

INDUCTION AND PERSISTENCE OF CHROMOSOME ABERRATIONS IN HUMAN LYMPHOCYTES EXPOSED TO NEUTRONS *IN VITRO* OR *IN VIVO*: IMPLICATIONS OF FINDINGS IN 'RETROSPECTIVE' BIOLOGICAL DOSIMETRY

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Abstract — The induction and persistence were evaluated of chromosome aberrations in lymphocytes exposed *in vitro* to highly efficient 1 MeV monoenergetic neutrons and in patients who received fast neutrons as therapy for tumours. For the *in vitro* studies, lymphocytes were exposed to various doses of neutrons and cultured for one or 20 cell cycles. Aberrations were quantified in painted chromosome pairs 1, 2, or 4. These 1 MeV neutrons were highly efficient in inducing aberrations, and dicentrics as well as one-way and two-way translocations increased as a linear function of dose. About 30% of the aberrant metaphases displayed complex aberrations. After multiple *in vitro* cell divisions, virtually all asymmetrical aberrations had been eliminated from the cell population, and the frequency of one-way translocations was reduced dramatically. In contrast, most two-way translocations apparently survived through multiple cell divisions and still displayed excellent correlation with dose after 20 cell cycles. Classical methods were used to evaluate persistence of aberrations in patients who received fractionated neutron therapy to tumours located in many different sites. Neutron induced dicentrics and rings disappeared from the peripheral circulation within the first three years after exposure, while translocations persisted for more than 17 y. However, considerable variability in numbers of aberrations were observed between patients who had received similar 'average bone marrow doses'. Results of these studies are discussed in relation to the possible use of translocations as retrospective doseimeters in persons exposed to radiation many years ago.

INTRODUCTION

Historically, cytogenetic biodosimetry assays have been based on quantifying asymmetrical aberrations (dicentrics, centric rings and acentric fragments) in mitogen-stimulated T-lymphocytes in their first mitosis after radiation exposure. To obtain accurate estimates of dose, it has been necessary to obtain blood samples for cytogenetic study within a few weeks after exposure, since T-cells have a finite lifespan in the peripheral circulation and because asymmetrical aberrations are lethal during cell division which eliminates aberration bearing cells from the stem cell pool.

Symmetrical chromosome aberrations are more 'stable' in proliferating cell populations than their asymmetrical counterparts, and it has been known since the early 1960s that these types of aberrations persist in the human lymphoid cell compartment⁽¹⁾ and show positive correlations with 'dose' many years after radiation exposure (e.g. References 2–4). However, classical techniques for identifying and enumerating symmetrical aberrations are tedious and require a great deal of skill and experience and these methods have not been applied routinely by the radiation cytogenetics community. With the advent of

fluorescence *in situ* hybridisation (FISH) methodology and the widespread availability of commercial whole-chromosome painting probes, interest in symmetrical aberrations and their potential use in 'retrospective' dosimetry has been rekindled.

Our laboratory in Oak Ridge is one of several that have used FISH methods to quantify stable aberrations such as translocations in persons having radiation exposures in the recent or distant past (e.g. References 5–12) and to evaluate the persistence of aberrations in lymphocytes exposed to low LET radiation *in vitro*^(13–15). Recently, two studies were completed in which the induction and persistence were evaluated of aberrations in lymphocytes exposed *in vitro* to highly efficient 1 MeV monoenergetic neutrons and in patients who received fast MeV neutrons as fractionated, localised therapy for tumours. In this report details are presented of our findings regarding persistence of aberrations in human lymphocytes exposed to neutrons *in vitro* or *in vivo*. These data and earlier published results from our laboratory are then utilised to address several issues related to the possible use of translocations as retrospective biodoseimeters in persons exposed to radiation many years ago.

IN VITRO STUDIES TO EVALUATE ABERRATION PERSISTENCE IN LYMPHOCYTES EXPOSED TO A MeV NEUTRONS

IN VIVO STUDIES TO EVALUATE ABERRATION PERSISTENCE IN LYMPHOCYTES OF PATIENTS WHO RECEIVED RADIATION THERAPY WITH FAST NEUTRONS

Radiation exposures

Heparinised blood from an adult woman was suspended in RPMI 1640 culture medium and purified mononuclear cells were collected using density gradient centrifugation. Lymphocytes were resuspended in medium supplemented with autologous plasma (15%), dispensed into six centrifuge tubes, pelleted to a cell volume of less than 0.5 ml, and exposed to 0, 0.05, 0.1, 0.2, or 0.4 Gy monoenergetic 1 MeV neutrons (dose rate 1.75 Gy.h⁻¹) at the Radiobiological Research Facility at Columbia University.

Culture methods

Within 24 h cells were transported to laboratories in Oak Ridge, TN and Burlington, VT. Our standard methods were used to establish and harvest short-term (48 h) lymphocyte cultures⁽¹⁶⁾. To establish long-term cultures, irradiated and non-irradiated lymphocytes were dispensed into cell culture multiwell dishes in 2 ml RPMI containing 20% nutrient medium HL1, 5% supplemented bovine calf serum and 1 µg.ml⁻¹ PHA (HA17). Cells were incubated to allow adherence of B cells and monocytes to the surface of the dishes and mitogenic activation of the T cells. Non-adherent cells consisted of ~99% T lymphocytes. After 48 h incubation the cells were put into long-term culture in medium containing interleukin II and lethally irradiated lymphoblastoid cells as described previously⁽¹⁷⁾. Cells were incubated at 37°C in an atmosphere of 5% CO₂ for 48 h, after which the lymphocytes were subcultured by dispensing 4 × 10⁵ lymphocytes (1 ml) into individual wells containing 4 ml medium (12 well plate). The population doubling time for lymphocytes cultured under these conditions is approximately 22 h, thus they undergo doubling every day. Irradiated and non-irradiated lymphocytes were subcultured every 2–3 days and maintained in continuous culture for 20 days before harvest for cytogenetic evaluation. Harvest methods were similar to those used for the 48 h cultures.

Slides were prepared for FISH with whole chromosome paints for chromosome pairs 1, 2, and 4⁽¹⁶⁾ and scored for aberrations as described⁽¹¹⁾. Chromosome pairs 1, 2, and 4 represent 22.34% of the DNA content in the female genome (see Reference 15) and this combination of painted chromosomes detects 34.7% of the chromosomal exchanges occurring in the complete genome as estimated by the method described by Lucas *et al*⁽⁵⁾.

Patient treatment protocols

As part of a pilot epidemiological study to evaluate late effects in patients who received neutron therapy, cytogenetic evaluations were performed on cultured lymphocytes from tumour patients who received radiotherapy with fast neutrons for cancers of the head and neck, thorax, or pelvis. Between 1989 and 1991, blood samples were obtained from 13 patients on the day of their last treatment and on at least two occasions after therapy was completed. Neutron therapy or neutrons in combination with conventional therapy (i.e. 'mixed beam') was administered at the M.D. Anderson Cancer Center. Neutrons were generated by bombarding a beryllium target with 42 mV protons. Typically, patients received neutron tumour doses of 20.4 Gy in 12 fractions of 1.7 Gy per fraction, generally administered 3 days per week. When mixed beam therapy was included, patients received both neutron and conventional therapy on the same day. Average bone marrow doses were calculated as previously described⁽¹⁸⁾. In addition, single blood samples were obtained from 33 cancer patients who had been treated with 42 or 50 mV neutron therapy 1.4 y to 17.5 y previously (1973–1991).

Lymphocyte cultures were initiated to evaluate radiation-induced chromosome aberrations (see Reference 4 for details of culture methods). From the 13 patients who had just completed therapy, 200 first division metaphases were scored from each culture to determine the frequency and cellular distributions of asymmetrical aberrations at various time intervals after exposure. In post-treatment cultures from the 33 patients, asymmetrical aberrations were scored and classical 'group analysis' techniques^(3,4) were used to quantify the frequencies of translocations that persisted for up to 17 years after exposure. In all cultures, translocations were scored only in those metaphases that did not display asymmetrical aberrations.

RESULTS

In vitro studies

Table 1 displays our findings of the frequencies of chromosome aberrations observed in lymphocytes that had undergone either one or approximately 20 *in vitro* cell divisions, respectively, after irradiation with 1 MeV monoenergetic neutrons. When compared to expected frequencies based on DNA content, under-representation of chromosome 2 and over-representation of chromosome 4 was observed in both sets of preparations, but deviations from expected frequencies were relatively small (data not shown). Thus, results are

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presented as calculated percentages of aberrations in the total genome. In metaphases that had completed one cell division post-exposure (Table 1(a)) the frequencies of aberrant metaphases ranged from 14.9 to 80.2% in lymphocytes exposed to 0.05 to 0.4 Gy. At each dose, approximately one third of the aberrant metaphases contained complex aberrations (i.e. 3 or more breaks in 2 or more chromosomes). Dicentrics as well as all classes of translocations increased as a linear function of dose. The total frequencies of translocations (two-way + one-way) exceeded the frequency of dicentrics at each dose. On average, more than 40% of the observed translocations were one-way and often these were found in 'complex metaphases' that had other aberrations as well.

In general, excellent preparations were obtained from T lymphocytes that had been stimulated to undergo multiple *in vitro* cell divisions using recombinant interleukins and other growth factors. All cultures exhibited an elevated frequency of chromatid gaps and breaks (~10%, data not shown). Since chromatid breaks and gaps were observed in non-irradiated control cells and their frequency showed no correlation with neutron dose, it appears that these aberrations are not manifestations of neutron-induced genomic instability. It is speculated that the increased expression of chromatid aberrations may have resulted from rapid cycling of lymphocytes in conditioned medium or other unknown factors related to the cell culture method. In these cultures which had completed approximately 20 *in vitro* cell divisions, the frequency of aberrant metaphases carrying typical radiation-induced chromosome aberrations

had decreased dramatically and the proportion of metaphases with asymmetrical aberrations such as dicentrics, centric rings and acentrics had dropped precipitously (Table 1(b)). Also, the proportion of metaphases with one-way translocations and the proportion with complex aberrations had decreased dramatically at all doses. On the other hand, the proportion of cells with two-way translocations was only marginally decreased compared to the frequency observed after one cell division.

For comparison of the dose-response relationships for different types of aberrations in lymphocytes that had completed one or multiple cell divisions after exposure to neutrons, linear regression curve fittings were performed using iteratively reweighted least squares methods. Dose-response coefficients for dicentrics, total, one-way and two-way translocations are expressed per 100 genome equivalents per Gy (Table 2).

In vivo data

The frequencies of dicentrics and centric rings observed in cultured lymphocytes from 13 cancer patients who received tumour therapy with fast neutrons are shown in Table 3. The average bone marrow doses of neutrons in these patients ranged from 0.6 to 3.43 Gy, while total average marrow doses (including photon dose) ranged from 0.86 to 4.1 Gy. All patients received localised therapy to sites in the thorax or head and neck. At the end of their course of therapy the observed percentage of first division metaphases with one or more dicentric chromosomes varied from 23% to

Table 1. Aberrations in lymphocytes that completed one or 20 *in vitro* cell divisions after exposure to 1 MeV neutrons^(a).

Dose (Gy)	Painted metaphases	Genome equivalent	Aberrant cells	Complex cells (%)	Total dicent.	Total trans.	Two-way trans. ^(b)	One-way trans. ^(b)	Insertions	Centric rings	Acentrics ^(c) (del)
(a) One cell division											
0	1600	555	0.5	0	0	0.18	0	0.18	0	0	0
0.05	500	174	14.9	26.9	5.7	8.1	4.6	3.5	0.6	1.7	0
0.1	500	174	23.0	30.0	9.2	16.7	12.1	4.6	0.6	0.6	5.7
0.2	725	252	49.2	31.5	27.4	27.8	16.7	11.1	4.8	3.6	12.7
0.4	725	252	80.2	36.6	36.1	46.6	27.0	22.6	8.3	11.1	22.2
(b) Twenty cell divisions											
0	1600	555	1.6	11.1	0.4	1.6	0.7	0.9	0	0	0.5 (0.4)
0.05	500	174	5.2	11.1	0	2.3	2.3	0	0.3	0	0.6 (2.3)
0.1	500	174	6.3	0	0.6	5.2	4.0	1.2	0	0	0.0 (0.6)
0.2	925	321	18.1	10.3	0.3	16.5	14.0	2.5	0.6	0	2.8 (1.2)
0.4	325	113	28.3	3.1	0	29.2	20.4	8.8	0	0	1.8 (0.9)

^(a)The formula of Lucas *et al.*⁽¹⁵⁾ was used to estimate genome equivalents, aberration frequencies expressed as number per 100 genome equivalents.

^(b)Two-way translocations are apparently reciprocal, 'complete' translocations, one-way translocations are apparently non-reciprocal, 'incomplete' translocations, total = sum of two-way + one-way translocations.

^(c)Acentrics include 'excess' fragments and acentric rings; deletions of portions of arms of painted chromosomes were observed in chromosome preparations after lymphocytes had divided multiple times in culture.

Table 2. Comparison of slope coefficients for translocations observed in lymphocytes that had completed one or 20 cell divisions after exposure to 1 MeV neutrons.

Translocation type	Intercept/100 cells	Slope/100 cells/Gy*
Total (one cell division)**	0.02 ± 0.20	133.4 ± 8.6
Total (20 cell divisions)	1.38 ± 0.63	67.7 ± 10.7
Two-way (one cell division)	0.00	78.7 ± 9.2
Two-way (20 cell divisions)	0.66 ± 0.37	55.0 ± 7.5
One-way (one cell division)	0.17 ± 0.17	55.0 ± 5.8
One-way (20 cell divisions)	0.63 ± 0.52	12.1 ± 5.2
Dicentrics (one cell division)	0.00	105.2 ± 12.1

*Coefficients expressed per 100 genome equivalents per Gy.

**Total translocations = one-way + two-way.

Table 3. Persistence of cells with asymmetrical aberrations after neutron therapy in 13 patients.

NCI ID	Average marrow dose (Gy)			Cells	Dicent.	c. ring	Cells with dicentrics		Months after therapy
	Photon	Neutron	Total				number	(%)	
400	0.29	2.30	2.59	200	199	42	92	46	0
				200	190	39	100	50	3.5
				200	201	38	96	48	8.5
403	0.27	2.07	2.34	200	234	52	115	58	0.0
				200	227	57	120	60	3.5
407	0.16	0.70	0.86	200	91	23	64	32	0.0
				200	80	24	56	28	3.5
				200	78	14	58	29	7.3
413	0.25	2.13	2.38	200	212	44	104	52	0.0
				200	196	29	89	45	1.4
418	0.24	1.77	2.01	200	63	11	45	23	0.0
				200	83	6	38	19	2.9
414	0.25	1.90	2.15	200	268	47	92	46	0.0
				200	154	30	79	40	6.3
409	2.10	2.00	4.10	200	267	42	132	66	0.0
				200	227	55	100	50	4.0
				200	229	41	106	53	6.3
412	0.34	3.43	3.77	200	149	26	86	43	0.0
				200	167	31	98	49	3.9
416	2.50	0.83	3.33	200	230	32	90	45	0.0
				200	293	40	95	48	3.9
				200	190	39	67	34	8.1
401	0.72	0.60	1.32	200	114	25	64	32	0.0
				200	186	35	94	47	3.1
				200	148	27	78	39	6.1
				200	97	18	49	25	11.5
404	3.10	0.73	3.83	200	153	21	64	32	0.0
				200	183	38	85	43	2.5
410	2.30	0.43	2.73	200	127	16	64	32	0.0
				200	189	37	79	40	4.2
				200	158	32	68	34	7.2
				200	140	8	60	30	14.6
415	2.80	0.53	3.33	200	143	16	71	36	0.0
				200	130	24	67	34	3.3

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66%. The cellular distributions of dicentrics were highly overdispersed relative to the Poisson distribution (data not shown) and many aberrant metaphases carried multiple asymmetrical aberrations. One to three follow-up blood samples were obtained from these patients to evaluate the rate of loss of lymphocytes with dicentrics during the first year after therapy. In most patients, the frequency of lymphocytes with dicentrics had not decreased dramatically during time intervals up to 14.6 months after their exposure.

A single blood sample was obtained for evaluation of unstable and stable chromosome aberrations in 33 other tumour patients who had received similar courses of neutron therapy 1.4–17.5 years earlier. These patients received average bone marrow doses ranging from less than 1 to greater than 10 Gy. During the first 1–3 years after therapy, high frequencies of asymmetrical aberrations were observed in lymphocytes (Table 4). However, regardless of their total marrow doses, the pro-

portion of metaphases with dicentrics had decreased sharply by ~3–4 years after completion of therapy.

The frequencies of 'stable' aberrations (i.e. abnormal monocentrics including translocations and inversions) in cells without asymmetrical aberrations varied from <1 to 14.5% in preparations from the 33 radiation therapy patients, and in contrast to the observed abrupt decline in dicentrics, the range of cells with stable aberrations remained relatively constant throughout the 17 y period after therapy. When comparisons are made between patients, the numbers of translocations show very little correlation with average bone marrow dose (Table 4 and Figure 1).

DISCUSSION

The ease of obtaining blood and culturing lymphocytes is a great advantage in the use of this cell type as a biomarker in estimating recent radiation dose. However,

Table 4. Persistence of aberrations in 33 patients who received mixed beam or neutron therapy for tumours.

NCI	Cells	Dicent.	Rings	Total	per 100	Trans*	per 100	Gy**	Post therapy (y)	Rx site
73	200	59	13	72	36	16	8	4.91	1.4	prostate
65	200	29	2	31	15.5	12	6	7.44	1.4	lung
64	200	86	13	99	49.5	22	11	4.78	1.5	prostate
67	200	176	37	213	106.5	20	10	6.50	1.5	lung
63	200	124	22	146	73	15	7.5	4.28	1.7	prostate
206	200	52	16	68	34	9	4.5	3.76	1.9	prostate
312	200	105	23	128	64	15	7.5	3.15	2	prostate
71	200	22	7	29	14.5	11	5.5	1.12	2.1	tonsil
59	200	29	10	39	19.5	29	14.5	3.35	2.1	lung
293	200	39	5	44	22	15	7.5	4.07	2.1	soft tissue
297	200	19	9	28	14	10	5	5.54	2.2	prostate
311	200	44	9	53	26.5	20	10	2.99	2.4	cervix
290	200	15	3	18	9	5	2.5	2.49	2.6	lung
68	200	36	7	43	21.5	4	2	4.95	2.7	tonsil
245	200	4	2	6	3	4	2	0.16	3.4	cervix
60	200	21	3	24	12	9	4.5	3.59	3.7	limb
154	200	24	8	32	16	9	4.5	2.54	5.9	mouth
49	200	4	2	6	3	4	2	1.29	8.8	prostate
46	200	7	2	9	4.5	23	11.5	9.08	9.5	thigh
47	200	4	1	5	2.5	17	8.5	1.86	9.6	cervix
41	200	10	1	11	5.5	8	4	9.19	10.3	parotid
28	200	5	1	6	3	10	5	7.19	10.5	cervix
40	200	6	0	6	3	7	3.5	4.69	10.6	prostate
48	200	3	3	6	3	13	6.5	7.35	10.6	chest
35	200	5	2	7	3.5	14	7	7.97	10.7	prostate
26	200	3	0	3	1.5	4	2	1.62	11.2	pelvis
50	200	2	0	2	1	4	2	0.15	11.8	face
22	200	5	1	6	3	16	8	9.58	11.9	thigh
93	200	2	0	2	1	5	2.5	11.28	12.5	cervix
103	200	0	0	0	0	1	0.5	8.09	13.1	cervix
3	200	1	2	3	1.5	13	6.5	3.30	16.2	cervix
4	200	0	0	0	0	12	6	6.07	16.4	breast
6	200	7	1	8	4	29	14.5	10.22	17.5	pelvis
										cervix

*Translocations scored in metaphases without asymmetrical aberrations.

**Average bone marrow dose.

several variables may introduce complications in the use of stable aberrations in lymphocytes as 'retrospective' dosimeters. Consensus agreement needs to be reached on several technical issues such as what chromosomes should be painted and what aberrations should be scored. Perhaps more importantly, more information is needed on several biological variables that may have significant impact on interpretation of FISH data in relation to making biological dose estimates. For example, more data are needed on the range and variability in baseline frequencies of translocations among control subjects of different age, genetic background and from different locales. Lastly, much more information is needed on factors affecting 'persistence' of radiation induced aberrations with time after exposure. In the following paragraphs data from the ORISE laboratory are summarised that pertain to these important issues with emphasis on our current studies of persistence of aberrations in lymphocytes exposed to neutrons.

What chromosomes should be painted?

In our studies of radiation induced chromosome aberrations in lymphocytes (References 11, 12, 15, and current data) FISH has typically been performed using direct-label probes for chromosome pairs 1, 2 and 4 which cover approximately 23% of the human genome. All whole chromosome paints were conjugated with SpectrumOrange or SpectrumGreen fluorophores (Vysis, Downer's Grove, IL) and metaphases were counterstained with 4',6-diamidino-2-phenylindole (DAPI) to allow ready visualisation of chromosome centromeres. In several studies the frequencies of aberrations observed in painted chromosome pairs were compared

with the expected numbers based on DNA content of chromosomes. Typically, a slight over-representation was observed of breaks in chromosome 4 and a slight under-representation in chromosome 2; however, deviations from randomness have been minor. Estimates of total genomic frequencies of dicentrics calculated by the method of Lucas *et al*⁽⁵⁾ have yielded generally good agreement with frequencies observed using classical scoring methods.

What aberrations should be scored?

When considering the use of FISH in retrospective dosimetry, the flagship aberration is the translocation, since some symmetrical types of translocations can apparently persist in the progenitor lymphocyte pool for years after radiation exposure. When quantified using FISH, translocations may appear to be reciprocal (i.e. 'complete' or 'two-way') or non-reciprocal (i.e. 'incomplete' or 'one-way') exchange events. The PAINT system of nomenclature⁽¹⁹⁾ has been consistently used as a short-hand notation to precisely describe each aberration observed, but both one-way and two-way translocations have been counted as single 'events' which allows direct comparison of paint dose response data with data from classical scoring.

What are the control levels of translocations?

Data have been accumulated on translocations involving painted chromosome pairs 1, 2 and 4 in early harvest lymphocyte cultures from 50 control men and 3 women ranging in age from 28–55 years. Portions of these data have been published⁽¹¹⁾. For each culture, 1600 painted

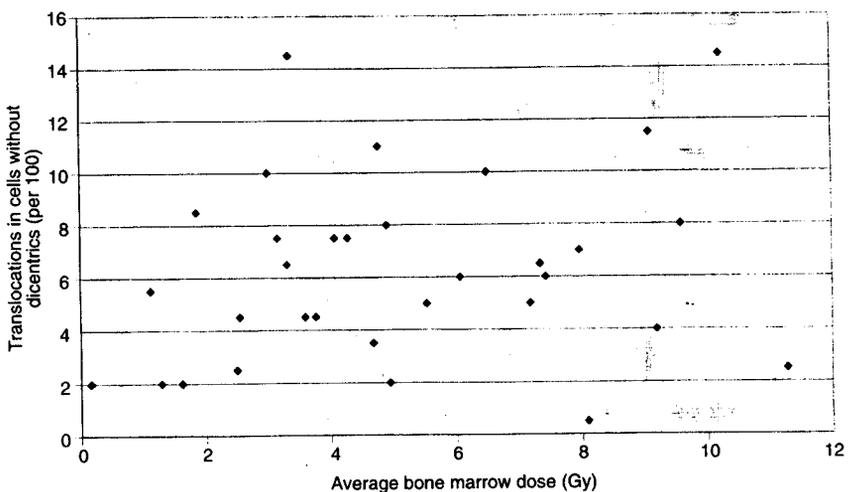


Figure 1. Translocations observed in metaphases without asymmetrical aberrations in cultured lymphocytes of 33 cancer patients. Blood samples were obtained 1.4–17.5 y after they received fractionated doses of fast neutrons to several different localised sites. Observed translocation frequencies are quite variable among patients having similar 'average bone marrow doses'.

metaphases were scored from each subject. For comparison with other data sets derived from painting different pairs of chromosomes, the observed frequencies of translocations were converted to numbers in the total genome. The mean frequency of translocations in ORISE control subjects is 7 / 1000 genome equivalents, about two-thirds of these were apparently reciprocal, i.e. two-way events. The baseline frequency of translocations varied widely among individual control subjects, and ranged from 0 to 23 translocations per 1000 genome equivalents. All subjects completed detailed work history and medical questionnaires at the time blood was drawn. No exposure or life-style variables that could account for the extreme variability observed could be identified, although translocations tended to increase with increasing age, and slightly higher average mean frequencies were observed among current smokers. Our data suggest that the sensitivity of FISH techniques in detecting low levels of exposure will be severely compromised as a result of the high mean frequency and wide variability in translocations observed among controls.

Do aberrations persist with time after exposure?

Regarding aberration persistence, it is possible that differing types of symmetrical aberrations will have differential rates of survival during mitotic cell division. Thus, it is imperative to understand which classes of 'stable' aberrations are transmitted to daughter cells during mitotic segregation and whether dose-response relationships remain intact over time.

As described in detail in this report, FISH methods were used to quantify aberrations in human lymphocytes exposed to several doses of 1 MeV neutrons to gain additional information on the induction and persistence of specific classes of radiation-induced chromosome aberrations during multiple mitotic cell divisions. Aberrations involving painted chromosomes 1, 2, and 4 were scored in cell populations that had completed one or approximately 20 *in vitro* cell cycles after exposure. These neutrons were highly efficient in inducing chromosome damage as has been observed using classical cytogenetic methods (e.g. Reference 20).

Several of our current findings using FISH to evaluate neutron-induced chromosome aberrations are in agreement with our earlier observations in lymphocytes exposed to 220 kV_p X rays (see Reference 15 for experimental results and review of related literature). For example, estimates of dicentrics induced in the total genome based on FISH data were slightly lower than estimates obtained from scoring dicentrics from Giemsa stained slides from lymphocytes exposed to these 1 MeV neutrons. Also, the total frequencies of neutron-induced translocations (one-way + two-way) slightly exceeded the frequencies of neutron-induced dicentrics at all radiation doses. After exposures to X rays, the frequencies of one-way translocations and metaphases

with complex aberrations were observed to increase with increasing dose. In contrast, the proportion of metaphases with one-way translocations, as well as complex aberrations, was observed to be relatively constant across all neutron doses.

Comparisons of data from lymphocyte populations in their first and 20th cycle demonstrated that asymmetrical aberrations (dicentrics, rings, acentrics, and insertions) had been virtually eliminated from the surviving population of irradiated cells after multiple cell divisions. Likewise, on average, more than two thirds of the cells with complex aberrations had disappeared. On the other hand, there was a differential survival of cells with differing types of radiation-induced translocations. Most of the one-way translocations had disappeared from the proliferating lymphocyte population, whereas the majority of the apparently reciprocal (i.e. two-way) translocations apparently survived, and showed excellent correlation with dose after 20 cell divisions (Tables 1(b) and 2). There was no evidence of clones of cells bearing identical chromosome aberrations involving the painted chromosome pairs. Although other interpretations are possible, our findings indicate that certain classes of neutron-induced translocations can be propagated through multiple mitotic cell divisions and that dose response relationships are maintained.

Since aberrations were evaluated only after 1 and 20 cell cycles there is no information on the rate of elimination of asymmetrical aberrations and one-way translocations after exposure to neutrons. In our earlier studies of aberration persistence in X irradiated lymphocytes that had completed 1, 2 or ≥ 3 *in vitro* cell divisions⁽¹⁵⁾ it was observed that dicentrics and fragments decline quite rapidly (~60% per cell generation) whereas translocations showed a more modest decline (13% per cell generation). Present data suggest that the decline in translocations may reach a plateau after multiple cell divisions possibly after selection has occurred for a population of translocation-bearing cells that suffers no mechanical impediment during mitotic segregation.

Thus, under conditions of growth factor-mediated T-cell proliferation *in vitro*, it appears that neutron-induced two-way translocations display amazing stability during multiple mitotic cell divisions. Our data further suggest that *in vitro* dose response functions for two-way translocations would be the most appropriate to use for retrospective dosimetry. However, in considering using stable aberrations to estimate dose many years after exposure, one must also consider the fate of lymphocytes bearing aberrations in the context of complex biological processes that may have an impact on 'aberration persistence' *in vivo*. If analysis of translocations in lymphocytes is to have utility for retrospective dosimetry in individuals, then it is of paramount importance that the population of lymphoid progenitors that sustains stable types of radiation-induced chromosome aberrations remains in a state of numerical 'equi-

librium' with aberration-free lymphoid precursors throughout the life-time of the exposed person.

To evaluate induction and persistence of neutron-induced chromosome aberrations in exposed persons, cytogenetic studies were performed in a group of patients who had received neutron therapy for various tumours. Data from lymphocyte cultures initiated on the last day of therapy in 13 patients demonstrated wide variation in the frequencies of lymphocytes with one or more dicentrics (i.e. 23–66%). Follow-up studies of these patients showed relatively little change in the proportion of their circulating lymphocytes with dicentric chromosomes during the first 1.4–14.5 months after therapy.

Evaluations of asymmetrical aberrations in cultured lymphocytes from 33 similarly treated patients 1.4 to 17 y after their neutron therapy demonstrated the vast majority of lymphocytes with dicentrics had disappeared from the peripheral pool by ~3–4 y in patients who had received widely different average bone marrow doses of neutrons. The disappearance rate of cells with dicentrics has been used by many others to estimate that the average 'life-span' of lymphocytes *in vivo* is in the range of <1–~3 years (for recent review see Reference 21). Biological factors that may affect lymphocyte 'life-span' are not understood. For example, it is not known whether these sub-populations of aberration bearing T-lymphocytes suffer apoptotic cell death, undergo clonal expansion as a result of antigen stimulation (which would eliminate dicentric bearing cells from the population), or whether they suffer some other fate related to their immunological function. For whatever biological reason, these cells and their accompanying dicentrics disappear from the peripheral pool, and cannot be used as biomarkers of radiation exposure after a finite interval.

To gain information on the persistence of lymphocytes with 'stable' aberrations in these patients, classical 'group analysis' methods were used to quantify abnormal monocentric chromosomes including translocations and inversions. Using these scoring methods it is possible to detect upwards of 70–80% of the translocations that occur in the total genome^(22,23). Abnormal monocentric chromosomes were selectively scored in metaphases that did not contain asymmetrical aberrations since it is this population of aberrant stem-cells that would be most likely to survive mitotic proliferation *in vivo*. The numbers of translocations observed in preparations from these 33 patients varied from 1 to 14.5%, and the relative frequency and range of translocations did not decrease (or increase) in any time-dependent manner over a period of 17 y). Review of these data also shows that the frequencies of induced dicentrics and persistent translocations are quite variable among different patients who received similar average bone marrow doses.

Data from these patients demonstrate that peripheral lymphocytes bearing neutron-induced stable aberrations

'persist' in the peripheral lymphoid compartment for many years after exposure as has been observed in numerous other studies of exposed populations^(1–11). However, it is obvious that these persistent translocations have little utility as a biological dosimeter for estimating average bone marrow dose in individual patients. Of course, interpretation of dose response data from partial-body radiation many years after exposure is quite complex, since patients typically receive multiple, fractionated doses to highly localised sites. Depending on the size of the radiation port and region of the body irradiated, differing percentages of lymphocytes and stem cells would be irradiated in different patients. Also, as a result of the partial-body exposure, lymphocyte populations in all these individuals are mixtures of two Poisson distributions of exposed and non-exposed cells. Because translocations occur in cells that have asymmetrical aberrations as well as those that do not, the 'linkage' of asymmetrical and symmetrical aberrations would have the effect of reducing the frequency of cells with translocations at times distant from exposure.

Data from these neutron-treated patients rather dramatically demonstrate the problems that will be encountered when attempting to correlate translocation frequency with radiation 'dose' in patients who received partial-body radiotherapy. Similar confounding variables would be expected to have an effect on retrospective dose estimates in persons having non-homogeneous accidental exposures.

In spite of the obvious complexities in attempting to interpret dose–response data from patients having partial-body exposures to different tumour sites, these studies are useful in demonstrating that lymphocytes with stable aberrations persist for decades after exposure. Based on our *in vitro* neutron data, one would expect that the population of surviving cells would have been selected for those that contain reciprocal, or two-way translocations as has been observed in short-term follow-up studies of radiation accident survivors using FISH methodology⁽²⁴⁾. Recent reports from FISH studies in exposed persons have suggested that stable two-way translocations may remain relatively constant for periods up to 10 y in situations involving whole-body exposures⁽¹⁰⁾. While this may be true in some instances, it is likely that inter-individual differences in complex immunological processes and responses could have an impact on long-term survival of sub-populations of lymphocytes carrying radiation-induced aberrations. Additional sequential studies of all classes of translocations in other exposed persons are needed to clarify questions regarding the usefulness of translocations in individual retrospective dosimetry.

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