10^5 cells/ml into 100 × 15-mm rubber-stoppered glass tubes. These samples were then pulsed with [^{3}H]thymidine, 6 μCi/ml, 25 Ci/mmole, for 1 hr at various times (1, 24, and 48 hr) after treatment with X-rays. The incorporation reaction was terminated by the addition of cold Hank's balanced saline. The cells were processed for cold tricholoracetic acid-precipitable material on Millipore filters

and the incorporated radioactivity was determined in a liquid scintillation counter. The effects of X-irradiation on DNA synthesis are expressed as a percentage of untreated control cultures.

Results. *Transformation of AT leukocytes with EBV.* Through use of isolated lymphocytes in the limiting dilution assay, the dilution of an EBV preparation at which 50% of the wells in a microtiter plate develop as morphologically transformed, has been shown to be directly related to the transformability of the mixed leukocyte population used (29). An EBV preparation known to be equal to or greater than 10^4 TD_{50} units/0.10 ml as measured on human unbilical cord leukocytes was used to successfully transform PBL from six patients homozygous for the AT gene. In five out of the six attempts the transformation efficiencies of the AT leukocytes were well below those expected from PBL isolated from normal, age-matched individuals without the AT gene (Table I). In each attempt the time needed before macroscopically visible AT colonies developed in the microtiter plates which could be subcultured (4–6 weeks) was longer than the time needed before normal colonies developed which could be subcultured (2–3 weeks). Attempts were also made to derive LCLs spontaneously from each AT patient without the addition of EBV, but in no instance was spontaneous transformation observed during these experiments in wells not inoculated with EBV. This was somewhat unexpected since the neoplasic diseases which develop in AT patients tend to originate in the lymphoreticular system (32, 33); and there is one report of an EBV-positive lymphoma developing in an AT patient (34). The reason for the reduced transformation efficiency of primary leukocytes from ataxia patients following the addition of exogenous EBV is not presently understood. Except for neonatal lymphocytes, donor age alone (up to 72 years) has been found to have little, if any, effect on the transformability of normal PBL; and the leukocyte numbers used in these transformation attempts, 2–3 $\times$ 10^5 cells/well, were well below the cell numbers needed to elicit "T cell"-mediated culture regression (35). It is therefore more likely that the reduced transformation efficiency observed in AT cultures is related to metabolic defects in the ataxia lymphocyte's potential for cell proliferation and not directly to susceptibility of AT lymphocytes to infection by exogenously added EBV.

Sensitivity of EBV-transformed LCLs to X-irradiation as measured by [^{3}H]thymidine incorporation or colony formation. With the development of the microtiter plating method for quantitating the potential for lymphoblast cell growth without the use of agarose (30), measuring LCL survival following various cy-

TABLE I. EPSTEIN-BARR VIRUS (B95-8)-INDUCED TRANSFORMATION EFFICIENCES OF PERIPHERAL BLOOD LEUKOCYTES (PBL) FROM INDIVIDUALS DIAGNOSED AS ATAXIA TELANGIECTASIA (AT)[a]

Experiment and date		Leukocyte donor		Transformation efficiency[b] (TD_{50})	Spontaneous transformation[c]
BB91	6/27/77	1	+/at	2.8	—
		2	+/at	2.4	—
		3	at/at	1.1	—
B40	5/5/78	1	+/+	3.5	—
		2	at/at	2.0	—
G25[d]	8/22/80	1	at/at (NBS)	1.0	—
G39	9/26/80	1	at/at	1.5	—
H43	1/18/82	1	+/at	3.5	—
		2	at/at	<0.5	—
		3	at/at	0.5	—
Normal (12) controls			+/+	2.5–3.5	—

[a] Determined in 96-well microtiter plates by limiting dilution of virus method.

[b] Transformation efficiencies were calculated by the Reed and Meunch formula, and were expressed as the positive log of the virus dilution at which 50% of the microtiter wells would yield transformed colonies (TD_{50}).

[c] 2 $\times$ 10^5 cells/well without addition of exogenous EBV.

[d] Nijmegen Breakage Syndrome (NBS).

totoxic treatments has been made simpler than measuring survival of anchorage-dependent fibroblasts. Consequently, we have used the microtiter method to analyze the effects of X-irradiation on ataxia and normal lymphoblasts under various conditions. Each of four LCLs established from patients with AT showed a greater decrease in post-X-irradiation colony-forming ability when compared to LCLs established from normal individuals (Fig. 1). The D_0 values calculated from the data in Fig. 1 are slightly lower than those values obtained for AT and normal fibroblasts (Table II) (2, 3), which may reflect our higher energy X-rays or a more general phenomenon

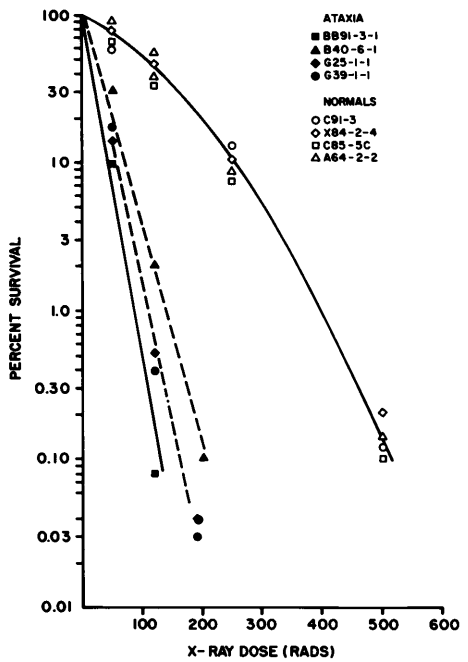

FIG. 1. Relative survival as a function of X-irradiation dose in air, for colony-forming ability in microtiter plates of lymphoblastoid cell lines (LCLs) established from EBV-infected peripheral blood leukocytes (PBL) from ataxia telangiectasia or normal adults. Each symbol represents the relative survival calculated from dividing the mean microculture initiating efficiency for the treated cells by the mean microculture initiating efficiency for untreated cells × 100% (30). The mean microculture initiating efficiencies were derived from six to eight selected diluted cell suspensions placed in each of six flat-bottom wells of a covered 96-well microtiter plate. Mean microculture initiating efficiencies for the untreated cells ranged from ≥1 to approximately 10%. X-irradiation dose rate was 125 rad/min at 50 cm from source.

TABLE II. X-RAY SURVIVAL IN EBV-TRANSFORMED LYMPHOBLASTOID CELL LINES (LCLs) ESTABLISHED FROM NORMAL ADULTS AND AT PATIENTS

	D_0 rad[a]	Host cell reactivation of X-irradiated herpes simplex virus[b]
Normal LCLs	70.8 ± 0.75	Normal
Ataxia LCLs	14.5–26.3	Normal
Nonstimulated human umbilical cord leukocytes[c]	50 ± 0.10	Not done

[a] Measured as the inverse of the slope of the straight line portion of the survival curve (3).

[b] Measured by infected centers on Vero cell monolayers (43).

[c] Measured by their potential for susceptibilty to morphologic transformation with EBV by transformed centers assay.

of lymphoid cell hypersensitivity to X-rays. Treatment with varying concentrations of leukocyte interferon before irradiation had no detectable effect on LCL survival.

Since it is known that the presence of oxygen in the system can influence cell killing by X-irradiation (36, 37), experiments were undertaken to assess the role of oxygen in LCL killing by X-rays. For this experiment some cells, with and without oxygen, were irradiated while frozen under liquid nitrogen in an attempt to limit, and therefore analyze, the effects of free radical formation on cell killing. LCLs were saturated with 95% nitrogen, 5% CO_2, or 95% air, 5% CO_2, either frozen in glass vials with 10% dimethylsulfoxide under liquid nitrogen or maintained as liquid cultures and then were X-irradiated (Table III). We observed that in the absence of oxygen (irradiation under nitrogen) X-irradiation was less cytotoxic for normal (E51-10F) as well at AT (BB91-3-1) lymphoblasts whether the irradiation was administered while the cells were in frozen or liquid state, although the protective effect of nitrogen was much more apparent if X-irradiation was carried out on liquid cultures. Freezing alone also had a protective effect against the cytotoxic effects of X-rays for AT irradiated in air. These data are consistent with the hypothesis that under our assay conditions DNA lesions produced during free radical formation in the presence of ox-

TABLE III. EFFECTS OF OXYGEN AND NITROGEN ON X-RAY (LOW DOSE)-INDUCED CYTOTOXICITY IN NORMAL AND AT LYMPHOBLASTS[a]

	X-irradiation dosage (rad)								
	Air					Nitrogen			
	0	50	100	250	375	0	50	100	250	375
	Frozen cultures[b] (−196°C)									
Normal (E51-10F)	1×10^2 (100)[c]	1×10^2 (100)	1×10^2 (100)	3.3×10^3 (3)	9.2×10^3 (1)	1×10^2 (100)	1×10^2 (100)	1×10^2 (100)	3×10^2 (30)	1×10^3 (10)
Ataxia (BB91-3-1)	3×10^3 (100)	9.2×10^3 (30)	2.7×10^4 (10)	8.3×10^4 (3)	$>2.5 \times 10^5$ (<1)	3×10^3 (100)	3×10^3 (100)	2.7×10^4 (10)	2.5×10^5 (1)	2.5×10^5 (<1)
	Liquid cultures									
Normal (E51-10F)	1×10^2 (100)	1×10^2 (100)	1×10^2 (100)	1×10^3 (10)	3×10^3 (3)	1×10^2 (100)	1×10^2 (100)	1×10^2 (100)	1×10^2 (100)	3×10^2 (30)
Ataxia (BB91-3-1)	1×10^3 (100)	3×10^3 (30)	2.7×10^4 (3)	2.5×10^5 (0.4)	$>2.5 \times 10^5$ (<0.4)	3.3×10^3 (100)	3.3×10^3 (100)	3.3×10^3 (100)	9.2×10^3 (30)	2.5×10^5 (1)

[a] Measured by cell growth in microtiter plates and expressed as minimum number of cells needed for colony formation.
[b] 10% dimethylsulfoxide.
[c] Percentage survival compared to that of unirradiated cells.

ygen are responsible for significant cell killing in AT and normal cells and it may be these lesions which AT cells are unable to recognize or respond to properly.

Inhibition of DNA synthesis by X-rays. The initial report by Edwards and Taylor (13) and the subsequent studies (14, 15, 38) showing that AT DNA synthesis was less sensitive to inhibition by gamma rays than was normal DNA synthesis prompted us to examine the effects of X-rays on DNA synthesis in normal and ataxia lymphoblasts. Table IV shows the rates at which X-rays inhibited [^{3}H]thymidine incorporation into normal and AT lymphoblasts. Initially, when measured 1 hr after X-ray treatment, there was less inhibition of DNA synthesis in ataxia lymphoblasts than in normal cells. However, at subsequent times the [^{3}H]thymidine pulses revealed inhibition of DNA synthesis in the X-irradiated ataxia LCLs. Significant is the relationship between X-ray dose and inhibition of DNA synthesis, determined by calculating the correlation coefficients. The correlation is nearly linear in both AT and normal cells (0.92 and 0.93, respectively) at 1 hr after X-irradiation. The loss of linearity at later times (48 hr), as indicated by coefficients of 0.69 and 0.66, may indicate that factors other than X-ray dose, such as cell growth or death, may contribute to these results.

Repair replication in LCLs. Experiments were undertaken which would both characterize DNA replication and quantitate the levels of DNa repair replication induced by X-irradiation in normal and ataxia LCLs as well as normal unstimulated PBL. Hydroxyurea was omitted from these experiments, to better analyze the effects of X-rays on DNA synthesis. In Fig. 2 are presented profiles of the first banding of phenol-extracted DNA on neutral cesium chloride gradients, DNA obtained from AT, normal LCLs, and normal nonstimulated PBL, following a protocol modified from Pettijohn and Hanawalt (39) classical experiments designed to measure DNA repair replication. Several facts emerge from these experiments. First, in our hands comparatively high doses of X-irradiation ($0.5–1.5 \times 10^5$ rad) are required to reduce semiconservative DNA replication in LCLs to a level which will allow induced levels of repair replication to be measured by this method

TABLE IV. INHIBITION OF LCL DNA SYNTHESIS BY X-RAYS AS MEASURED BY [^{3}H]THYMIDINE INCORPORATION

LCL treatment	1 hr after treatment		24 hr after treatment		48 hr after treatment	
	cpm[a]	% of control	cpm	% of control	cpm	% of control
Normal (C85-5)						
Control	24,700 ± 1500	1.00	38,200 ± 300	1.00	71,700 ± 13,200	1.00
100 rads	22,400 ± 4900	0.91	26,500 ± 8100	0.70	34,100 ± 8,200	0.48
200	20,700 ± 1400	0.84	24,900 ± 6600	0.65	26,400 ± 700	0.37
400	16,100 ± 9400	0.65	9,700 ± 1300	0.25	22,900 ± 5,200	0.32
800	13,400 ± 1000	0.54	8,100 ± 600	0.21	8,400 ± 2,500	0.12
		$r^{2\,b} = 0.93$		$r^2 = 0.80$		$r^2 = 0.66$
Ataxia (G25-1-3)						
Control	15,200 ± 1600	1.00	15,400 ± 2800	1.00	15,500 ± 3,100	1.00
100 rads	15,500 ± 1400	1.02	13,300 ± 2500	0.86	9,500 ± 30	0.61
200	13,500 ± 400	0.86	8,900 ± 900	0.58	7,500 ± 700	0.48
400	13,000 ± 200	0.86	6,800 ± 1400	0.44	5,700 ± 800	0.37
800	14,500 ± 5800	0.95	2,700 ± 30	0.18	4,200 ± 1,000	0.27
		$r^{2\,b} = 0.92$		$r^2 = 0.91$		$r^2 = 0.69$

[a] cpm ± standard deviation.
[b] Correlation coefficient determined by linear regression analysis.

without rebanding the DNA a second time in cesium chloride. Second, following a rebanding in cesium chloride the ataxia LCLs we have established following EBV-infection can be shown to be DNA excision repair proficient as measured by [^{3}H]thymidine:BrdUrd incorporation into unreplicated cellular DNA (Table V). In fact, following rebanding in cesium chloride, the specific activities of unreplicated DNA were consistently higher in the AT cells than in normals. The feature of initial increased resistance of what appears to be replicative DNA synthesis to X-irradiation observed in ataxia cells (Table IV) is indeed replicative DNa synthesis. This is evident in the profiles of cesium chloride gradients by the appearance of an increased amount of replicated hybrid (heavy–light) DNA in X-irradiated (at 0.5×10^5 rad) ataxia cultures. As expected, unstimulated PBL produced little hybrid-replicated DNA and had lower levels of DNA repair. Pulse chase experiments with [^{3}H]thymidine were carried out in LCLs following X-irradiation to determine the size of newly synthesized DNA in X-irradiated cells. As determined by sedimentation on 5–20% sucrose gradients, at higher irradiation doses (50 krad) low-molecular-weight DNA synthesis as well as chain elongation was enhanced in the AT LCLs when compared to that of normals (data not shown). These results are in agreement with those of Ford and Lavin (40).

Discussion. With the exception of xeroderma pigmentosum, ataxia telangiectasia (formerly also known as Louis–Barr syndrome) is perhaps the most intensely studied of the human genetic disorders predisposing towards cancer (for reviews see 12, 41). This is in large measure a result of the original findings of Taylor *et al.* (1) which demonstrated that cultured cells from AT patients were hypersensitive to gamma-irradiation as detected by decreased postirradiation colony formation, and which supported *in vivo* findings with AT patients. These experiments clearly demonstrated that following gamma-irradiation ataxia cells behaved similar to the way cultured xeroderma pigmentosum cells behaved after uv-irradiation. However, whereas demonstrating a deficiency in excision repair of un-damaged DNA in xeroderma cells (thus explaining the increased uv sensitivity) was possible, demonstrating a consistent molecular defect in ataxia cells (and thus explaining the hypersensitivity to ionizing radiation) has not been possible (12, 41). Although the exact enzymatic defect responsible for the observed phenotype presently remains elusive in both xeroderma and ataxia, a quantitative defect in ionizing radiation-induced DNA repair has been shown in some AT cell lines. This defect

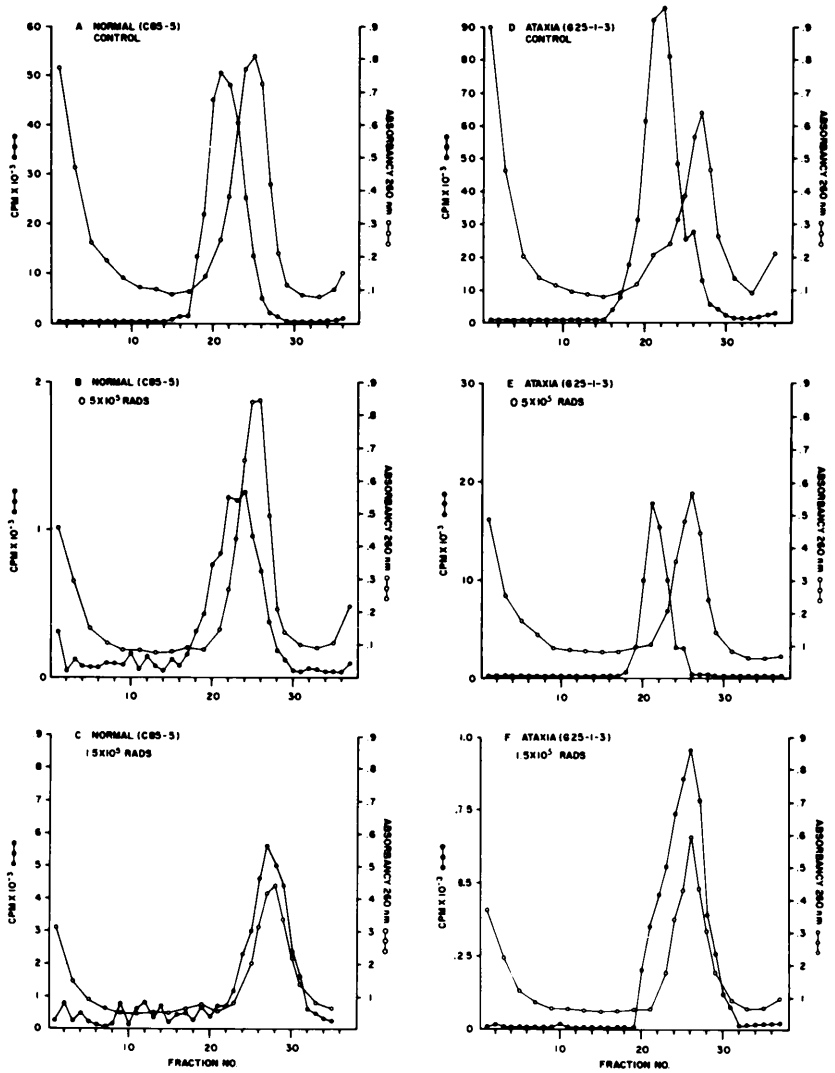

FIG. 2. (A and B) Graphs of neutral cesium chloride gradients of DNA extracted from control or X-irradiated LCLs or normal PBL. Approximately 2.5 × 10^7 exponentially growing lymphoblasts or unstimulated PBL were incubated with BrdUrd, 10 μg/ml, for 30 min, and then exposed to X-rays in plastic flasks at a dose rate of 5000 rad/min. Immediately after irradiation, [^{3}H]thymidine (49 Ci/m*M*, 25 μCi/ml) was added and the cultures were incubated for 4 hr at 37°C. Following cell harvest, DNA was extracted, banded and collected as described (31). Symbols: (○), profiles of absorbancy at 260 nm; (●), profiles of radioactivity (^{3}H). Increasing density from right to left.

has not been found in all AT cell lines having a similar increased sensitivity to ionizing radiation.

Ionizing radiation is both ubiquitous and carcinogenic in humans. It is therefore not surprising that patients with AT have an increased incidence of cancer. The finding that most of the malignancies seen in AT patients involve the lymphoreticular system has implications as to which cell type is the most representative type to study in ataxia in terms of malignant transformation.

Although lymphoblasts are being increasingly used, most studies up to this point have been done with fibroblasts. There has been recent interest in the disease ataxia telangiectasia from the viewpoint of the consequences of the immune deficiencies asso-

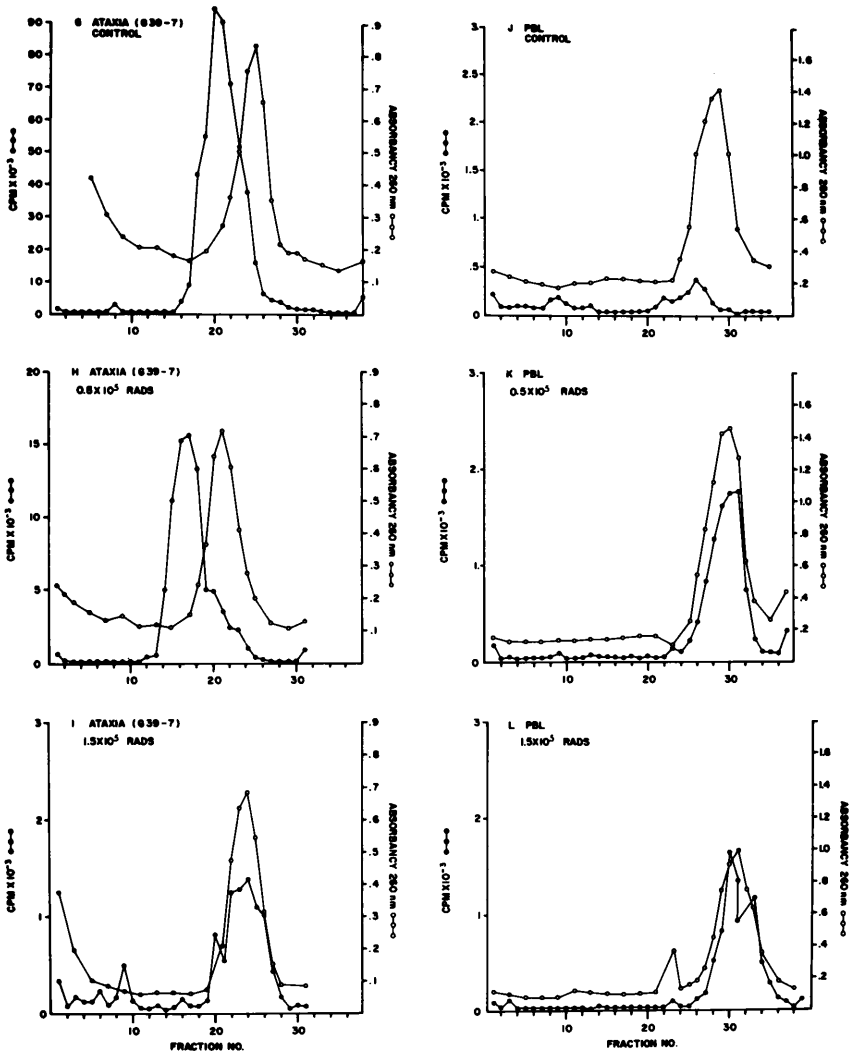

FIG. 2—*Continued.*

ciated with AT resulting in increased susceptibility to EBV-induced lymphoblast proliferation (42). In this regard, we have observed that the PBL isolated from five ataxia patients, none of which had undergone previous cytotoxic drug therapy, transformed less efficiently than controls following the addition of exogenous EBV. The morphologically transformed AT colonies also required a longer period of growth than did normals before they could be transferred to subcultures. The reduced transformation seen with ataxia lymphocytes most likely represents a reduced propensity of ataxia PBL to undergo DNA replication or cell division requisite for transformation following EBV-infection. However, the situation in the peripheral blood may not be totally representative of that found in the bone marrow. For instance, in our laboratory we have been able to occasionally induce transformation with EBV using cells from the bone marrow of some disorders when transformation could not be induced from the peripheral blood. In one experiment, an unsuccessful attempt was made to influence the transformation efficiency of ataxia PBL by exposing the AT leukocytes to low levels of X-irradiation (25–100 rad) before EBV infection. Following 25 rad of X-irradiation, the EBV-infected AT cells failed to transform,

TABLE V. X-RAY-INDUCED REPAIR REPLICATION IN LCLs ESTABLISHED FROM NORMAL ADULTS AND FROM AT PATIENTS[a]

Source of cells	Treatment[b]	cpm/μg DNA[c]
Normal LCL (C91-3)	(1) None	27
	(2) 50 krad	81
	(3) 150 krad	93
Ataxia LCL (BB91-3-1)	(1) None	35
	(2) 50 krad	164
	(3) 150 krad	132
Normal adult PBL (48-hr unstimulated)	(1) None	14
	(2) 50 krad	42
	(3) 150 krad	64

[a] Classic repair replication as measured by [^{3}H]thymidine:5-bromodeoxyuridine incorporation into rebanded nonreplicated DNA.

[b] Following mock or X-ray treatment, cultures were pulsed with 5-bromodeoxyuridine 33 μ*M*:[^{3}H]thymidine 25 μCi/ml, 49 Ci/m*M* for 3 hr.

[c] Calculated for five, peak, normal density fractions of cesium chloride rebands.

whereas the control unirradiated EBV-infected AT cells did transform. It may be that even these small doses of X-rays were cytotoxic to the ataxia lymphocytes, and thus prevented them from dividing after EBV infection. Therefore our results suggest that AT lymphocytes, which are within the subset of cells most likely to lead to transformation *in vivo,* are not inherently more transformable by EBV yet, once transformed, retain the phenotype characterizing AT cells.

There are reports that radiation-sensitive AT cell lines can be identified that are also excision repair deficient as measured by unscheduled DNA synthesis or BrdUrd incorporation into unreplicated DNA (11, 12). Other cell lines from ataxia, which are equally sensitive to killing by radiation, have been shown by the same methods to be excision repair proficient (38, 41). Our LCLs established from four AT patients also appear to be excision repair proficient as measured by [^{3}H]thymidine:BrdUrd incorporation in unreplicated DNA (Table V), although our methodology may not be sensitive enough to allow us to detect small defects in repair replication which may be of significance. This brings to light the question of the significance of the deficiency in the excision repair pathway seen in ataxia cell lines as it relates to the cellular phenotype presently accepted as being that which characterizes AT. Unfortunately, it has not been possible to obtain AT PBL in sufficient numbers to carry out extensive DNA repair replication experiments.

Recent results demonstrating gamma-ray- and bleomycin-resistant DNA synthesis in ataxia cell lines (13–16), as well as the studies showing lower levels of poly (ADP) ribosylation in ataxia cells when compared to normals after gamma-irradiation (13), raise the question that there is possibly a lesion in ataxia other than in enzymes involved directly in DNA repair. Thus, it has been proposed that in ataxia the defect may reside in DNA replication, or chromosomal structure and/or function. Our results showing that ataxia lymphocytes have lower than normal transformation frequencies following exposure to exogenous EBV and that the resulting ataxia LCLs, while being X-ray sensitive, are both X-ray excision repair proficient and capable of normal host cell reactivation of X-irradiated herpes simplex virus (43), support the concept that the defect in ataxia resides in mechanisms other than DNA repair.

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