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In the past twelve months, which covers the time of this progress report, continued progress has been made at the Radiation Effects Research Foundation (RERF) in the various research projects that are conducted to evaluate the late health effects, both somatic and genetic, resulting from radiation exposure of the survivors of the atomic bombs at Hiroshima and Nagasaki, Japan.

Considerable progress has been made in the collection and utilization of the various epidemiological data bases that are available at RERF. These include the Life Span Study (LSS) cohort, the Adult Health Study (AHS) cohort, the In Utero cohort, the leukemia registry and the F-1 Study population.

Important progress has been made in using RERF Tumor and Tissue Registry records for evaluation of cancer incidence and radiation risk estimates for comparison with cancer mortality and risk in the LSS cohort. At the present time, a manuscript on the incidence of solid tumors (1950-1987) is undergoing internal and external review for publication as an RFRF Technical report (TR) and for publication in a peer-reviewed scientific journal. In addition, manuscripts are in preparation on (1) a comprehensive report on the incidence of hematological cancers, including analysis of leukemia by cell type (1950-1987), (2) a general description of Tumor Registry operations and (3) a comparison of incidence- and mortality-based estimates of radiation risk in the LSS cohort.

The first two parts (part I and Part II) of Life Span Study Report 11 (Report 9 as published in Radiation Research) entitled: "Studies of the Mortality of A-Bomb Survivors 9. Mortality, 1950-1985: Part 1. Comparison of Risk Coefficients for Site Specific Cancer Mortality Based on the DS86 and T65DR Shielded Kerma and Organ Dose" and Studies of the Mortality of A-Bomb Survivors 9. Mortality, 1950-1985: Part 2. Cancer Mortality Based on the Recently Revised Doses (DS86) have been published in Radiation Research 118,502-524 (1989) and 121, 120-141 (1990) respectively. Part 3 of the Life Span Study series, entitled: "Life Span Study 11. Part 3. Noncancer Mortality, 1950-1985" is in the final stages of its review and preparation as RERF Technical Report 02-91. It will be submitted for publication soon in Radiation Research. The evaluation of the noncancer mortality data base indicates an increase, although small, in the relative risk for mortality due to cardiovascular disease in the high dose group (2+ to 3+ Gy) of females, but not males, who were less than 40 years of age at the time of the bomb (ATB). The mortality rate for digestive organ disease was also high. The dose-response information on noncancer disease in the LSS cohort has stimulated considerable discussions in RERF and in Scientific Council

deliberations about the incidence of cardiovascular diseases and possible pathologic mechanisms and/or competing risks that might be involved in such observed effects.

Although it is a fact that radiation, as well as chemicals, induce cancer in experimental animals and, through epidemiological studies, cancer incidence is increased in human populations that have been exposed to radiation, questions remain as to the mechanism(s) that is/are involved in transforming a normal cell to the malignant state. To better understand the mechanism(s) underlying radiation-induced carcinogenesis, RERF is developing a multi-disciplinary research activity in molecular epidemiology or epidemiological oncology which will include basic and radiation studies at the molecular and cellular level of organization to determine whether "molecular fingerprints" might be found in selected oncogenes or tumor suppressor genes that are involved in the establishment of cancer. These studies will, in the beginning, involve studies of human tumors known to be associated with specific carcinogens with later studies utilizing archival pathological material that is available at RERF. As a part of the implementation of this inter-disciplinary program, an inventory of existing archival material at RERF and assessment of its quality will be made, and an active program will be initiated to obtain various kinds of fresh malignant and benign tumor and non-tumor tissue from A-bomb survivors. At the present time, RERF, Hiroshima University and the U.S. National Cancer Institute are collaborating in a project to determine whether a correlation exists between the history of exposure to radiation and lung cancer and the mutational spectra of the tumor suppressor gene, p-53. Dr. Takeshima of Hiroshima University is presently in Dr. Curtis Harris laboratory at NCI for an extended period of time, and Dr. Itoh from the Department of Radiobiology at RERF is spending a month at NCI to learn new techniques needed for the conduct of this new effort at RERF.

The genetics program at RERF involves two separate research activities. These involve efforts to measure genetic changes at the chromosome (cytogenetics program) and DNA (biochemical genetics program) levels that might have been induced by exposure to radiation from the atomic bombs. In the cytogenetics program, the main objective is to determine the relationship between stable chromosome aberrations and radiation dose in the A-bomb survivors. Because of the difficulty of identifying stable chromosome changes, efforts are in progress to introduce the fluorescent in situ hybridization (FISH) technique that was developed at the Lawrence Livermore National Laboratory (LLNL) for detection of translocations between chromosomes that have been differentially labeled with fluorescent dyes. This permits detection of stable aberrations that have persisted for decades and can be used for dose-response relationships, correlation with other radiation-induced biological end-points, such as somatic mutations, and for use in potential biological dosimetry activities.

In the biochemical genetics program, work is continuing in the collection of blood samples and preservation of cell lines from 500 "exposed" families (one

or both parents were exposed to radiation and all available children) and 500 "non-exposed" families (neither parent exposed and all available children) and in the development of methods for the detection of various genetic changes (point mutation, deletion, insertions and translocations) at the DNA level. The cells from the family "trios" (Father, Mother and at least one child) are transformed by the Epstein-Barr virus, allowed to proliferate and are then preserved in liquid nitrogen. Aliquots of intact lymphocytes and polymorphonuclear leukocytes are also preserved in liquid nitrogen for later use. To date, cell lines have been established on 2,500 individuals (725 trios).

In the development of methods for the potential measurement of mutations at the DNA base level, three promising approaches have been chosen for examination. Since it is believed that radiation induces predominately deletions, insertions and rearrangements (D/I/R), two of these techniques are especially designed to measure these end points. The techniques are: (1) Denaturing Gradient Gel Electrophoresis of DNA fragments which have been amplified by the Polymerase Chain Reaction (PCR-DGGE), the technique of choice for small variations (deletions of less than 50 base pairs as well as base pair substitutions) in genomic DNA. For detection of variations in messenger RNA (mRNA), a modification of this technique is used. In this method, mRNA is copied into its complimentary DNA by reverse transcriptase, subsequent fragments are amplified by the polymerase chain reaction and separated by the denaturing gradient gel electrophoresis (RT-PCR-DGGE). Target sequences of varying base pair length have been examined from the human (coagulation) factor IX gene (F9). In this pilot test, 100 families with 124 children were examined, and 17 variants were detected in the children, all of which were inherited from one or the other of their parents. Using the RT-PCR-DGGE test of the same families, no variants in cDNA of phosphoglycerate kinase-1 (PGK1) gene, and one variant was detected in the retinoblastoma susceptibility (RB) gene. (2) the second technique is a modification of the Southern Blotting approach which is primarily for the detection of large D/I/Rs. Using this technique with the same families as above, one child showed an abnormal band which appeared to be homozygous. When the parents (who were found to be cousins) were examined, the abnormal band was present in both parents; therefore, the child inherited the band from both parents.

In addition to the above techniques, a new method has been developed in the biochemical genetics laboratory using high performance liquid chromatography (HPLC). This technique is capable of measuring a 50% loss of a gene, however, the effectiveness of this technique for mass screening has not yet been determined.

Progress continues to be made in the Department of Radiobiology in the development of somatic mutation assays that can be used as potential biological dosimeters for measuring radiation-induced changes in the DNA of somatic cells. To date, four assays have been developed and tested. They are: (1) the glycophorin A (GPA) assay, (2) the T-Cell Receptor (TCR) assay, (3) the human-leucocyte-associated antigen (HLA-A) assay and (4) the hypoxanthine quanine phosphoribosyl transferase (HPRT) assay. Of these assays, only the GPA assay can be used as a "life-time" biological dosimeter. The others tend to have a short biological half life which reduces their usefulness as biological dosimeters.

Research is also being conducted, using the Severe Combined Immune Deficiency (SCID) mouse, to identify the mechanism and gene control of high radiosensitivity in these mice. Hybridization of the SCID mouse cell with human fibroblast cells permitted the establishment of a human-mouse hybrid cell line which were exposed to 1 Gy of X-rays/day for 10 days and radiation resistant cells were selected. Since human chromosomes disappear with time from the hybrid cells, the colonies of cells were subcultured until the human chromosomes contained in each colony of cells became stable. The cells were further irradiated and radiation resistant cell lines again isolated. All resistant cell lines were found, by appropriate analysis, to contain human chromosome number 8. When this chromosome is lost from an established resistant cell line, the cell reverts to the high radiation sensitivity observed in the original SCID cell line. These studies indicate that the gene which confers resistance to the hyper sensitive SCID cell is located on human chromosome 8.

The long-term follow-up of the survivors of the atomic bombs has necessitated continuing collection of mortality and morbidity data and an extensive capability to develop appropriate data bases for use by the epidemiologists and statisticians in the important analysis of the data and estimation of radiation risks. To accomplish this, it has been necessary to develop a strong computer network at RERF in support of these activities.

Actions initiated in the past year represent the start of a multi-year effort to develop a new computing environment at RERF to provide the computer-related resources that will meet user needs, be compatible with future technology and be cost-efficient. The replacement of the NEC mainframe computer last November with a newer, less expensive model has led to a significant reduction in operating costs. The savings are being used to subsidize, in part, the expense of the new computer system. Ultimately the new system will consist of a network of work stations and personal computers which will eventually eliminate the need for the mainframe computer which is expensive to maintain and does not provide the computing power nor the features required by the users. It is the intent to complete the establishment of the new system by 1995-1996 or sooner if possible.

At the present time, an ethernet backbone cabling system has been installed for establishment of a network in the building which houses the Departments of Epidemiology, Epidemiologic Pathology, Statistics and the Research Information Center (the Computer Center). Three Sun SPARCstation2 units and one NEC EWS unit have been acquired and connected to the network. Selected PCs in the Research Information Center have also been connected to the network. Current activities have consisted of preparing and testing work station-networking resources that will be made available to the users.

In addition to the relatively brief summarization of work that has been accomplished during the past year, twenty-six manuscripts are in various stages of internal and external peer review at RERF. In addition, a number of

manuscripts have been published as RERF Technical Reports, 54 articles (see attached list) have been published in the scientific literature and a number of manuscripts have been approved for publication as Technical Reports (copies of summaries attached). It should be pointed out that the National Academy Press has just published a book entitled "The Children of Atomic Bomb Survivors: A Genetic Study", edited by Drs. James V. Neel and William J. Schull, which contains a selection of papers (see attached list) that have been published on the results obtained in the genetic program that has been conducted since 1947 at the Atomic Bomb Casualty Commission (ABCC) and RERF. In addition this book contains the authors assessment of the significance of the findings in this long-term genetic study.

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Overview of immunological studies on A-bomb survivors. M Akiyama, Y Kusunoki, S Kyoizumi. *J Radiat Res* (Tokyo) 32S:301–9, 1991.

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Multiple myeloma among atomic bomb survivors. M Ichimaru, K Mabuchi. *J Radiat Res* (Tokyo) 32S:168–71, 1991.

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An estimate of the magnitude of random errors in the DS86 dosimetry from data on chromosome aberrations and severe epilation. R Sposto, DO Stram, AA Awa. *Radiat Res* 128:157–69, 1991. (RERF TR 7-90)

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Calibration of Mg_2SiO_4(Tb) thermoluminescent dosimeters for use in determining diagnostic x-ray doses to Adult Health Study participants. K Kato, S Antoku, S Sawada, WJ Russell. *Am Assoc Phys Med* 18:928–33, 1991. (RERF TR 11-89) ❑

Somatic cell mutations in atomic bomb survivors. M Akiyama, N Nakamura, M Hakoda, S Kyoizumi, J Kushiro, Y Hirai, Y Kusunoki. *J Radiat Res* (Tokyo) 32S:278–82, 1991.

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Mortality and cancer risk among the offspring (F_1) of atomic bomb survivors. Y Yoshimoto, K Mabuchi. *J Radiat Res* (Tokyo) 32S:294–300, 1991.

Approved Technical Reports

Mortality among the offspring (F_1) of atomic bomb survivors, 1946–85. Y Yoshimoto, WJ Schull, H Kato, JV Neel. **RERF TR 1-91.**

We compare deaths occurring in the years 1946–85 in a cohort of 31,159 children born to parents, one or both of whom were exposed to the atomic bombing of Hiroshima or Nagasaki, and who received a combined (i.e., joint) gonadal dose of 0.01 Sv or more, with deaths in a comparable control group, totaling 41,069 children. The average combined gonadal dose equivalent for the exposed parents was 0.435 Sv. Gonadal doses were calculated using the recently established DS86 system, supplemented by an ad hoc system for those children for whom a DS86 dose could not be computed for one or both parents. At the end of 1985, those members of the study groups born in 1946 had reached 39 years of age, whereas those born in the years 1966 through 1984 had not yet reached their 20th birthday. The mean age of living members of the cohorts was 28.8 years.

When a linear relative risk model is fitted to the data, no statistically significant increase in the risk of mortality attributable to diseases other than neoplasms is noted following parental exposure, the excess relative risk being 0.030 (±0.046) per sievert based on the subset of individuals with DS86 doses, assuming the RBE of neutrons to be 20. For fatal cancer, in confirmation of an earlier report on cancer incidence below the age of 20 in this same group, again no statistically significant effect as parental radiation dose increased was observed. Finally, although the present method of analysis using Poisson regression and person-years at risk of death seems more appropriate now, particularly as the cohort ages, since earlier analyses of mortality in the F_1 cohort have been based on a simple linear regression of the frequency of death on parental dose, this model was also fitted to the data used in the relative risk estimate to provide some continuity with the past. The results give an intercept of 0.0420 (±0.0015) and a linear regression coefficient of 0.00169 (±0.00157) per sievert. This leads to the calculation of a (statistically nonsignificant) excess relative risk of 0.040, in good agreement with the excess obtained by fitting the relative risk model. An analysis based on the full sample, using not only the DS86 dose group but also the ad hoc dose group, yields essentially the same result as the analysis restricted to the DS86 dose group.

Life Span Study Report 11, Part 3. Noncancer mortality in the years 1950–85 based on the recently revised doses (DS86). Y Shimizu, H Kato, WJ Schull, DG Hoel. **RERF TR 2-91.**

Deaths in the RERF Life Span Study (LSS) sample have been determined for the years 1950–85 and an analysis of cancer mortality using the revised DS86 doses has been described separately (LSS Report 11, Parts 1 and 2). In this report we examine the relationship to dose of deaths from all diseases other than cancer.

Although the evidence is still limited, there seems to be an excess risk from noncancer death at high doses (2 or 3 Gy and over). Statistically, a pure quadratic or a linear-threshold model (the estimated threshold dose is 1.4 Gy [0.6–2.8 Gy]) is found to fit better than a simple linear or linear-quadratic model. This increase in noncancer mortality is statistically demonstrable, generally, after 1966 and among the younger survivors at the time of the bombing (<40), suggesting a sensitivity for this age group. For specific causes of death, an excess in relative risk at the high dose level, that is, 2 Gy or more, is seen in circulatory and digestive diseases. The relative risk is, however, much less than that for cancer.

These findings, based as they are on death certificates, have their limitations. Most significant, perhaps, is the possible erroneous attribution of radiation-related cancer deaths to other causes. At present, the contribution such errors may make to the apparent increase in noncancer deaths at the higher doses cannot be estimated as rigorously as is obviously desirable. However, even now, this increase does not appear to be fully explicable in terms of classificatory errors.

Further follow-up of mortality in this LSS cohort as well as disease revealed by the biennial physical examinations of the morbidity subsample (the Adult Health Study) of the LSS cohort will be needed to confirm this suggestion of a radiation-related increase in mortality from causes other than cancer, and to determine whether it results in a demonstrable life-shortening among the heavily exposed A-bomb survivors.

Development of a flow-cytometric HLA-A locus mutation assay for human peripheral blood lymphocytes. J Kushiro, Y Hirai, Y Kusunoki, S Kyoizumi, Y Kodama, A Wakisaka, A Jeffreys, JB Cologne, N Nakamura, M Akiyama. **RERF TR 3-91.**

A flow-cytometric technique was developed to measure the frequency of mutant lymphocytes lacking expression of human leukocyte antigen (HLA) A2 or A24 allele products among donors heterozygous for HLA-A2 or A24.

It was found that the mutant frequency of lymphocytes in peripheral blood was on the order of 10^{-4} and increased with donor age. Molecular analyses of mutant clones revealed that about one-third were derived from somatic recombinations and that the remaining two-thirds did not show any alterations after Southern-blotting analysis. A small-scale study on atomic bomb survivors did not show a significant dose effect.

An in vitro mutagenesis study showed that the mutant frequency at the HLA-A24 locus increased at a rate of roughly 2×10^{-4}/Gy, about 10 times greater than that reported at the X-chromosomal hypoxanthine phosphoribosyltransferase locus in lymphocytes. These mutants were found to be mostly derived from large chromosomal deletions.

The effect of diagnostic misclassification on noncancer and cancer mortality dose response in the RERF Life Span Study. R Sposto, DL Preston, Y Shimizu, K Mabuchi. **RERF TR 4-91.**

We performed analyses of cancer and noncancer mortality in the RERF Life Span Study (LSS) to determine whether the observed increased risk of noncancer death due to radiation exposure could be attributed solely to misclassification of causes of death on death certificates. Cancer and noncancer misclassification rates and their dependence on age at death were estimated from a series of autopsies performed on LSS participants between 1961 and 1975. The crude misclassification rates were 20% for cancer and 2.8% for noncancer. Although the noncancer dose response remained significant, correcting for this amount of misclassification reduced the estimated noncancer excess relative risk (ERR) at a 1-Gy exposure by 21% and the number of excess noncancer deaths in the cohort by 23%. The estimated cancer ERR at 1 Gy was increased by 12% and the excess cancer deaths by 16% as a result of the correction. The statistical significance of the noncancer dose response was relatively insensitive to underestimating the cancer misclassification rate, but sensitive to assuming that cancer misclassification was positively associated with dose.

We discuss implementation of the EM algorithm for adjusting for misclassification, and extensions of the method to more than two causes of death.

Thyroid cancer incidence among atomic bomb survivors in Hiroshima and Nagasaki, 1958–79. S Akiba, J Lubin, H Ezaki, E Ron, T Ishimaru, M Asano, Y Shimizu, H Kato. **RERF TR 5-91.**

One hundred and twelve cases of thyroid cancer diagnosed during the period 1958–79 among the extended Life Span Study cohort in Hiroshima and Nagasaki were studied. There was a statistically significant association between thyroid cancer incidence and exposure to atomic bomb radiation. The adjusted excess relative risk (ERR) per gray was 1.1 (95% confidence interval = 0.3; 2.5) and the adjusted absolute risk per 10^4 Gy was 0.59 (95% confidence interval = 0.2; 1.7). Based on a comparison of the deviances obtained from relative and absolute risk models, a simple linear relative risk model appeared to fit the data better than an absolute relative risk model, however, it would not be appropriate to conclude that the data conform strictly to a relative risk pattern.

The incidence of thyroid cancer among the members of the Adult Health Study (AHS) population, who have been medically examined biennially at the RERF clinics since 1958, was 70% higher than that among the rest of the extended LSS cohort after adjustments for city, sex, log age, calendar year, and DS86 dose. There was no significant difference between the slope of the dose-response curve for AHS and non-AHS participants, although the estimated ERRs at 1 Gy for the AHS and non-AHS populations were 1.6 and 0.3, respectively. The elevated risk appeared to be confined to women, and there was an increasing risk with decreasing attained age at exposure.

Measurement of $CD4^-8^-$ T cells bearing T-cell receptor αβ chains by flow cytometry: I. Results for a normal population including two cases with unusually high frequencies. Y Kusunoki, Y Hirai, S Kyoizumi, M Akiyama. **RERF TR 6-91.**

Detection of rare, possibly abnormal, T cells bearing CD3 surface antigen and T-cell receptor αβ chains but lacking both CD4 and CD8 antigens (viz., TCR $\alpha\beta^+CD4^-8^-$ cells as determined by flow cytometry) was performed. The TCR $\alpha\beta^+CD4^-8^-$ T cells were detected at a mean frequency of 0.63 ± 0.35% (mean ± standard deviation) in peripheral blood TCR $\alpha\beta^+$ cells of 119 normal individuals. Two unusual cases besides the 119 individuals showed extremely elevated frequencies of TCR $\alpha\beta^+CD4^-8^-$ T cells, viz., approximately 5% to 10% and 14% to 19% in whole TCR $\alpha\beta^+$ cells. Both were males who were otherwise physiologically quite normal with no history of severe illness, and these high frequencies were also observed in blood samples collected 2 or 8 years prior. The TCR $\alpha\beta^+CD4^-8^-$ T cells of the two individuals were found to express mature T-cell markers such as CD2, 3, and 5 antigens as well as natural killer (NK) cell markers, viz., CD11b, 16, 56, 57 antigens when peripheral blood lymphocytes were subjected to three-color flow cytometry. Lectin-dependent or redirected antibody-dependent cell-mediated cytotoxicities were observed for both freshly sorted and TCR $\alpha\beta^+CD4^-8^-$ cells and in vitro-established clones. Nevertheless, NK-like activity was not detected. Further, Southern blot analysis of TCR β and γ genes revealed identical rearrangement patterns for all the TCR $\alpha\beta^+CD4^-8^-$ clones established in vitro. These results suggest that the TCR $\alpha\beta^+CD4^-8^-$ T cells from these two men exhibit unique characteristics and proliferate clonally in vivo.

Detection of a length polymorphism in the 5′ flanking region of the human β-globin gene in a Japanese population with denaturing gradient gel electrophoresis. N Takahashi, K Hiyama, M Kodaira, C Satoh. **RERF TR 7-91.**

An analysis of the ATTTT repeat polymorphism located approximately 1,400 base pairs upstream from the β-globin structural gene was carried out by denaturing gradient gel electrophoresis (DGGE) of RNA:DNA duplexes. Genomic or cloned DNAs were digested with restriction enzymes and hybridized with P-32-labeled RNA probes, and resulting RNA:DNA duplexes were examined by DGGE. A difference in the number of repeat units was recognized by differences in duplex mobility on the DGGE gel. In this study of 81 unrelated Japanese from Hiroshima, a sequence heteromorphism was observed at this site. Alleles with 5 and 6 repeats of the ATTTT unit, which had already been reported, were found in polymorphic proportions. In addition, two unreported alleles, one having 7 repeats and the other having an A-to-G nucleotide substitution in the fifth repeat, were detected. Family study data showed that the segregation of these four types of variants is consistent with an autosomal codominant mode of inheritance. This study also demonstrated that DGGE of RNA:DNA duplexes is a sensitive tool for detecting variations in DNA.

Radon concentrations in residential housing in Hiroshima and Nagasaki. T Aoyama, EP Radford, H Yonehara, H Kato, M Sakanoue. **RERF TR 8-91.**

A survey of indoor radon (Rn-222) concentrations in Hiroshima and Nagasaki was carried out to assess the variability of exposure expected among atomic bomb (A-bomb) survivors. Two hundred dwellings (100 from each city), chiefly of members of the Life Span Study population, were selected for this survey. We used two types of alpha-track detector: a Terradex detector type SF and a bare-track detector improved by Yonehara et al. Comparative measurements showed that although there was an adequate correlation between the values obtained using the two detectors, the geometric mean value for the bare-track detector was 45% lower than that for the Terradex detector. This difference was considered to be due to differences in the calibration methods and sensitivities of the detectors to thoron (Rn-220).

The geometric mean values of the radon concentrations for 193 locations in Hiroshima and 192 locations in Nagasaki measured by Terradex SF detectors were 51.8 Bq/m^3 and 26.5 Bq/m^3, respectively. The large difference is attributable to the different geological environments of the two cities.

Correlating factors with the indoor radon concentrations were also studied. The geometric mean concentration was significantly higher in wooden houses with mud walls than in other types of house. This tendency was especially remarkable in Hiroshima.

The difference between the estimated dose equivalents for exposure to radon progeny in dwellings in Hiroshima and Nagasaki over the last 30 years might amount to 0.8 Sv; however, no statistically significant difference was observed in lung cancer mortality in the low-dose range in either city. Nevertheless, the indoor radon concentrations estimated in this survey could have a significant influence on the dose-response relationship for A-bomb exposure.

Differential effects of atomic bomb irradiation in inducing major leukemia types: Analyses of open-city cases including the Life Span Study cohort based upon updated diagnostic systems and the Dosimetry System 1986. M Tomonaga, T Matsuo, RL Carter, JM Bennett, K Kuriyama, F Imanaka, S Kusumi, K Mabuchi, A Kuramoto, N Kamada, M Ichimaru, AV Pisciotta, SC Finch. RERF TR 9-91.

From 1945 through 1980, 766 cases of leukemia were reported to the Atomic Bomb Casualty Commission and its successor, the Radiation Effects Research Foundation, among the open-city sample of atomic bomb survivors who were within 9 km of the hypocenters at the time of the bombings (ATB). Only 249 of these cases occurred among the Life Span Study (LSS) cohort. In this paper, we use data from the additional 517 cases from the leukemia registry together with the LSS cohort data to study the effects of atomic bomb irradiation on major leukemia types. All available hematological specimens of registered leukemia cases were reviewed. The French-American-British classification and other improved diagnostic methods were used to reclassify cases into 21 categories, including new disease entities such as adult T-cell leukemia (ATL). These categories were then grouped into four major types for analysis: (1) acute lymphoid leukemia (ALL), (2) acute myeloid leukemia (AML) including myelodysplastic syndromes (MDS), (3) chronic myeloid leukemia (CML), and (4) other types including ATL (OTHER). Analyses of radiation effects were based on the updated Dosimetry System 1986 (DS86).

Incidence rates of all four leukemia types increased with increasing exposure level. The effects of radiation exposure were significantly greater on the incidence of ALL and CML than on that of AML and OTHER. Exposures of 50 mGy and probably as low as 10 mGy apparently produced excess cases of ALL and CML, whereas exposures of 50 mGy and probably at least 220 mGy were required to produce excesses in AML. This differential effect disappeared in time as incidence rates returned to or toward background levels.

In the two lowest dose categories (1–49 and 50–499 mGy), estimated incidence either remained constant or increased slightly as the population observed aged. In the two highest dose categories (500–999 and ≥1,000 mGy), however, estimated incidence rates of all types declined. An excess of AML and ALL, but not CML and OTHER, remained through the final study period (1976–80) in the ≥1,500-mGy dose category.

Among unexposed persons, the estimated risk of CML in Nagasaki relative to Hiroshima was significantly less than that of AML, whereas that of OTHER types was significantly greater, because ATL cases occurred only in Nagasaki. The city effect on background rates appeared to explain the generally higher incidence of leukemia (except for ATL) in Hiroshima.

Also in unexposed persons, incidence in older groups (16–35 years ATB, ≥36 years ATB) relative to the youngest group (0–15 years ATB) was less for ALL than AML, but greater for CML and OTHER types than for AML. The risk of ALL remained relatively constant with age ATB whereas that of AML, and to a greater extent CML and OTHER, increased with age ATB.

The time to onset of ALL, AML, and CML declined with increasing dose. The rate of decline, however, was greater for ALL and CML than for AML. The resulting differences at high doses reflect shorter incubation times for atomic bomb-induced ALL and CML than for AML.

Skin cancer incidence among atomic bomb survivors in Nagasaki based on the DS86 dosimetry system. N Sadamori, M Otake, T Honda. **RERF TR 10-91.**

The effects of exposure to ionizing radiation on the incidence of skin cancer in a cohort of A-bomb survivors in the Nagasaki Life Span Study extended (LSS-E85) sample were investigated using the Dosimetry System 1986 (DS86). Among a total of 25,942 survivors at risk whose DS86 dose estimates were available, 47 cases of skin cancer including malignant melanoma were confirmed from the Nagasaki Tumor Registry during the period from 1 April 1958 to 31 December 1985. The dose-response relationship of skin cancer based on an additive relative risk model showed linearity without threshold, not a linear-quadratic curve. The excess relative risk (ERR) of 2.2/Gy in the LSS-E85 sample was highly significant (95% confidence limits: 0.5 to 5.0/Gy). On the other hand, the ERR of 3.1/Gy in the AHS sample was also significant (95% confidence limits: 0.6 to 20.3/Gy). When dose equivalents based on a relative biological effectiveness of neutrons of 10 were used, the ERR in the former sample decreased to 2.0/Sv (0.7–4.5), and the risk in the latter group also declined, to 2.7/Sv (0.6–17.8). The ERR did not differ significantly between males and females in the LSS-E85 and AHS samples, but a highly significant increase was observed for the ERR of age at exposure and time trend since exposure. The ERR of skin cancer cases including and excluding four malignant melanoma cases for the LSS-E85 sample (there were no malignant melanoma cases in the AHS sample) showed almost the same linear dose response.

X-ray induction of micronuclei in human lymphocyte subpopulations differentiated by immunoperoxidase staining. S Ban, M Nakano, JB Cologne. **RERF TR 11-91.**

In this report, we sought to confirm the radiosensitivity of human peripheral blood lymphocytes using a micronucleus assay. Mononucleated cells isolated from peripheral blood were irradiated with X rays. After being cultured for 3 days, the cells were fixed and stained using the immunoperoxidase staining technique. Lymphocyte subpopulations were characterized by means of the monoclonal antibodies Leu4 (CD3), Leu2a (CD8), and Leu19 (CD56).

Dose-response curves were obtained by scoring the number of micronuclei in binucleated cells that reacted with specific antibody and were then stained. The dose response of CD^+ (suppressor/cytotoxic) cells was quite similar to that of $CD3^+$ (pan T) cells. In comparison, $CD56^+$ (natural killer) cells were significantly less sensitive, although scorable binucleated $CD56^+$ cells made up less than 4% of the total number of binucleated cells.

A simple, quick method for HLA-DQA genotyping by PCR-SSCP analysis. T Hayashi, T Seyama, T Ito, Y Kusunoki, Y Hirai, N Nakamura, M Akiyama. **RERF TR 12-91.**

A simple, quick method for characterizing polymorphisms at the HLA-DQA locus has been developed. The procedure involves the selective amplification of the polymorphic second exon of the DQA locus by the polymerase chain reaction (PCR), followed by analysis of the amplified DNA with nondenaturing polyacrylamide gel electrophoresis after heat-denaturation of the amplified DNA (PCR-SSCP analysis). HLA-DQA alleles were classified into four major groups: DQA1, DQA2, DQA3, and DQA4 using this PCR-SSCP analysis. It would be feasible to use this PCR-SSCP analysis for detecting point mutations at various positions in a fragment as well as new HLA-DQA genotypes.

Brain abnormalities among the mentally retarded prenatally exposed atomic bomb survivors. WJ Schull, H Nishitani, K Hasuo, T Kobayashi, I Goto, M Otake. **RERF TR 13-91.**

An increased occurrence of severe mental retardation, with or without accompanying small head size, at specific gestational ages has been the most conspicuous effect on brain development of prenatal exposure to the bombings of Hiroshima and Nagasaki. A variety of biological mechanisms could be responsible for this finding, including cell killing and mismanaged neuronal migration. We describe here the findings on magnetic resonance imaging of the brains of five of these mentally retarded individuals, all of whom were exposed in the 8th through the 15th weeks following fertilization, the gestational period shown to be the most vulnerable to radiation-related damage. In the two cases exposed at the 8th or 9th week following fertilization, large areas of ectopic gray matter are seen, strong evidence of a failure of the neurons to migrate to their proper functional sites. The two individuals exposed in the 12th or 13th week show no readily recognized ectopic gray areas, but do show mild macrogyria, which implies some impairment in the development of the cortical zone. Moreover, both have mega cisterna magna. Finally, the one individual seen who was exposed still later in development, in the 15th week, shows none of the changes seen in the other four individuals. This person's brain, though small, appears to have normal architecture.

These findings are discussed in terms of the embryological events transpiring at the time of the prenatal exposure of these individuals to ionizing radiation.

A longitudinal study of the association between ABO blood phenotype and total serum cholesterol level in the Adult Health Study, 1958–86. FL Wong, K Kodama, H Sasaki, M Yamada, HB Hamilton. **RERF TR 14-91.**

This study examines the relationship between ABO blood phenotype and total serum cholesterol level in a Japanese population to determine whether an elevated cholesterol level is associated with phenotype A, as has been demonstrated consistently in many West European studies. Studies of this nature in non-white populations are scarce; available findings have generally failed to demonstrate the relationship, suggesting racial heterogeneity. Cross-sectional data of various racial groups with age categories ranging from neonates to adults exhibited varying results, including nonsignificant ABO–cholesterol associations. This raised the question of an age effect as a possible explanation for the discrepancies. It has also been suggested that the ABO–cholesterol association may not be apparent in populations with low fat intake or low mean cholesterol levels. We addressed these hypotheses by examining long-term data on total serum cholesterol levels collected serially from survivors of the atomic bombings of Hiroshima and Nagasaki who were participants of the Adult Health Study program at the Atomic Bomb Casualty Commission–Radiation Effects Research Foundation between 1958 and 1986. A longitudinal statistical method of growth curve analysis for serially measured response is used to model age-dependent changes in cholesterol levels within individuals. The effects of the ABO polymorphism in modifying the resultant growth curve are also examined. We demonstrate that total serum cholesterol levels were elevated on average by about 4 mg/dL in phenotype A compared to non-A phenotypes in the Japanese ($p = .027$), and that this relationship is maintained from early to late adulthood. Thus, phenotype A individuals may be predisposed to cardiovascular disease through one of its major risk factors. This is the first study of the ABO–cholesterol association in the Japanese, and the first that is based on a cohort with longitudinally collected total serum cholesterol data.

Combining diagnostic categories to achieve improved agreement between death certificate and autopsy classifications of cause of death of atomic bomb survivors, 1950–87. RL Carter, E Ron, K Mabuchi. **RERF TR 15-91.**

Several investigators have observed less than desirable agreement between death certificate and autopsy diagnoses for most specific causes of death, and even for some causes grouped by major disease category. Our results from data on 5,130 members of the Life Span Study (LSS) cohort of atomic bomb survivors in Hiroshima and Nagasaki who were autopsied at the Atomic Bomb Casualty Commission–Radiation Effects Research Foundation prior to September 1987 were equally discouraging. Among diseases with more than 10 cases observed, confirmation rates ranged from 13% to 97% and detection rates from 6% to 90%. Both were greater than 70% for only 6 of 60 disease categories studied and for only 1 of 16 categories defined by major ICD classifications (neoplasms). This deficiency suggests cautious interpretation of results from studies based on DC diagnoses.

To determine whether any groupings of diagnoses might meet acceptable accuracy requirements, we applied a hierarchical clustering method to data from these 5,130 cohort members. The resulting classification system had 10 categories: breast cancer; other female cancers; cancers of the digestive organs; larynx cancer; leukemia; nasal, ear, or sinus cancer; tongue cancer; external causes; vascular disease; and all other causes. Confirmation and detection rates for each of these categories were at least 66%. Although the categories are broad, particularly for nonneoplastic diseases, further divisions led to unacceptable accuracy rates for some of the resulting diagnostic groups.

Using the derived classification system, there was 72% agreement overall between death certificate and autopsy diagnoses compared to 53% agreement for a second system obtained by grouping strictly by major disease category. Eighty-seven percent agreement was observed for a similar classification system with vascular disease grouped with all other nonneoplastic diseases. Further agglomeration achieved very little additional improvement. Accuracy rates for some of the categories of the 10 category diagnostic system defined above varied with various covariates. For example, accuracy decreased with increasing age at death for most of these categories. Thus, subpopulations exist for which accuracy rates can be expected to be either better or worse than for the whole population. While these results do not necessarily dictate which diseases and/or populations should be studied in future cause-specific mortality investigations, they do provide investigators with useful information pertinent to the planning of their study, analysis of data, and interpretation of results.

Evidence for increased in vivo somatic mutations in T-cell receptor genes in lymphocytes but not in the glycophorin A gene in erythrocytes of Thorotrast patients. S Umeki, S Kyoizumi, Y Kusunoki, N Nakamura, M Sasaki, T Mori, Y Ishikawa, JB Cologne, M Akiyama. **RERF TR 16-91.**

Recent discoveries of cancer suppressor genes have strengthened the somatic mutation theory of carcinogenesis. Since exposure to ionizing radiation is a well-recognized risk factor for cancer among other human health defects, and since ionizing radiation can induce mutations, an accurate way of measuring somatic mutation frequencies could be a useful tool for evaluating cancer risks. In the present study, we have examined in vivo somatic mutation frequencies at the erythrocyte glycophorin A (GPA) and T-cell receptor (TCR) loci in 18 Thorotrast patients. These patients have been continuously irradiated with alpha particles emitted from the internal deposition of thorium dioxide and thus have increased risks of certain malignant tumors. When compared with controls, the results showed a significantly higher frequency of mutants at the lymphocyte TCR loci but not at the erythrocyte GPA loci in the Thorotrast patients. The discrepancy between the results of the two assays is discussed.

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