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FOREIGN TRIP REPORT

ORNL/FTR-2840

DATE: April 7, 1988

SUBJECT: Report of Foreign Travel of R. Julian Preston
Senior Research Staff Member, Biology Division

TO: Alex Zucker

FROM: R. Julian Preston

PURPOSE: To participate in a Workshop at the Radiation Effects Research Foundation (RERF), Hiroshima, Japan (March 18-20, 1988) on "Radiation Susceptibility," and to provide recommendations to RERF on future studies designed to address the identification of possibly susceptible population sub-groups.

To visit Dr. Masao Sasaki, Radiation Biology Center, Kyoto University, to discuss current research on the role of specific chromosomal and molecular alterations in cell transformation.

SITES VISITED:	March 17-20, 1988	RERF, Hiroshima, Japan
	March 22-23, 1988	Kyoto, Japan

ABSTRACT: The traveler was a participant in a workshop at RERF that was established to determine if current data or future studies could be utilized to address the question of whether radiation-sensitive individuals could have been over-represented in the A-bomb non-survivors in Hiroshima and Nagasaki and thereby affect the cancer incidences. The topic was addressed by presentations by RERF staff on their current studies pertinent to radiation susceptibility; round-table discussions among panel members and observers; a written series of recommendations prepared by the workshop members and presented to the RERF council.

DISCLAIMER

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The Radiation Effects Research Foundation has established a series of workshops to take place over the next 2-1/2 years to address a variety of questions that arise from the long-term studies of A-bomb survivors and on the future directions that might address these. It is particularly important to determine what additional studies might be important because of the limited time that is now available due to the age of the exposed population. The Workshop attended by the traveler was the first in the series and addressed the question of radiation susceptibility, i.e. is the surviving population more representative of a cancer resistant population than the general population, making the estimates of radiation-induced cancer artificially low?

The first day of the Workshop consisted of presentations by the staff of RERF to provide background information on previous studies and data collection and also results from ongoing studies of the Radiation Biology and Cytogenetics Groups that were designed to attempt to address the question of radiation susceptibility.

Dr. Finch, Chief of Research, RERF, provided a summary of the data on late effects that had been collected over the past 40 years with particular reference to the effect that the new dosimetry (DS86) had on the conclusions, and on the comparisons between Hiroshima and Nagasaki. In general, it can be concluded that no differences in cancer rates or chromosome aberrations can be observed in persons exposed in Hiroshima and Nagasaki. This is in rather good agreement with the expected outcomes based upon the similarity of doses for both gamma rays and neutrons for the two cities (DS86). In addition, Dr. Finch described the Tumor Registries that were established in the 1950's. These now contain a large and valuable collection of tumor information. RERF has recently also become

involved in the establishment of a Cancer Registry for Nagasaki Prefecture. RERF also maintains a bank of biological materials for additional studies, and is currently expanding this. Peripheral blood samples are stored, and since 1985 some EBV-transformed permanent lymphoblastoid cell lines have been established for the analysis of mutant frequencies. Human skin and lung fibroblasts, together with a range of tumor cell lines, are also stored. Thus, it is possible to consider devising studies that would address the question of radiation susceptibility utilizing some of these cell samples and lines.

The Cell Biology Group has already initiated a study of cell killing and mutation induction in lymphocytes, thyroid cells, and skin fibroblasts in exposed and control persons to determine if there are variations in radiation sensitivity to X rays and/or neutrons. The cell killing studies with lymphocytes have been performed with T-lymphocytes and recombinant interleukin-2. The individual variation in radiosensitivity is generally smaller than that observed for skin fibroblasts, and increased radiosensitivity is observed when cloning efficiency is low. A similar response has been observed by other laboratories for mutants at the hgprt locus. No conclusions are yet available on the ranges of sensitivity for persons who had received different estimated A-bomb exposures. Mutants have also been analyzed in erythrocytes and T-lymphocytes of control and A-bomb exposed persons, utilizing the glycophorin A method (MN loci) and thioguanine resistance (HGPRT locus). It is also possible to further analyze the HGPRT mutants by Southern blot analysis (point mutation or deletion) and by using probes for the T cell antigen receptor gene to determine if multiple mutants are independent events or the result of clonal expansion from a single induced mutation. There is a general dose response for glycophorin mutants in exposed persons, with some clear outliers that might result from fluctuations in the

number of mutants in the stem-cell pool. However, the nature of these outliers needs to be studied in greater detail. One drawback of this assay is that since erythrocytes are analyzed for variant forms, it is not possible to determine if these are true mutations.

Mutations at the HGPRT locus have been analyzed in 30 exposed persons (> 1 rad) and 17 age- and sex-matched controls (< 1 rad). In general, mutant frequency correlates with revised dose estimates (DS86). A very small subset of individuals (3 exposed; 2 controls) have been further studied to determine the nature of mutations and the independence of induction. The method requires clonal expansion of mutant colonies using interleukin-2 followed by Southern blot analysis and T cell receptor gene analysis. The only conclusion of note to date is that one A-bomb survivor had a 100 times higher than control mutant frequency. All the mutant T-cell colonies possessed the same HGPRG gene alteration, but different rearrangements of the T cell receptor gene. This result almost certainly arises from a single mutation in an immature stem cell. Thus the complete analysis of mutant colonies allows for a very specific interpretation of the mode of induction, and an accurate measure of induced mutation frequency. One drawback to the use of the HGPRT assay is that since it is an X-linked gene, deletions that include surrounding loci that are essential for viability will not be recovered, and observed mutant frequency will be an underestimate of the induced frequency. It was recommended that additional loci be studied, for example, the APRT locus and HLA mutations.

Cell killing in thyroid cells can be assessed by growth *in vitro* or by grafts in athymic Nu/Nu mice. Preliminary studies have shown that the method is feasible, using normal thyroid cells from persons treated surgically for malignant or benign thyroid tumors irradiated *in vitro*. The thyroid cells are

more sensitive than most non-hematologic cells (D_o = 92.9 $\pm$ 2.8 cGy). Additional preliminary studies on cells from A-bomb exposed persons have been performed, but the sample sizes are too small for conclusions to be drawn, although no clear increase or decrease in X ray sensitivity was observed for these samples.

A large study has been conducted on the sensitivity of skin fibroblasts to X ray- and Cf-252-induced cell killing and chromosome aberration induction. The cell lines studied were from control samples with or without breast cancer and A-bomb exposed persons with or without breast cancer. The hypotheses being tested are: (1) Is the exposed group more resistant to radiation, and (2) are cells from breast cancer patients more or less sensitive than those without? The study is fraught with some technical difficulties, although in concept it is good. The authors concluded that there were no cell lines in any group that were clearly radio-sensitive, although perhaps of interest (and not mentioned) is that there is a wide range of sensitivities to killing by X rays, and rather less following neutron irradiation. The authors' conclusion that cells from exposed persons with breast cancer contained more stable chromosome aberrations than those without breast cancer or non A-bomb exposed did not appear to be supported by the data presented.

A very small study has been initiated on cytogenetic and somatic mutation outliers, i.e. individuals whose aberration or mutant frequencies were much higher or lower than the calculated dose response curve for all individuals studied. No results are yet available to establish the nature of these outliers. However, it might well be that they could represent examples whereby the issue of radiation susceptibility could be addressed experimentally. This was the basis for one of the Workshop Group's recommendations.

The studies described by the RERF were both rather extensive and well-conducted. However, although they were intended to address the question of radiation susceptibility, it was difficult to see how they would indeed provide appropriate data for drawing conclusions pertinent to the question. Of course, the ideal approach would be to base studies on individuals that could be matched to the non-survivors, but this is difficult or impossible to do. Thus the Workshop members moved to the next stage, to devise practical approaches to determine factors that could influence radiation susceptibility.

On the second day, there was considerable discussion of ongoing studies and on different approaches for addressing the issue of radiation susceptibility. These discussions formed the basis for a series of recommendations for future research.

Recommendations by category only:

1. DNA Damage and Repair
2. Acute Radiosensitivity and Cancer Susceptibility
3. Correlation among end-points
   - (a) Mutations vs. Chromosome Aberrations
   - (b) Mutations vs. Cancer
   - (c) Marker Differences
     - (i) HLA
     - (ii) ARPT
4. Cell Bank
5. Outliers ("Sensitive" and "Resistant")
6. F_1 Study
7. DNA Damage and Repair - oxygen radicals
8. Chromosome Specific Staining
9. G_2 Chromosome Hypersensitivity

10. Skin Cancer.

These recommendations were presented in draft form for submission to the RERF Scientific Council. They will be described more fully for final submission.

The Workshop provided an excellent opportunity for exchange of ongoing research and ideas for future directions. It proved to be a valuable experience, and the end-product should provide the basis for addressing the question of possible individual differences in radiation susceptibility and perhaps provide opportunities for collaborative research. The question being addressed is an important and worthy of a fairly extensive research effort. In addition, animal models could be developed for studying radiation susceptibility and are worthy of consideration.

The traveler visited Dr. Sasaki at Kyoto University for discussions of current research. The studies that Dr. Sasaki has been conducting on the nature of the production of retinoblastoma appears to be related to studies of the mechanism of chromosome aberration production by a recombination mechanism involving repetitive DNA sequences being conducted by the traveler at Oak Ridge. It was also particularly informative to compare the results from the two laboratories on the possible role of specific chromosome alterations in the induction or progression of tumors. Dr. Sasaki has access to human tumor samples, while the traveler is utilizing animal models to address similar questions. The information obtained will be shared with colleagues at ORNL.

APPENDIX

Itinerary

March 15-17, 1988	Travel from Oak Ridge, Tennessee to Hiroshima, Japan
March 18-21, 1988	RERF Workshop, Hiroshima, Japan
March 22-23, 1988	Visit with Dr. Masao Sasaki, Kyoto University, Kyoto, Japan
March 23, 1988	Travel from Kyoto, Japan, to Oak Ridge, Tennessee

Literature Acquired

Preprints of research discussed at RERF Workshop

APPENDIX II

10 February 1988

RADIATION SUSCEPTIBILITY WORKSHOP

Place: Radiation Effects Research Foundation
5-2 Hijiyama Park, Minami-ku, Hiroshima
732 Japan

Time and date:

Day 1: 09:00-17:00 hours, 18 March 1988 (Friday)
Day 2: 08:30-17:00 hours, 19 March 1988 (Saturday)
Day 3: 08:30-17:00 hours, 20 March 1988 (Sunday)

List of Expected Participants

Workshop Members:

Dr. Hiraku Takebe (workshop co-chairman), Professor, Department of Experimental Radiology, Faculty of Medicine, Kyoto University

Dr. Taisei Nomura, Professor, Department of Fundamental Radiology, Medical Schoo, Osaka University

Dr. Hiroshi Kasai, Senior Investigator, Biology Division, National Cancer Center Research Institute

Dr. Kouichi Tatsumi, Associate Professor, Department of Molecular Oncology, Faculty of Medicine, Kyoto University

Dr. Richard B. Setlow (workshop co-chairman), Biology Department, Brookhaven National Laboratory

Dr. Kenneth Kraemer, Laboratory of Molecular Carcinogens, National Cancer Institute

Dr. Robert Painter, Associate Director, Laboratory of Radiobiology and Environmental Health, University of California, San Francisco

Dr. R. Julian Preston, Cytogenetics, Biology Division, Oak Ridge National Laboratory

RERF Science Councillors:

Dr. Masanori Kuratsune, Dean, Nakamura Gakuen University and College

Dr. Toshiyuki Kumatori, Chairman, Radiation Effects Association

Dr. Shigefumi Okada, Professor, Radiation Biology Center, Kyoto University

Dr. Barry R. Bloom, Chairman of Microbiology and Immunology, Albert Einstein College of Medicine

List of Expected Workshop Participants (continued)

Dr. Mortimer L. Mendelsohn, Associate Director for Biomedical and Environmental Research, Lawrence Livermore National Laboratory

Dr. Robert W. Miller, Chief of Clinical Epidemiology, National Cancer Institute

Dr. Arno G. Motulsky, Professor of Medicine and Genetics, School of Medicine, University of Washington

Dr. Donovan J. Thompson, Professor Emeritus, University of Washington

Observers:

Dr. Masao Sasaki, Professor, Radiation Biology Center, Kyoto University

Dr. Kenjiro Yokoro, Professor, Research Institute for Nuclear Medicine and Biology, Hiroshima University

Ministry of Health and Welfare representative(s)

Ministry of Foreign Affairs representative

Dr. Robert W. Wood, Acting Associate Director for Health and Environmental Research, Office of Energy Research, Department of Energy

Dr. Alvin G. Lazen, Executive Director, Commission on Life Sciences, National Research Council

Dr. Charles W. Edington, Associate Director for International Affairs, Commission on Life Sciences, National Research Council

Dr. James E. Norman, Assistant Associate Director for International Affairs, Commission on Life Sciences, National Research Council

Mr. Paul Sullivan, Office of the Comptroller, National Academy of Sciences

Dr. Richard W. Getzinger, Counselor for Scientific and Technological Affairs, U.S. Embassy

Mr. Robert M. Jackson, Senior Representative, Department of Energy, U.S. Embassy

Mr. Seymour Jablon, former Associate Director for International Affairs, Commission on Life Sciences, National Research Council

Dr. Gilbert W. Beebe, Clinical Epidemiology Branch, National Cancer Institute

List of Expected Workshop Participants (continued)

Dr. Seymour Abrahamson, Professor, Department of Zoology, University of Wisconsin

Dr. James V. Neel, Professor, Department of Human Genetics, University of Michigan

Dr. Howard B. Hamilton, former Chief, Department of Clinical Laboratories, RERF

RERF Directors:

Dr. Itsuzo Shigematsu, Chairman [Former Director, Department of Epidemiology, Institute of Public Health]

Dr. J.W. Thiessen, Vice Chairman [Former Deputy Associate Director for Health and Environmental Research, Office of Energy Research, U.S. Department of Energy]

Dr. Stuart C. Finch, Permanent Director and Chief of Research [(on leave from) Professor, University of Medicine and Dentistry of New Jersey - Rutgers Medical School]

Dr. Fumihiko Munakata, Permanent Director [Former Director, Health Center Division, Public Health Bureau Ministry of Health and Welfare]

Mr. Tomoyuki Kono, Permanent Director and Chief of Secretariat [Former Director, Social Insurance Training Institute, Ministry of Health and Welfare]

Dr. Tsutomu Sugahara, Visiting Director [Professor Emeritus, Kyoto University]

APPENDIX III

(Tentative Program) 3 March 1988

Radiation Susceptibility Workshop
Hiroshima RERF
March 18 - 20, 1988

Day 1: March 18 (Friday)

9:00 - 9:15	Executive session	Drs. Setlow and Takebe
9:15 - 9:25	Greetings by the RERF Chairman	Dr. Shigematsu

Morning Program Chairman: Dr. Takebe

9:25 - 10:05	Summary of radiation effects, availability of biological materials & definition of the problems	Dr. Finch
10:05 - 10:15	Discussion	
10:15 - 10:30	Coffee break	
10:30 - 11:10	Population samples and DS86 cancer risk estimates	Dr. Kato
11:10 - 11:20	Discussion	
11:20 - 12:00	Lymphocyte, thyroid and fibroblast radiation sensitivity studies	Drs. Nakamura and Ban
12:00 - 12:10	Discussion	
12:10 - 12:25	Group photograph	
12:25 - 1:30	Lunch	

Afternoon Program Chairman: Dr. Setlow

1:30 - 2:10	Current and proposed micronucleus studies at RERF	Dr. Ban
2:10 - 2:20	Discussion	
2:20 - 3:00	Somatic mutation (HPRT, glycophorin A) studies	Drs. Akiyama and Nakamura
3:00 - 3:10	Discussion	
3:10 - 3:25	Coffee break	
3:25 - 4:05	Chromosome radiosensitivity and cancer	Drs. Awa and Ban
4:05 - 4:15	Discussion	

4:15 - 4:35	Cytogenetic outliers	Dr. Awa
4:35 - 4:40	Discussion	
4:40 - 5:00	Somatic mutation outliers	Dr. Akiyama
5:00 - 5:10	Discussion	
6:00	Reception and dinner	

Day 2: March 19 (Saturday)

Morning Program Chairman: Dr. Takebe

8:30 - 10:00 Consideration of hypothesis and population effects

- Possible relationship between acute radiosensitivity and carcinogenesis
- Effective radiation dose range
- Relationship between survival and late effects
- Estimation of maximum effect on surviving populations

10:00 - 10:15 Coffee break

10:15 - 12:00 Radiation sensitivity and carcinogenesis

- Probability of demonstrating significant variation in radiation sensitivity in survivors
- Possible relationship between increased radiation sensitivity and carcinogenesis
- Possible radiation sensitivity studies (repair, free radicals, oxygen protection, etc.)

12:00 - 1:30 Lunch

Afternoon Program Chairman: Dr. Setlow

1:30 - 3:00 Somatic mutation and carcinogenesis

- Types of A-bomb somatic mutation with possible cancer implications (e.g. glycophorins, chromosomes, HLA, DNA, etc.)
- Significance and types of somatic mutations induced by irradiating cells of A-bomb survivors (e.g. micronuclei, DNA mutations of many types, sister chromatid exchange, chromosome aberrations, etc.)

3:00 - 3:15 Coffee break

3:15 - 5:00 Resistance - sensitivity mechanisms

- Evaluation of free radicals scavengers
- Generation of free radicals in response to radiation, peroxidants, etc.
- Evaluation of repair systems (enzymes, etc.)
- Oncogene activation

6:00 Dinner for members of the Workshop only

Day 3: March 20 (Sunday)

Morning Program Chairman: Dr. Takebe

8:30 - 10:00 Open for discussion or special topic presentations

10:00 - 10:15 Coffee break

10:15 - 12:00 Closed session of Workshop for organization of recommendations

12:00 - 1:30 Lunch

1:30 - 3:00 Closed session of Workshop for organization of recommendations

3:00 - 3:15 Coffee break

3:15 - 4:15 Closed session of Workshop for organization of recommendations

4:15 - 4:30 Preliminary recommendations (Drs. Takebe and Setlow)

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