RADIATiON RESEARCH 139.40--46 (1994)

Chromosome Aberrations in Lymphocytes from Women Irradiated for Benign and Malignant Gynecological Disease

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Kleinerman, R. A., Littlefield, L. G., Tarone, R. E., Sayer, A. M., Cookfair, D. L., Wactawski-Wende, J., Inskip, P. D., Block, A. W., Ramesh, K. H. and Boice, J. D., Jr. Chromosome Damage in Lymphocytes from Women Irradiated for Benign and Malignant Gynecological Disease. *Radiat. Res.* 139, 4046(1994).

Excess leukemias have occurred after partial-body radiotherapy for cervical cancer and benign gynecological disease (BGD). However, the level of risk is nearly the same in both groups, about twofold, despite a tenfold difference in average dose to active bone marrow (8 Gy vs 0.7 Gy, respectively). High-dose cell killing has been postulated as one explanation for this apparent inconsistency. To examine whether chromosome aberration rates observed in lymphocytes many years after exposure might serve as population markers of cancer risk, blood samples were taken from 60 women treated for BGD (34 with radiation) and cytogenetic data compared with previous results from 96 women irradiated for cervical cancer. Remarkably, the rate of stable aberrations, which reflects nonlethal damage in surviving stem cells, was only slightly higher among the cancer patients. Thus the lower-dose regimens to treat benign disorders resulted in much higher aberration yields per unit dose than those for cervical cancer. Assuming that the fraction of cytogenetically aberrant stem cells that survive radiotherapy contributes to the leukemogenic process, these data are then consistent with the epidemiological observations of comparable overall leukemia risks seen in these two irradiated populations. Accordingly, for patient populations given partial-body radiotherapy, stable aberrations at a long time after exposure appear to serve as biomarkers of effective risk rather than as biomarkers of radiation dose received.

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INTRODUCTION

Radiation-induced chromosome aberrations of the symmetrical (i.e. stable) type may be detected in cultured lymphocytes several decades after persons received partial-body exposures, and within several irradiated cohorts of patients, stable aberration frequencies have a relationship with radiation dose (1,2). We previously postulated that these persistent chromosomal rearrangements in peripheral T lymphocytes may be useful as surrogate biomarkers that gauge the relative levels of genetic damage sustained by the surviving fraction of irradiated hematopoietic stem cells (3). Because it is these same stem cells that give rise to leukemia. and because chromosomal rearrangements are relevant to and play a role in leukemogenesis, we thought that the frequencies of radiation-induced chromosome aberrations observed many years after exposure would be correlated positively with the risk for radiation-induced leukemia in populations of exposed persons. Women irradiated for benign and malignant disease were studied because similar twofold risks of leukemia have been reported despite a difference of over tenfold in dose (4-8).

To determine whether the levels of persistent radiation-induced chromosome aberrations in lymphocytes correlate with leukemia risk in women who received localized radiotherapy to the pelvis. we compared aberration rates in women with benign gynecological disease (BGD) who were irradiated more than 40 years ago with data for cervical cancer patients exposed more than 20 years ago. Given the similar leukemia risks reported for the two cohorts, the proportion of lymphocytes with chromosome aberrations should be similar in both groups of women in spite of the fact that they received widely disparate average radiation doses to the bone marrow (0.7 Gy vs 8 Gy). Because radiation risks are

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related to dose, and to the extent that cytogenetic abnormalities are similarly related to dose. a correlation would be expected between risk and aberrations. However. for pelvic irradiation, risk does not appear to be related to dose in a simple linear manner. We considered it of interest to learn whether persistent cytogenetic aberrations are more related to dose or to risk. We report here on cytogenetic findings in 34 women irradiated for BGD and 26 nonirradiated comparison subjects. These data are compared with our earlier findings in lymphocyte cultures from 96 irradiated and 26 nonirradiated cervical cancer patients *(1).*

MATERIALS AND METHODS

Study Population

Women irradiated for benign gynecological disease or treated with other modalities for similar conditions between 1925–1960 at either of two medical centers located in the Northeast [Roswell Park Cancer Institute (RPCI) and Rhode Island Hospital] were eligible for study if they lived within driving distance of either of these two centers. Three hundred women (195 irradiated. 105 nonirradiated) were approached to participate in the study, and 121 (62%) irradiated and 57 (54%) nonirradiated subjects refused. Many subjects were elderly and hesitant to travel, so it was decided to send nurses to the subjects' homes to draw blood from qualifying subjects (i.e. women who had never been diagnosed with cancer, or received any chemotherapy or radiotherapy for other than BGD, or undergone any diagnostic radiation procedure other than dental or chest X rays). At the time of screening, 32 (16.4%) irradiated and 15 (14.3%) nonirradiated subjects were determined to be ineligible for the study. Between December 1987 and March 1990. blood samples were obtained from a total of 42 irradiated and 33 nonirradiated women. All women provided written consent.

The majority of the irradiated BGD patients were treated with only radium implants. The treatment typically consisted of radium capsules inserted into the uterine cavity or cervical canal for between 12 and 24 h *(4).* In addition to radium, six subjects (two from Rhode island and four from RPC1) also received external-beam irradiation which involved a single given dose of 6 Gy of orthovoltage X rays (200 kVp. 0.9 mm Cu HVL)

TABLE I
Selected Characteristics of BGD Patients

Characteristic	Radiation exposure Yes	Radiation exposure No
Number of subjects	34[a]	26[b]
Mean age at treatment	41	35
Mean time since treatment (years)	42	41
Mean age at venipuncture	83	76
Mean dose to active bone marrow (Gy)	0.7[c]	0
Range of dose	0.13–1.6	NA

[a]Thirty irradiated subjects had cultures initiated at ORAU; four irradiated subjects had cultures initiated at RPCI.
[b]Two subjects had cultures initiated at ORAU; 24 subjects had cultures initiated at RPCI.
[c]One irradiated subject had unknown dose.

TABLE II
Summary of Cytogenetic Findings in BGD Patients

Proportion of metaphases[a] with:	Radiation exposure Yes	Radiation exposure No
Stable aberrations[b]	2.06 (0.25)	0.74 (0.19) $P = 0.002$[c]
Translocations	1.51 (0.21)	0.59 (0.17) $P = 0.015$
Inversions	0.08 (0.03)	0.05 (0.03) $P = 0.85$
Deletions	0.60 (0.09)	0.12 (0.05) $P = 0.026$
Unstable aberrations[d]	0.66 (0.13)	0.30 (0.10) $P = 0.18$
Dicentrics	0.45 (0.09)	0.19 (0.08) $P = 0.25$
Fragments	0.26 (0.12)	0.09 (0.09) $P = 0.16$

[a]Mean percent of metaphases containing aberrations; standard error in parentheses.
[b]Includes translocations, inversions and chromosomes with deleted segments.
[c]All analyses adjusted for age at blood drawing.
[d]Includes dicentrics, rings and fragments.

RESULTS

BGD Subjects

We were able to score more than 10,000 metaphases from lymphocyte cultures from 34 irradiated and 26 nonirradiated subjects. Our success rate in culturing lymphocytes for these elderly women was less than we usually achieve in younger subjects, possibly due to changes in the composition of the mononuclear cell compartment of human peripheral blood and decreased responsiveness of T lymphocytes to the mitogen, PHA, which have been observed in aged subjects (22, 23). Nonetheless, lymphocyte cultures from blood samples from 60 of these older women did yield metaphase preparations having sufficient mitotic indices and chromosome morphology for detailed cytogenetic evaluations. For most of the cultures. fewer than 5% of lymphocytes had proceeded to their second metaphase by 47 to 48 h harvest time; thus the cytogenetic data were obtained predominantly from evaluations of first-division metaphases.

Basic descriptive data for each treatment group are presented in Table I. The irradiated subjects were older (41 years) at treatment compared with nonirradiated subjects (35 years), although the mean elapsed time from diagnosis to blood drawing was similar (42 years vs 41 years). Irradiated subjects were on average 83 years old at time of blood drawing and the nonirradiated subjects were younger, 76 years old.

Table 11 presents the mean proportion of metaphases bearing stable and unstable aberrations by treatment group with estimates of error adjusted for age at blood drawing. The proportion of cells with any stable aberrations per 100 cells in the irradiated group exceeded the proportion per 100 cells in the nonirradiated group (2.06 vs 0.74; $P = 0.002$). The majority of the stable aberrations observed in cultures from irradiated as well as nonirradiated BGD subjects were translocations. For cells with unstable aberrations, the proportion was greater in the irradiated group compared with the nonirradiated group, but the difference was not significant (0.66 vs 0.30; $P = 0.18$).

We observed duplicate aberrations (in each instance, two cells with apparently identical translocations) in cultures from four irradiated women. None were detected in nonirradiated subjects.

Comparison of BGD and Cervical Cancer Patients

Table III compares selected characteristics of the irradiated BGD arid cervical cancer patients. For these comparisons, we restricted data from the BGD patients to include only those 30 irradiated women whose lymphocytes were cultured at the ORAU laboratory, because culture methods were identical to those we had used previously for the cervical cancer patients. Although age at treatment was similar for the two study populations, almost twice as many years had elapsed since treatment for BGD (43 years vs 23 years), so that the subjects in the BGD cohort were older at the time of blood drawing (83 years vs 67 years). All but two BGD subjects from Rhode Island received radium implants only, whereas all of the cervical cancer patients received a combination of both radium implants and external-beam therapy. Doses averaged over the entire bone marrow were tenfold greater for cervical cancer patients and ranged from 1.3 to 17 Gy (average = 8 Gy) compared with doses ranging from 0.13 to 1.6 Gy (average = 0.7 Gy) for BGD patients. A twofold relative risk for leukemia was reported for both cohorts based on a simple yes/no radiation comparison, but the average excess relative risk per gray was greater for the BGD patients (1.66 vs 0.14).

The mean frequencies of lymphocyte metaphases bearing one or more types of stable chromosome aberrations were similar in the two groups of women, i.e., an average of 2.85

TABLE III
Comparison of Selected Characteristics of Irradiated BGD and Cervical Cancer Patients

Characteristic	Study population: BGD	Study population: Cervical cancer
Number of subjects	30[a]	96
Age at irradiation	41	44
Years since treatment	43	23
Age at blood drawing	83	67
Local radiation site	Pelvis	Pelvis
Average bone marrow dose and range (Gy)	0.74 (0.13–1.6)	8.1 (1.3–17)
Mean proportion of cells with stable aberrations	2.21 (0.29)	2.85 (0.24)
Relative risk (RR) for leukemia	2.00[b]	2.02[c]
Average excess RR per gray for leukemia	1.66[d]	0.14[e]

[a]Includes only those irradiated patients whose cultures were initiated at ORAU.

[b]Ref. (*5*), based on a simple yes/no radiation comparison.

[c]Ref. (*8*), based on a simple yes/no radiation comparison.

[d]Average excess RR per gray was calculated as RR − 1 divided by 0.6 Gy (which is the mean dose for the entire BGD population followed for leukemia risk, ref. *5*).

[e]Average excess RR per gray calculated as RR − 1 divided by 7.10 Gy (which is the mean dose for the entire cervical cancer population followed for leukemia risk, ref. *8*).

per hundred metaphases among the 96 women treated for cervical cancer and 2.21 in cultures from 30 women who received localized pelvic irradiation for benign disease (Table III). [n all cultures, we also quantified the numbers of stable aberrations in each metaphase that displayed one or more abnormal monocentric chromosomes. Since aberrations were identified using classical "group analysis" scoring methods *(1, 13–16)* rather than more precise banding techniques, we may have underascertained the numbers of symmetrical exchanges in cells carrying multiple aberrations. Nonetheless, these data are useful for making comparisons between the two irradiated cohorts. The cellular distributions of stable aberrations in 19,061 lymphocyte metaphases from the cervical cancer patients and 5,934 metaphases from the BGD patients are shown in Table IV. In women treated for BGD, 91% of those metaphases bearing stable aberrations had a single translocation, inversion or deletion compared to 79% in the cervical cancer patients. The cellular distributions of aberrations were overdispersed relative to Poisson expectations in both groups of women. Such overdispersion is observed typically in individuals who receive a highly localized exposure to a portion of the body because the dose distribution to lymphocytes and lymphocyte progenitors is not homogeneous. The greater degree of overdispersion observed in the pooled data set from the cervical cancer patients was as expected because the doses received by these women varied widely, and because some women received very large doses, i.e. >17 Gy. The total number of stable aberrations observed in the cervical cancer patients was slightly higher than in the BGD patients (i.e., 3.6 vs 2.4 per hundred), but the difference was small compared to the difference in their doses.

DISCUSSION

Radiation-induced stable chromosome aberrations in T lymphocytes are sensitive biomarkers of exposure in populations who received whole-body or partial-body exposures up to several decades ago *(1, 2, 25, 24, 25)*. Within groups of patients having similar types of exposure, aberration frequencies show positive dose–response relationships. However, frequencies of persistent aberrations do not necessarily show strict correlations with dose when data are compared between populations who received different types of exposure, even when lymphocytes are cultured at similar times after exposure and evaluated using identical scoring techniques. When we compared cytogenetic findings in cultured lymphocytes from cervical cancer patients (mean marrow dose of 8.1 Gy) with data from A-bomb survivors (mean marrow dose 1 Gy), it was apparent that higher frequencies of persistent stable aberrations resulted per unit dose from the acute whole-body radiation received by the A-bomb survivors than for the partial-body exposure received in multiple high-dose-rate fractions by the cervical cancer patients (2). We speculated that such differences in frequencies of persistent aberrations may result from differences in dose distribution (i.e., partial-body vs whole-body exposures). dose rate or by a dose wastage effect if extensive cell killing

TABLE IV
Observed Distributions of Stable Chromosome Aberrations in Pooled Data from Cultured Lymphocytes of 96 Women Who Received Localized Pelvic Radiation for Cervical Cancer and 30 Women Who Received Localized Pelvic Irradiation for Benign Gynecological Disease (BGD)

Cohort	Number of stable aberrations	Stable aberrations per 100 cells	Distribution of cells with *n* stable aberrations: 0	1	2	3	≥4
Cervical cancer	676	3.6	18,527	420	90	21	3
BGD	141	2.4	5,807	116	9	1	1

occurs in the radiation field. Since a lower leukemia risk has also been observed in cervical cancer patients compared to A-bomb survivors, it appeared that persistent chromosome aberrations in peripheral T lymphocytes many years after radiation exposure may show better correlations with ultimate risk for development of leukemia than with actual dose received.

To gain additional information on possible correlations between persistent chromosome aberrations in lymphoid cells and the risk of radiation-induced leukemia, we initiated studies to compare our findings in cervical cancer patients with data from a group of women who received localized radiotherapy to the same region of the body for benign gynecological disease. Age at treatment was similar for the two groups of women; however, almost twice as many years have elapsed since treatment for BGD (43 years vs 23 years), so that the women in the BGD cohort were older than the cervical cancer patients at the time blood was drawn.

Since data from several earlier studies have suggested that baseline frequencies of stable and/or unstable types of chromosome aberrations may show an increase with age *(3, 26-28),* we initially compared cytogenetic findings in lymphocyte cultures from the two groups of nonirradiated women. In lymphocyte cultures from the 26 nonirradiated BGD patients whose mean age was 76 at the time of blood collection, an average of 0.74 ± 0.19 metaphases carried one or more stable types of chromosomal aberrations, while 0.30 ± 0.10 metaphases contained one or more unstable aberrations. These aberration frequencies are similar to those we observed previously in 26 nonirradiated women with cervical cancer whose average age was 65 at the time of blood collection (i.e. 0.69 ± 0.11 and 0.40 ± 0.09 metaphases with stable and unstable aberrations, respectively). Thus. even though the BGD patients were somewhat older than the cervical cancer patients at the time their blood was collected for lymphocyte culture and cytogenetic evaluation. baseline frequencies of chromosome aberrations in the two groups of nonirradiated women were similar, suggesting that differences in age should not affect data comparisons between the two elderly cohorts of irradiated patients. Nonetheless, all statistical comparisons took age into account.

Compared to findings in nonirradiated BGD patients, we observed that the number of lymphocytes carrying one or more symmetrical types of aberrations was increased by about threefold in women with similar nonmalignant diseases who had received intracavitary radiation exposures more than 40 years ago. This finding extends our earlier observations which have demonstrated that radiation-induced stable aberrations in mitogen-responsive lymphocytes are sensitive biomarkers of exposures in populations of persons who received partial-body irradiation many years previously (1, 2). The mean half-life of lymphocytes has been estimated to range from 130 days (29) to up to 3 years *(30).* Thus it is probable that most of the lymphocytes that were evaluated in these BGD patients for radiation-induced chromosome aberrations were progeny of lymphoid progenitors that had repopulated the peripheral lymphoid compartment during the several decades that had elapsed between their exposure and collection of blood for cytogenetic evaluations. The increase in the number of cells carrying asymmetrical types of aberrations in the irradiated BGD patients is not significant but may represent a small proportion of immunocompetent T lymphocytes surviving without undergoing mitotic cell division for up to 50 years after exposure. Although we have not previously detected increased frequencies of asymmetrical aberrations in our long-term follow-up studies of other cohorts of patients who received partial-body radiotherapy (3), long-term survival of lymphocytes with asymmetrical aberrations has been noted in the Hiroshima and Nagasaki A-bomb survivors as well as ankylosing spondylitis patients *(15, 25).* Alternatively, the increase in unstable aberrations in the irradiated BGD subject might reflect more recent radiation exposures than we were able to identify in the screening interview, although both treatment groups were screened similarly.

A primary purpose of our study was to compare the frequencies of residual chromosome aberrations in cultured lymphocytes in women who received localized irradiation for benign and malignant gynecological diseases more than two decades ago. We observed that the mean frequencies of lymphocytes bearing stable types of chromosome aberrations were quite similar in preparations from the two groups of women. despite the fact that cervical cancer patients had received on average a tenfold higher bone marrow dose than received by women treated for BGD. If mitogen-responsive lymphocytes bearing symmetrical aberrations several decades after exposure are indeed progeny of irradiated lymphoid stem cells, then our findings suggest a larger fraction of chromosomally aberrant stem cells survived per unit dose in subjects irradiated for benign disease than in those patients who received high, localized doses to the same region of the body for cervical cancer. Several radiobiological factors, such as total dose, dose deposition, dose rate and protraction of dose, could have contributed to cell sterilization or a disproportionate survival of stem cells carrying chromosome aberrations in these two populations.

Regarding the total radiation dose received by the two groups of women, the cervical cancer patients were treated with external-beam radiotherapy to the whole pelvis (total average accumulated dose 40 Gy), plus one or two intracavitary radiation insertions delivering an additional 10 Gy to the pelvic sidewall *(1).* At such high local doses, it is likely that most hematopoietic stem cells that were present in the radiation field would have received lethal doses; thus

most of the dose was "wasted" in terms of causing late effects (2. 8), whereas the estimated mean dose of *y* radiation to marrow in the pelvic bone of BGD subjects was typically 1.5 Gy (range: 0.38–4.08 Gy) *(5).*

For both the cervical cancer and BGD patients, one might speculate that some proportion of the surviving fraction of irradiated stem cells of both the lymphoid and myeloid lineages would be expected to carry biologically relevant chromosome rearrangements that could be crucial events in cancer genesis. Our observation of similar residual burdens of cytogenetic damage in cultured lymphocytes of these two groups of women who have similar risks for leukemia in spite of the fact that their mean dose differed by an order of magnitude suggest that persistent stable aberrations in lymphoid progenitors may be a useful surrogate biomarker of the relative levels of cytogenetic injury sustained by other populations of hematopoietic stem cells. The cytogenetic data are indeed consistent with epidemiological observations of a twofold leukemia risk in both of these populations. Moreover, a higher average excess relative risk per gray for acute leukemia and chronic myeloid leukemia related to mean bone marrow exposure has been observed in the BGD cohort (1.66/Gy) *(5),* compared with the cervical cancer patients (0.14/Gy) *(8).*

In our comparisons of data from these two groups of irradiated women, we attempted to control for as many potential confounding variables as possible. Samples of blood were collected using the same supplier of heparin and vacutainer tubes, and samples were shipped overnight under identical shipping conditions. In culturing the lymphocytes for cytogenetic evaluations, we used identical culture techniques and reagents and used the same approach for microscopic analysis of metaphase aberrations. The same scorers prepared and evaluated slides from both groups of women. Our finding that the two groups of women exhibit similar frequencies of stable aberrations in their cultured lymphocytes many years after exposure thus provides support for our hypothesis that persistent stable aberrations in lymphoid stem cells correlate more closely with cancer risk than with the actual partial-body radiation dose.

ACKNOWLEDGMENTS

We are grateful to Ms. Shirley Colyer, Ms. Josephine Outlaw and Ms. Linda Dry of Oak Ridge Institute for Science and Education for providing cytogenetic support; Dr. Arvin R. Glicksman and Ms. Fran Laurie of Rhode Island Hospital for their clinical support: Ms. Marilyn Stovall of M. D. Anderson Cancer Center for providing dosimetry; Mr. Karl Wende of Roswell Park Cancer Institute for field support; Ms. Mollie Miedzinski of Westat, Inc. for data management; and Ms. Cathy Drzyzgula and Mr. Joe Barker of IMS for computer support. This study was supported by an Interagency Agreement YO1-CP-80505 between the National Cancer Institute and Department of Energy; contract DE-AC05760R00033 between Department of Energy and Oak Ridge Associated Universities: and contracts NO1-CP9-5614 and NO I-CP8-5604 from the Division of Cancer Etiology.

Received: November 8, 1993: accepted: February 21, 1994

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