放影研 RERF update
Radiation Effects Research Foundation News and Views
Hiroshima and Nagasaki, Japan
Volume 21, Issue 1, 2010

Radiation Effects Research Foundation

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This newsletter is published by the Radiation Effects Research Foundation (formerly the Atomic Bomb Casualty Commission), established in April 1975 as a private, nonprofit Japanese foundation. It is supported by the government of Japan through its Ministry of Health, Labour and Welfare and that of the United States through the National Academy of Sciences under contract with the Department of Energy.

RERF conducts research and studies—for peaceful purposes—on the medical effects of radiation on humans with a view toward contributing to the maintenance of the health and welfare of atomic-bomb survivors and to the enhancement of the health of all mankind.

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Editorial Policy

Contributions to Update *receive editorial review only and do not receive scientific peer review. The opinions expressed herein are those of the authors only and do not reflect RERF policies or positions.*

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From the Editors

Yoku irasshaimashita!

We welcome you back with the enthusiastic greeting that is shouted by staff as you enter a typical Japanese restaurant. The characteristic and beautiful song of the Uguisu (Japanese bush warbler) in the Hijiyama air once again reminds us that spring is ending, that the sakura have finished blooming, and that Scientific Council has ended (see RERF News) and preparations for the annual Board Meeting are underway. In this first 2010 *Update* you will learn about three somewhat special events for RERF. First, in November RERF hosted for the first time the 52nd Annual Meeting of the Japan Radiation Research Society (see Reports on Conferences and Workshops). Second, with planning and active participation by RERF employees, including pounding rice to make mochi (see cover photo) and breaking open the new barrel of sake (kagami-wari), staff ushered in January with a successful New Year Party. And third, in February, a National Geographic Television film crew spent a long day filming throughout RERF in preparation for a documentary film about the A-bombing of Hiroshima that will include some coverage of RERF research activities.

Breaking open the new barrel of sake at the New Year Party

We are especially pleased to announce that four scientists joined RERF since the last *Update*; please read their self-introductions. A science article describes the goals of a new, large RERF contract with significant support from an institute within the U.S. National Institutes of Health. The contract represents a unique opportunity for RERF scientists, in collaborations with scientists and institutions in Japan and the U.S., to test a hypothesis that radiation enhances the effects of aging on the immune system. A second science article begins a series of *Update* reports that will highlight and summarize the recent findings and ongoing activities of RERF departments. The first article in the series focuses on the research activities of the Department of Clinical Studies.

As always, we invite you to send your questions, comments, or recommendations for how we might improve *Update*. One such inquiry has led to the discovery that ABCC might have influenced the life of an adventurous Belgian physician (check out Human Interest Notes). So until the next issue—Mata oidekudasai (goodbye and please come again)!

Evan B. Douple
Editor-in-Chief

Yuko Ikawa
Technical Editor

Report on the 37th Scientific Council Meeting

The 37th Scientific Council (SC) met from March 3 to 5, 2010 in Hiroshima, Japan to review RERF's scientific programs. It was co-chaired by Dr. Takashi Yanagawa and Dr. John Mulvihill. Dr. Kazuo Sakai of the National Institute of Radiological Sciences (Chiba) joined us as a new member of the SC. To enhance this year's in-depth review of the Department of Clinical Studies (see a summary of the highlights of this department on page 24 of this issue of *Update*), the SC was augmented by special *ad hoc* scientific councilors, Drs. Koji Maemura (Hiroshima University), Yukihito Higashi (Nagasaki University), and David Rush (Tufts University), who added expertise in cardiovascular research, clinical studies, and clinical epidemiology.

Dr. Toshiteru Okubo, Chairman of RERF, opened the meeting with a warm welcome to all in attendance and emphasized the importance of the SC's work to RERF. He explained that RERF must convert to an approved Public Interest Incorporated Foundation to

continue to qualify for funding under the Public Interest Corporation Reform Bill. Other major administrative activities have included employee training in communication skills, leadership, and problem-solving; continued implementation of Senior Review Panel recommendations; hosting, for the first time, the annual meeting of the Japan Radiation Research Society; and continued meetings with the Local Liaison Councils in Hiroshima and Nagasaki.

Following Dr. Okubo's comments, Dr. Roy Shore, Chief of Research at RERF, provided responses to the recommendations of the 2009 SC and an overview of new research developments at RERF. A number of improvements and changes have been made in response to the 2009 SC recommendations: We have begun to use presentations of Initial Concept Proposals (before formal research proposals) to help direct the research focus to program priorities. Research milestones and timelines are beginning to be introduced, and theme-oriented research working groups continue to facilitate interdepartmental planning and communication. Departmental seminars with invitations extended to members of other departments are being encouraged to promote more cross-fertilization of ideas within RERF. To keep in step with advancing technologies, an initial pilot study using large-scale genomic sequencing has been planned. A Data Management and Documentation Committee was formed, with input from all departments, to recommend procedures for quality-control, documentation, and transparent storage and retrieval of data throughout RERF. That Committee will interface with the Committee on Biological Samples to ensure consistency and comprehensiveness in the management of data and biosamples. The Department of Statistics has been upgrading its capabilities in bioinformatics and is working with researchers in the basic science departments to address issues such as handling high-dimensional microarray data.

New or continuing research developments presented at the SC meeting include:

- Efforts to enlarge the numbers of individuals in the Adult Health Study clinical examination program who were under age 10 at the time of the bombs are nearly completed, with about 1,800 newly recruited participants.
- A longitudinal clinical follow-up of about 12,000 children of A-bomb survivors is being planned and should be underway during 2010. The primary purpose is to determine whether parental radiation exposure has an effect upon the incidence of multifactorial diseases in adulthood in the F_1 generation, an issue about which essentially no information is currently available.
- In concert with the aforementioned clinical study, new Genetics Department studies of the F_1 generation of humans and of mice are beginning to determine if radiation dose-related increases in genomic deletions and duplications can be detected with high-density microarrays.
- Investigators in the Department of Radiobiology/Molecular Epidemiology recently obtained a substantial five-year contract from the National Institute of Allergy and Infectious Diseases (NIAID) of the U.S. National Institutes of Health to conduct basic and applied immunological studies in order to further delineate determinants of radiation-induced immunological changes and to assess their health-related effects. RERF has established collaborations with scientists in seven U.S. and six Japanese institutions in order to bring the latest technologies and expertise to bear on the goals of this large project (see summary of this project on page 13 of this issue of *Update*).

A synopsis of the general recommendations of the SC is:

- The SC looks forward to a "road map" establishing research priorities, resource allocations, and research project timelines in relation to RERF's stated five-year goals.
- More collaboration among departments and utilization of interdepartmental working groups is encouraged.
- A centralized, comprehensive database is needed that integrates all the information on study participants and is linked to available biosamples.
- Increased bioinformatics support needs to be developed and coordinated among departments.
- The SC encourages a focus on major first-authored publications, both for individual career enhancement and for the overall reputation of RERF.
- Plans for the training and mentoring of junior scientists at RERF would be useful.

Highlights of a few of the notes and recommendations for departments and committees or working groups are:

- It is recommended that attention be focused on the mechanisms that underlie the radiation-associated excesses of cardiovascular diseases and cataracts. RERF scientists are urged to collaborate with other groups with such expertise.
- The SC recommended that our initial study of the lifetime risk of stroke starting at middle age be extended to younger ages and to other cardiovascular diseases.
- With regard to cardiovascular disease, the Adult Health Study should consider including echocardiography and additional biomarkers for

endothelial dysfunction.
- New plans to assess late cognitive dysfunction among those exposed *in utero* or in early childhood are strongly supported.
- Continuation of the biodosimetry program with measurements of chromosome aberrations in blood and carbonate radicals in teeth is encouraged, particularly at lower exposure levels.
- Preliminary studies of the use of direct sequencing are useful in relation to detecting potential genetic effects.
- Further work is recommended to characterize the radiation dose response for immunological aging and to investigate the role of such aging in disease risk.
- Studies of DNA methylation in pathology specimens will need to carefully distinguish radiation-associated changes from pre-cancerous changes.
- Further studies of *RET/PTC* and *ALK* mutational rearrangements in radiation-related thyroid cancers are useful, and linkage with histopathological and clinical parameters would provide further clarity.
- Additional prioritization of work in the epidemiology program is recommended. This will facilitate the timely publication of important mission-oriented papers in international scientific journals.
- Continued work to develop an inventory and database of pathology specimens held by the Epidemiology Department is supported.
- Increased publication of Statistics methodological papers is encouraged. Development of a flexible methodological framework for the use of individual data for assessing the relationship of radiation dose and disease will be particularly useful.
- Developing a concerted data management and documentation strategy for RERF is a very important effort that is central to the mission of RERF, and critical for the maintenance of its world heritage resource.
- The improvements being implemented in radiation dosimetry are a high priority and should be expedited. When those are completed, new analyses should be conducted to determine if the dosimetry alterations appreciably affect risk estimates.
- The recently developed primer on radiation and health is highly commended. It is recommended that additional strategies be developed to reach out to members of the RERF cohorts, the community, and the international news media.

In summary, the SC felt that RERF is making progress on a number of fronts, and they encourage additional attention to the prioritization and focus of research activities.

RERF Scientific Councilors

Dr. Takashi Yanagawa, Co-chairperson, Professor, The Biostatistics Center, Kurume University

Dr. John J. Mulvihill, Co-chairperson, Professor of Pediatrics, University of Oklahoma Health Sciences Center

Dr. Yoshiharu Yonekura, President, National Institute of Radiological Sciences

Dr. Katsushi Tokunaga, Professor, Department of Human Genetics, Division of International Health, Graduate School of Medicine, The University of Tokyo

Dr. Kiyoshi Miyagawa, Professor, Laboratory of Molecular Radiology, Center for Disease Biology and Medicine, Graduate School of Medicine, The University of Tokyo

Participants of the 37th Scientific Council meeting held at the Hiroshima Laboratory

Dr. Kazuo Sakai, Director, Research Center for Radiation Protection, National Institute of Radiological Sciences

Dr. Marianne Berwick, Professor and Chief, Division of Epidemiology, Associate Director, Cancer Research and Treatment Center, University of New Mexico

Dr. David G. Hoel, Distinguished University Professor, Department of Biostatistics, Bioinformatics and Epidemiology, Medical University of South Carolina

Dr. Michael N. Cornforth, Professor and Director of Biology Division, Department of Radiation Oncology, University of Texas Medical Branch

Dr. Sally A. Amundson, Associate Professor of Radiation Oncology, College of Physicians and Surgeons of Columbia University

Special Scientific Councilors

Dr. David Rush, Emeritus Professor of Nutrition, Community Health (Epidemiology), and Pediatrics, Tufts University

Dr. Koji Maemura, Professor, Department of Cardiovascular Medicine, Course of Medical and Dental Sciences, Graduate School of Biomedical Sciences, Nagasaki University

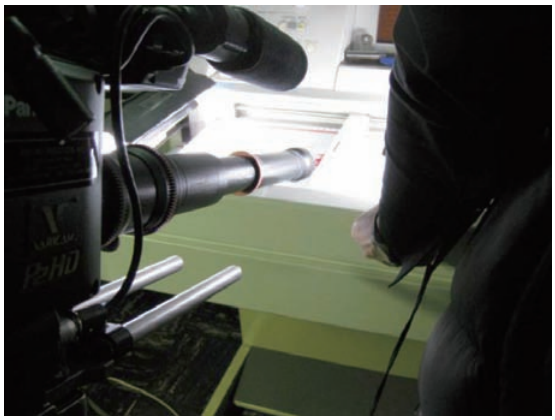
Dr. Yukihito Higashi, Associate Professor, Department of Cardiovascular Physiology and Medicine, Division of Molecular Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University

National Geographic Television Films at RERF

Thursday, 18 February: A busy day started for RERF at 7:00 with the arrival of a filming crew from National Geographic Television (NGT). While the magazine *National Geographic* is known around the world for its remarkable photography and excellent journalism, Americans are also familiar with the award-winning film documentaries and journalism created by NGT. The company produces 120 hours of documentary television each year for the National Geographic Channel, PBS, and other broadcasters worldwide. Last year, NGT officials contacted RERF and indicated that they were producing a new series of scientific documentaries focusing on the earth's major catastrophes. They had selected the atomic-bombing of Hiroshima as the topic of one of their programs and were interested in including the historic and scientific contributions of ABCC and RERF during the 65 years following the bombing. Drs. Roy Shore (RERF Vice Chairman) and Evan Douple (Associate Chief of Research) met with the co-producers, Pamela Wells and Holly Taylor, in December 2009 at the NGT headquarters in Washington, DC, and they came away impressed with the plans for the documentary project that NGT officials described during the preliminary meeting.

The film crew started filming the project in San Francisco, where they interviewed A-bomb survivors. The crew then traveled to Hiroshima, where they spent about one week filming. Ms. Taylor spent a couple of hours on 16 February 2010 touring RERF

NGT team filming medical examinations of an AHS participant at the clinic



Special close-up lens used by NGT filming a recording of tooth ESR signals

and discussing potential filming sites. The six-person crew, including Rob Lyall, Director of Photography, and Gabe Monts, sound man, arrived on 18 February and spent 13 hours setting up their equipment and filming in high-definition TV. The filming included scenes of a medical exam of an Adult Health Study (AHS) participant with the assistance of Dr. Kazuo Neriishi and nurses Michiko Kuwamoto and Junko Hino, the extensive cabinets containing files in the clinical records room, ESR (electron spin resonance) tooth dosimetry measurements with the assistance of Toshie Inoue and Drs. Nori Nakamura and Yuko Hirai, storage of lymphocytes in liquid nitrogen with the assistance of Dr. Tomonori Hayashi, interviews with Drs. Neriishi and Douple about the research studies and history of ABCC and RERF, and several views within the RERF facilities.

After darkness returned to Hijiyama and the many containers of equipment had been loaded back into the NGT vans, the film-crew members expressed their feelings of appreciation for the cooperation of so many RERF employees and for all they had learned about RERF. They were very impressed with RERF's many dedicated employees, the important contributions of the A-bomb survivors, and the unique and historic work that has been done here. Those of us who were impressed by the professional and creative skill of the dedicated filming crew members are confident that the NGT team's efforts will result in a quality product, in the form of an hour-long documentary, that we hope will help to communicate a positive image of RERF around the world. That product is expected to be completed this summer and should appear on television in October or November. We thank all RERF employees who provided assistance or altered their work schedules to accommodate this important public-relations activity.

Staff News

We are pleased to report that four scientists have joined RERF since the last issue of *Update*. **Seishi Kyoizumi** was appointed Project Research Scientist in the Laboratory of Immunology, Department of Radiobiology/Molecular Epidemiology (RME) effective April 1, 2010. He is no stranger to RERF since he started his career as a research scientist in RERF in 1982. He served as Chief of the Immunology Laboratory between 1994–2003 in what was the Department of Radiobiology and he served as Assistant Chief of the Department of RME 2003–2004 before accepting a position as Professor of Nutritional Sciences in Yasuda Women's University. **Junko Kajimura** accepted a Research Scientist position in the Laboratory of Immunology of RME effective April 1, 2010. She was most recently a research associate at Yasuda Women's University and prior to that was a research fellow in the Research Institute for Radiation Biology and Medicine at Hiroshima University from 2005–2007. Two statisticians have recently joined the Department of Statistics. **Ravindra Khattree** began work as a senior scientist at RERF April 26 and comes to RERF from Oakland University in Rochester, Michigan where he has been a Professor of Mathematics and Statistics since 1991. **Robert D. Abbott**, formerly Professor of Biostatistics in the Division of Biostatistics and Epidemiology at the University of Virginia School of Medicine in Charlottesville since 1982, started work as a senior scientist at RERF May 10. Both statisticians were recruited by the National Academy of Sciences and RERF is especially fortunate to have two very experienced scientists join its research team. We have asked the four new members to introduce themselves in this issue of *Update*.

Harry M. Cullings was promoted from Acting Chief to Chief of the Department of Statistics effective April 15, 2010. He began his employment at RERF in October of 1999 as a National Academy of Sciences recruit. Since then, he has been involved in providing extensive statistical consultation to other departments at RERF in support of various types of studies and he was especially instrumental in the implementation of the DS02 system for calculating survivors' radiation doses for use in RERF studies.

Seishi Kyoizumi


Seishi Kyoizumi, Project Research Scientist

For the purpose of joining the National Institute of Allergy and Infectious Diseases (NIAID)-funded project, I came back to RERF for the first time in six years. I spent those years on the faculty of the Department of Nutritional Management, Yasuda Women's University, but I feel that I am more adapted for life centering on research activities than for education-centered life.

Even though I felt anxious about a period of six years away from the cutting edge of scientific research, I was able to readjust my brain into research mode in early April. (Of course, I experience slowing

of thought appropriately for my age.) The scientific research community is basically competitive. Researchers must make important findings ahead of other researchers in order to be praised. In the field of biology, methods that can efficiently narrow down targets with the use of genome-wide high-speed screening have become more common. A high-budget approach can obtain the outcome faster than low-budget ones. When employing such methodology, originality in research will be shown in terms of either a subject of research (phenomenon) or how to approach the subject phenomenon with the use of collected data. In the case of A-bomb survivor studies, the sample itself is a very valuable population. Meanwhile, aging of the population is noticeable. The mission of RERF research scientists is to verify hypotheses as efficiently as possible and obtain correct findings. Specifically, the NIAID project, which aims at elucidation of effects of immunological aging and radiation exposure on disease development mechanisms on the basis of epidemiological findings regarding diseases, is highly original. I hope to make humble contributions to the aforementioned project.

Junko Kajimura

Junko Kajimura, Research Scientist

By way of introduction, my name is Junko Kajimura, and I started work at the Department of Radiobiology/Molecular Epidemiology on April 1 of this year. As a matter of fact, this is not the first time for me to work at RERF, since I belonged to the aforementioned department as a visiting scientist during the period from 2005 through 2007. After working at the Yasuda Women's University starting in 2007, I came back to RERF for the purpose of joining the NIAID-funded project.

I will introduce my background in brief. After I received a Ph.D. at Hiroshima University, I was employed as a postdoctoral fellow at the Uniformed Services University of the Health Sciences (USUHS) in Maryland, U.S. and conducted research activities with a focus on microbial glycosylation for three years. After returning home, I was employed as a research scientist at the Research Institute for Radiation Biology and Medicine, Hiroshima University and engaged in research on translesion DNA synthesis, while serving as a visiting scientist at the Department of Radiobiology/Molecular Epidemiology. Then, I served as a research associate at the Yasuda Women's University before returning to RERF.

In my childhood, I lived in the vicinity of Hijiyama, and a park behind the RERF facilities, called "Hijiyama amusement park," was one of my favorite playgrounds. RERF's unique quonset-hut buildings seemed very mysterious to me at that time, and I used to wonder what happened inside the facilities with much interest. Now I am given an opportunity to be engaged in research activities as a full-time research scientist at RERF, my childhood "mysterious research institute." Even though areas surrounding Hijiyama are noticeably different now due to the Danbara district redevelopment project, I am going to work on research every day at the buildings, which are maintained in their original state. I feel this is a kind of fateful link.

Since I am new to my post and short of perfection, I hope to perform day-to-day research operations under the direction of, and with support from, research scientists and the staff. I would be grateful for your support.

Ravindra (Ravi) Khattree

Ravindra Khattree, Senior Scientist

All my life I have been interested in statistics and various aspects of it. I received an M. Stat. from the Indian Statistical Institute and, in 1985, received a Ph.D. from the University of Pittsburgh. I started my academic career as an Assistant Professor at the North Dakota State University. I spent two years at BFGoodrich Chemical Groups as a Senior Research and Development Statistician working on development of several projects and various environmental and safety issues. While there, I also served as an adjunct faculty member of Case Western Reserve University. I moved to Oakland University in 1991 where I served as an associate and then full professor for the last 19 years, with a one-year leave in between to work as a professor in the Department of Epidemiology and as Biostatistics Group Leader at the Biomedical Research and Informatics Center, both positions in the College of Human Medicine, Michigan State University. My academic research interests have been in multivariate and longitudinal data analysis and in bioequivalence problems, and my interdisciplinary research mostly focuses on cancer and mental health-related problems.

I have always wanted to work in areas where I can contribute, in some substantive way, to the good of the society at large. That was a main motivation for me to join RERF as I consider the implications of the research done here to be far reaching and very relevant to the efforts of making this world a better place to live. I look forward to getting involved in as many projects as possible. I love challenges and I love statistics so I invite all of you to discuss with

me the statistical aspects of your research projects if you think that I can be of help.

Although I have been here only a few days, I am already beginning to love Hiroshima. With alternating sunny days and rainy days and the smell of the soil, it reminds me of my childhood days in India. The densha (streetcar) services remind me of my youth in Kolkata and the clean environment and organizational efficiency of the city is reminiscent to that in Michigan, USA. I enjoyed going to the Flower Festival. The richness of the Japanese culture and the sincere efforts to practice and preserve it, were very evident during the Flower Festival.

I am looking forward to working with all of you during the years to come and learning from you. Apart from learning about various research projects, another thing which I would like to learn more about, while in Japan, is Buddhism as practiced in Japan. I would like to visit as many places of Buddhist heritage as possible.

Robert D. Abbott

After graduating from the University of Michigan with a Ph.D. in biostatistics, I joined the staff at the U.S. National Heart, Lung, and Blood Institute in 1980. During that time, I became involved in two long-term follow-up studies (similar to the RERF Adult Health Study). One study was the Framingham Study, and the other was the Honolulu Heart Program (HHP). The HHP was created as part of the NI-HON-SAN study (the HON part) to help explain the changing risk of stroke and heart disease as Japanese migrated from Japan to Hawaii and California. The NI part included the RERF Adult Health Study.

Robert D. Abbott, Senior Scientist

Although I later accepted a faculty position at the University of Virginia in 1989, I continued my HHP involvement. During frequent visits to Japan, I participated in comparisons between the HHP and cohorts in Japan that were genetically homogeneous but environmentally different. Those collaborations continued during a sabbatical year at Shiga University of Medical Science (2006–2007) through an Invitation Fellowship from the Japan Society for the Promotion of Science. My host scientist was Hirotsugu Ueshima. At the conclusion of that rewarding experience, my goal was to return to Japan on a permanent basis. With a long-term familiarity with the RERF and its proud history in statistics and epidemiology, I knew that an appointment at the RERF would be a highlight in my career. The timing was perfect when RERF openings were announced in 2009. I am now grateful and most fortunate to be a part of this prestigious organization.

2010 Distinguished Lecture Series

The first Distinguished Lecturer of 2010 was **Peter W. Laird, Ph.D.** who delivered the keynote address to open the International Workshop on Epigenetics in Radiation Effects among A-bomb Survivors and their Children, March 17, 2010. The title of his lecture was "The Cancer Epigenome: Origins and Applications."

Dr. Laird is Associate Professor of Surgery and of Biochemistry and Molecular Biology at the University of Southern California (USC), Keck School of Medicine. He is Director of the USC Epigenome Center, Director of Basic Research for the Department of Surgery, and Program Leader of the Epigenetics and Regulation Program at the USC Norris Comprehensive Cancer Center. Dr. Laird received his Ph.D. from the University of Amsterdam. He pioneered the use of mouse models to investigate the causal contribution of DNA methylation to cancer and invented two DNA methylation assays, COBRA and MethyLight. In his lecture he provided an introduction to the revolution in DNA methylation analysis technology that has taken place over the past decade and suggested some approaches that RERF scientists might consider as they explore applications of next-generation sequencing (see P. W. Laird, Principles and challenges of genome-wide DNA methylation analysis. *Nature Reviews/Genetics* 11:191–203; 2010).

Report of the 52nd Annual Meeting of the Japan Radiation Research Society

Kazunori Kodama, Chief Scientist and Chairman of the Planning Committee for the 52nd JRRS Meeting

The 52nd Annual Meeting of the Japan Radiation Research Society (JRRS) was held with great success at the Hiroshima Minami Ward Community Cultural Center on November 11–13, 2009. A total of 224 presentations including poster presentations were submitted and as many as 461 people participated in the meeting. RERF made foundation-wide efforts to organize this meeting, with many RERF staff providing support either at the Center or from RERF. I would hereby like to take this opportunity to provide a brief description of the 52nd JRRS meeting.

The first JRRS meeting was held in Tokyo in 1959. A total of six meetings had been held in Hiroshima (from 1962 until 2005), all of which were hosted by Hiroshima University. RERF has studied health effects from radiation for over 60 years since the days of ABCC, and it was believed that hosting the JRRS meeting would provide RERF with an opportunity to inform JRRS members of its research activities and that supporting such meetings was one of RERF's duties. For those reasons, RERF Chairman **Toshiteru Okubo** decided that RERF would promote itself as a candidate for organizer of the meeting. In meetings of the JRRS Board of Councilors and general assembly, RERF was thus chosen to be the organizer for the 52nd meeting.

The meeting's working committee and program committee, including leading external members, were established under the leadership of Chairman Okubo, who served as President of the meeting. I was appointed as chairman of the working committee, and **Nori Nakamura**, RERF Chief Scientist, as chairman of the program committee.

Prior to the meeting, the working committee met 11 times to make careful planning and preparations, and the RERF staff members appointed to the meeting's secretariat traveled numerous times to the Hiroshima Minami Ward Community Cultural Center to inspect the venue and discuss the meeting details with the staff there. It was decided that RERF would develop an in-house computer system for management of the meeting. A registration system for enrollment and presentations via the website of the meeting was developed by the Department of Information Technology (ITD), under the supervision of Department Chief **Hiroaki Katayama**. ITD also developed an operation and management system for the meeting venue (e.g., registration of participants and presentation of slides during the meeting).

The scientific program for the meeting was carefully developed under the leadership of Dr. Nakamura, chairman of the program committee, with a focus on the main theme "Radiation Health Effects and Epidemiology—From DNA Repair to Epidemiology." Many fresh ideas were incorporated into the scientific program developed by Dr. Nakamura and others. The program was composed of many facets including two special lectures, one special program, one special session, three luncheon seminars, three symposia, eight workshops, 78 general presentations (oral presentations), and 146 poster presentations. A number of RERF research scientists also made presentations. The following are some of the highlights of the meeting.

Although many JRRS members are fairly knowledgeable about RERF's study results, they seemed to have less understanding of the process by which such results are generated. It was therefore decided to take this opportunity to provide a brief description about RERF's epidemiological studies.

In the session "A Quick Lesson on Epidemiological Studies of A-bomb Radiation Effects," Dr. Okubo delivered a lecture titled "The Outline of Epidemiological Studies at the Radiation Effects Research Foundation and Changes in the Dosimetry System." **Yuko Hirai**, Senior Scientist of the Genetics Department, talked about "Biodosimetry and Its Implications for Epidemiological Studies." Both presentations were designed to explain the methodology of the epidemiological studies conducted at RERF. **Kotaro Ozasa**, Chief of the Epidemiology Department, and **Saeko Fujiwara**, Chief of the Clinical Studies Department, then outlined study results by providing lectures titled "Epidemiological Study: Key Points of the Results" and "Clinical Study: Key Points of the Results," respectively. Although this session started after 5:00 p.m., many JRRS members turned up to listen to the lectures with great interest. I believe that one of our initial goals was thus fully achieved.

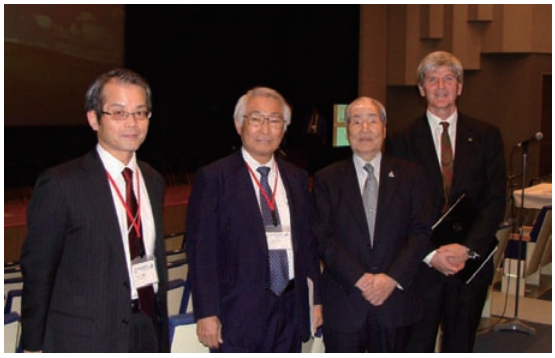
On the second day of the meeting, **John J. Mulvihill**, RERF Scientific Councilor and Professor at the University of Oklahoma Health Sciences Center, gave a special lecture titled "Genetic Epidemiology of Offspring of Cancer Survivors: A Window on Human Germ Cell Mutagenesis." While providing comparisons with past results from the studies of the children of A-bomb survivors, he reported that a large-scale study of offspring of

cancer survivors did not show any high risk of genetic effects.

It was decided, based on the strong desire of Dr. Okubo, to include a session only possible in Hiroshima. Hence the special program "Message from Hiroshima" was held. In this session, **Sunao Tsuboi**, Chairman of the Hiroshima Prefectural Confederation of A-bomb Sufferers Organization, delivered a moving lecture titled "Close to Death," in which he talked about the health problems experienced by A-bomb survivors based on his own experience as a survivor and expressed his fervent desire for the elimination of nuclear weapons.

The next lecture, titled "Chance to Eliminate Nuclear Weapons—Why President Obama Needs Japan," was given by **Steven Lloyd Leeper**, Chairman of the Board of Directors, Hiroshima Peace Culture Foundation. He provided his insight on the world situation pertaining to nuclear weapons and the necessity of nuclear abolition. His lucid explanation for such a complex issue resulted in the entire audience listening attentively to his lecture.

On the first and second days of the meeting, luncheon seminars focusing on the theme "Peace Activities and International Contributions of Hiroshima" were given. In these seminars, the audience was able to listen while enjoying lunch. **Hiroo Dohy**, Director of the Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, representing the Hiroshima International Council for Health Care of the Radiation-exposed (HICARE), and **Shizuteru Usui**, President of the Hiroshima Prefectural Medical Association, representing the International Physicians for the Prevention of Nuclear War (IPPNW) Japan Office, talked about the activities of HICARE and the IPPNW Japan Office, respectively. In addition, HICARE and the Hiroshima Peace Culture Foundation both set up panel exhibitions.



(From left) Takanobu Teramoto, RERF Permanent Director, Toshiteru Okubo, RERF Chairman, Sunao Tsuboi, Chairman of the Hiroshima Prefectural Confederation of A-bomb Sufferers Organization, and Steven Lloyd Leeper, Chairman of the Hiroshima Peace Culture Foundation

On the last day of the meeting, a lecture open to the public was hosted jointly with HICARE at the Hiroshima International Conference Hall. **Kenzo Oshima**, Vice President of the Japan International Cooperation Agency (JICA) and the former Permanent Representative of Japan to the United Nations, delivered a lecture titled "Recent Situation Concerning Support for Radiation-exposed by International Organizations." Although this venue and the Minami Ward Community Cultural Center are separated by some distance, there was a good turnout of about 150 people.

To the best of my knowledge, this is the fourth annual meeting of a national academic society RERF has organized. Although RERF now has accumulated significant experience in organizing academic meetings, this was the first annual meeting of a national academic society for which RERF made foundation-wide efforts. I would like to express my sincere appreciation for the hard work and dedication of the entire RERF staff.

International Epigenetics Workshop

Evan B. Douple, Associate Chief of Research

The "**International Workshop on Epigenetics in Radiation Effects among A-bomb Survivors and Their Children**" was held at Hiroshima Laboratory on March 17–18, 2010. It included 13 invited speakers from six Japanese universities (Waseda, Hiroshima, Kumamoto, Kyushu, Tokyo Medical and Dental, and Sapporo Medical), RIKEN Kobe Institute, RERF, and two U.S. universities (Southern California and Brown). The workshop was officially opened with words of welcome and appreciation from RERF Chairman **Toshiteru Okubo**. He explained that the goal of the workshop was to have experts introduce RERF scientists to the emerging frontier of epigenetics, a field that has grown exponentially in recent years, and to enable RERF speakers to introduce leading experts in the field of epigenetics to RERF's research results, ongoing and future plans, and unique biosample resources. He concluded his opening remarks by explaining that the workshop was dedicated to **Donald G. MacPhee**,

former RERF Research Advisor and Chief of the Department of Radiobiology, in recognition of his active encouragement of RERF scientists to explore the field of epigenetics.

The workshop was organized into four major sessions and the first session was titled "Epigenetics: Overview and background of the field and of RERF and its resources." The opening keynote lecture was delivered by **Peter W. Laird** who provided an introductory overview of epigenetics—the study of inherited changes in phenotype (appearance) or gene expression caused by mechanisms other than changes in the underlying DNA sequence. The inherited changes may remain through several cell divisions or for the remainder of the cell's life, and they may also be transmitted to multiple generations. In other words, non-genetic factors cause the organism's genes to behave or "express themselves" differently. Dr. Laird explained that it has become clear that the origin of health and susceptibility to disease are, in part, the result of epigenetic regulation of the genetic blueprint and such effects may explain a number of human diseases including cancer. A form of epigenetics called genomic imprinting (the differential expression of a gene depending on whether it was inherited from the father or mother), may explain a number of human diseases and may be influenced by factors such as nutrition and exposure to environmental agents including low doses of ionizing radiation. Dr. Laird summarized his talk with the following points:

- Epigenetics determines gene expression potential, rather than gene expression state.
- In mouse model systems such as ApcMin mice, sufficient levels of DNA methyltransferase expression are as essential for tumor formation as is the predisposing mutation in the Apc gene.
- An epigenetically defined subtype of colorectal cancer, referred to as CpG island methylator phenotype (CIMP), is very tightly associated with BRAF mutation. IGFBP7 is necessary and sufficient for BRAF-induced apoptosis and senescence. Epigenetic silencing of IGFBP7 by CIMP is likely a prerequisite to acquisition of a BRAF mutation in colorectal cancer.
- An epigenetically defined subtype of brain glioma, referred to as G-CIMP, is very tightly associated with IDH1 mutation. G-CIMP patients are younger, and have better survival, than non-G-CIMP patients.
- Genes occupied by Polycomb repressors in stem cells are predisposed to acquiring cancer-specific DNA methylation.
- It is now technically feasible to perform a whole-genome, shotgun-approach bisulfate sequencing to generate DNA methylation profiles at single-basepair resolution.
- Sensitive techniques, such as Digital Methy-Light, can be used to detect abnormal DNA methylation patterns in the bloodstream of cancer patients.

RERF's Chief of Research, **Roy E. Shore**, presented background about RERF in his talk "RERF: A unique laboratory for the study of human radiation epigenetic effects and disease." He pointed out six criteria that the Adult Health Study's cohort met that made RERF a unique and unparalleled laboratory for the study of human radiation epigenetic effects and disease: (1) having a well-defined prospective cohort with a large range of accuarately determined radiation exposure levels; (2) having a substantial number of disease cases; (3) having appropriate types of biosamples to permit assays for various epigenetic endpoints; (4) having biosamples that predate the development of the disease(s); (5) having a variety of exposure ages and both genders; and (6) having information on lifestyle and other exposure to adjust for those confounders and/or to evaluate them as modifiers of the radiation effect.

Hiroyuki Sasaki closed the first session with a review of genomic imprinting—epigenetic programming in the germline of mammals that causes parental-origin-specific monoallelic expression of a small subset of genes through differential DNA methylation. His laboratory had previously shown that the *de novo* DNA methyltransferase Dnmt3a is responsible for establishment of the germ-line specific methylation imprints. He proposed a model in which the Piwi-interacting RNA (piRNA) and its target RNA play a critical role in methylation and imprinting of the mouse Rasgrf1 locus.

A second session focused on: "Imprinting, development, and fetal origins of adult disease." **Hideoki Fukuoka** described the fetal origin of adult disease (FOAD) theory and its importance for understanding the patho-physiology of developing lifestyle-related adult disease that has been changing to be called the developmental origins of health and disease (DOHaD). The mechanism for the FOAD theory is believed to be epigenetic modification caused by intra-uterine malnutrition. The one carbon metabolism regulates

Chairman Toshiteru Okubo (right) opens the International Epigenetics Workshop.

the epigenetic modification with DNA methylation and folate vitamins B6 and B12 are key nutrients for the metabolism. **Jun-ichi Nakayama** described a role of chromodomain (CD) proteins in the epigenetic gene regulation and DNA damage response. He and his colleagues have shown that the CD, an evolutionarily conserved protein module, binds to methylated histones and thus plays diverse roles in epigenetic gene regulation and chromatin dynamics. MRG15 is one example that binds to Lys36-methylated histone H3 and is linked to cellular senescence in human cells. The author's recent results suggest that MRG15 is a key molecule in epigenetic gene regulation and regulates homology-directed repair of chromosomal breaks, and possibly is a molecular linker between BRCA1 and BRCA2.

A third session focused on "Epigenetics and carcinogenesis." Although a family emergency prohibited **Olga Kovalchuk** from attending the workshop, she provided her recent publication (*Environmental and Molecular Mutagenesis* 49:16–25, 2008) in which she hypothesized that changes in global and regional DNA methylation and regulatory microRNAs play pivotal roles in radiation responses and radiation-induced genomic instability. **Toshiya Inaba** pointed out that epigenetic dysregulation can abolish the expression of anti-oncogenes allowing the development and/or promotion of malignant transformation. He posed the question "Is epigenetic suppression of the anti-oncogenes equivalent to genetic deletion in oncogenesis?" Monosomy 7, or deletion of the long arm of chromosome 7 (-7/7q-) is the most frequently observed chromosome anomaly in myeloid leukemia among irradiated persons, as well as in the general population. Inaba's group has recently identified Samd9L gene on chromosome band 7q21 as a promising candidate for the responsible gene for -7/7q-. He described recent studies in which results suggest that genetic deletion of genes and transcriptional/epigenetic downregulation play different biological roles in oncogenesis.

Mitsuyoshi Nakao reported his finding that the high mobility group A (HMGA) proteins are overexpressed, directly inhibit RB, and maintain malignant features in cancers. He concluded that DNA methylation and chromatin factors are important for cell regulation and deregulation.

Karl T. Kelsey described the work in his laboratory using array platforms to delineate the extent of variation in normal tissues, including variation associated with age and with environmental exposures. They have shown that reduction in the extent of methylation of the repetitive LINE1 region, detectable in peripheral blood, is associated with an increased risk of sporadic head and neck cancer and bladder cancer. They have proposed that risk may be modified by exposures or diet.

DNA methylation plays a critical role in silencing cancer-related genes and **Minoru Toyota** and his colleagues have shown that subsets of colorectal cancers (CRCs) result in genome-wide methylation defects that they call CIMP. New methods for detecting DNA methylation have enabled his group to create epigenetic profiles of CRCs and to classify them into three distinct subgroups based on genetic and epigenetic alterations: CIMP1, CIMP2, and CIMP negative. They have hypothesized that genes inactivated by DNA methylation are involved in the BRAF and p53 signaling pathways. Methylation of IGFBP7 was correlated with BRAF mutations, an absence of p53 mutations, and the presence of CIMP. Thus, epigenetic inactivation of IGFBP7 appears to play a key role in the carcinogenesis of CRCs with CIMP by enabling escape from p53-induced senescence.

To clarify the role of miRNA in gastric carcinogenesis, **Yasuhito Yuasa** and his colleagues performed miRNA microarray analyses and investigated expression changes of miRNAs in a gastric cancer cell line. The miR-181c exhibited CpG islands in their upstream sequences, and their up-regulation was verified by reverse transcription-polymerase chain reaction (RT-PCR) analysis. Their results indicated that miR-181c may be silenced through methylation and play important roles in gastric carcinogenesis through its target genes such as NOTCH4 and KRAS. They also examined the methylation status of six tumor-related genes in primary gastric carcinomas by methylation-specific PCR and compared them to the past lifestyles of the patients. Significant association was found between a decreased intake of green tea and methylation of CDX2 and BMP-2, and more physical activity was correlated with a lower methylation frequency of CACNA2D3. They are examining further whether the methylation states of blood leukocyte DNA can be a surrogate marker for liability to cancer.

The final session, "Brainstorming: A role for RERF in epigenetic research," included presentations by four RERF scientists who described their preliminary results and future research plans:

- **Norio Takahashi** asked the question: "How can we best utilize the trio-samples in future epigenetic studies?" As background information, he summarized the studies of trans-generational effects of A-bomb irradiation that have been conducted by the Genetics Department, and he described the archival specimens stored in the department.
- **Reiko Ito** described results of a pilot study: "Epigenetic alterations in colorectal cancer among atomic-bomb survivors." The study focused on microsatellite instability (MSI)-related CRC, and analyzed both epigenetic and genetic alterations of MLH1 and selected Ras-signaling related genes, as well as CIMP.

Although CIMP status significantly correlated with MLH1 methylation, no significant association was found with MSI-H and radiation exposure.

- **Yasuharu Niwa** discussed the establishment and validation of assay systems for evaluation of radiation- and aging-related epigenetic alterations in blood cells, including changes in gene-specific methylation and H3K9 acetylation frequencies. Combined bisulfite restriction analysis (COBRA) and enzyme-linked immunosorbent assay (ELISA) methods were combined for detection of DNA methylation and histone acetylation, respectively. Global DNA methylation was evaluated with methylation frequency in the long interspersed nuclear element 1 (LINE1), and gene-specific methylation was assessed for several aging-related genes including TUSC3. Histone H3 lysine 9 (H3K9) acetylation was also examined.
- **Kazuo Neriishi** presented results of annual exams that included measuring systolic blood pressure and systolic hypertension in 1,014 adolescent A-bomb survivors (at ages 9–19) who were exposed *in utero*. For the measurements of systolic blood pressure, the dose effect was an increase of 2.09 mmHg per Gy and was significant ($p = 0.017$). A significant radiation dose effect was found for the second trimester ($p = 0.001$) with an estimated 4.17 mmHg per Gy. For the prevalence of systolic hypertension, the radiation dose effect was significant ($p = 0.009$); the odds ratio at 1 Gy was 2.23 (95% confidence interval: 1.23, 4.04).

RERF scientists received valuable information, encouragement, and advice on how best to determine whether epigenetics might have an important role in the increased health effects observed in the A-bomb survivors and how best to use RERF's valuable databases and biosamples to address this question. The participants actively and frankly exchanged their views, and some of the invited experts expressed an interest in exploring future research collaborations with RERF scientists. A summary of their major observations includes the following points:

- Epigenetics is likely to mediate some aspects of susceptibility to radiation-associated effects, including the cancers, but also the cardiovascular and other non-cancer health effects recorded in the A-bomb survivors.
- Array-based platforms are well suited to biomarker discovery in cancer and non-cancer health effects.
- The RERF cohorts and the biosample resources represent an extraordinary opportunity for exploration of epigenetic hypotheses.
- Additional efforts to obtain tumor specimens are warranted.
- The *in utero*-exposed cohort is particularly important for exploration of epigenetic hypotheses.

Participants of the International Epigenetics Workshop held at the Hiroshima Laboratory

A Special Opportunity for RERF to Expand Studies of Immunosenescence and Other Late Effects of Acute Ionizing Radiation Exposure in A-bomb Survivors

Yoichiro Kusunoki,[1] Tomonori Hayashi,[1] Evan B. Douple,[2] and Kei Nakachi[3]

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Abstract

A new project has begun at RERF that is based on the results of RERF's studies of the effects of radiation on the immune system of A-bomb survivors conducted over two decades. Funding provided by the U.S. National Institute of Allergy and Infectious Diseases (NIAID) will enable RERF researchers to strengthen their efforts to define effects of ionizing radiation on aging of the human immune system (immunosenescence) and elucidate underlying responsible mechanisms. The proposed approach rests on three critical and unique advantages: (i) utilization of the large, unique database and biosamples from a 63-year follow-up of A-bomb survivors by RERF; (ii) integration of complementary *in vitro* and *in vivo* mechanistic studies (including a humanized mouse model) to help guide the studies in humans; and (iii) synergistic efforts of co-investigators from five U.S. and four Japanese institutions who bring key expertise and methodologies to assist RERF in analyses and attaining study objectives. The research program consists of four focused projects with explicit goals of assessing mechanisms and effects of radiation on immune aging and dysfunction: (1) the role of hematopoietic stem cells and their microenvironment in T-cell development; (2) dendritic cell numbers, responsiveness, and function; (3) immune responsiveness to influenza vaccination; and (4) a multivariable assessment of immune patterns to create an integrated scoring system of immune competence. Project 4 will incorporate RERF's previously and newly studied parameters with novel findings from projects 1–3, in order to provide new analytical tools for radiation effects, aging, and disease outcomes. The results of the proposed studies will not only provide a wealth of fundamental biological information on the process of—and the impact of radiation on—aging of the immune system, but may also provide insights that lead to amelioration of immune dysfunction and other adverse health outcomes in A-bomb survivors and radiation-exposed older adults in general.

A. Background Relative to Immunosenescence and Radiation

RERF's mission is to "conduct research, for peaceful purposes, on the medical effects of radiation on man, with a view to contributing to the health and welfare of the atomic-bomb survivors and to the enhancement of the health of mankind." RERF scientists and the scientists of its predecessor institution, the ABCC, have been evaluating, documenting, and publishing for the past 63 years the long-term health effects associated with the ionizing radiations emitted by the A-bombs in Hiroshima and Nagasaki (see for example mortality studies summarized in refs.1–6). Much of the research of ABCC, and now RERF, has focused upon characterization of the effects of the A-bomb radiation exposure on the immunological parameters in the survivors. Those studies are being conducted in an aging population in one of the longest-living and most homogeneous populations in the world. So when NIAID identified an important need for more information on the effects of radiation on the immune system and immunosenescence, RERF was in a unique position to address basic science questions that are of direct relevance to understanding effects of radiation exposure on the immune system across the lifespan.

Immunosenescence is the gradual deterioration of the immune system with aging as illustrated in the Figure on the next page. As a natural physiological process, immunosenescence is involved in a range of vital activities, including: (i) degradation of host capacity to respond to infections; (ii) diminished development and retention of long-term immune memory, including the one generated by vaccination; (iii) alteration of immune cell turnover; and (iv) an imbalance between innate and adaptive immunity, potentially causing enhanced and persistent inflammatory responses. Immunosenescence is a major contributory factor to the increased frequency of morbidity and mortality among the elderly from various aging-related diseases as also illustrated in the Figure.

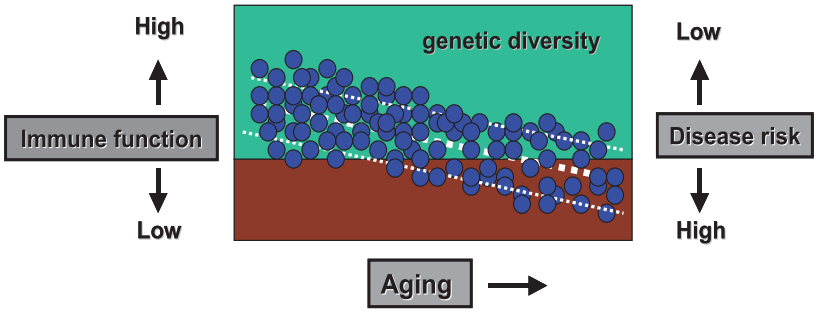

Figure. Immunosenescence as a natural aging process

Understanding the effects of radiation on immunosenescence should lay a foundation to mechanistically explain some of the long-term health effects that have been previously documented in the A-bomb survivors. That knowledge will help develop a more robust database for radiation hygienic purposes, especially in terms of protecting exposed populations.

NIAID and RERF held a workshop 27–29 November 2007 in Hiroshima that was organized with the guidance of RERF's Associate Chief of Research, Thomas Seed. The workshop goal was to review the RERF's extensive information regarding immunological effects in the A-bomb survivors and to explore areas where additional scientific information was needed (see workshop report by Y. Kusunoki in *Update* Volume 19, Issue 1, 2008). In 2008, NIAID issued a request for proposals (RFP) that solicited research proposals that would explore the effects of radiation exposure on immunosenescence. RERF responded to the NIAID RFP and the scientific work outlined in the resultant proposal clearly is consistent with the research mission of RERF.

Several major study groups (cohorts) have been established and tracked by ABCC-RERF scientists over several decades relative to the long-term health effects associated with the A-bomb irradiations. The "Life Span Study" (LSS) cohort is the major cohort and includes some 120,000 individuals. Based on intensive follow-up studies of those cohorts, dose-dependent relationships between radiation exposure and subsequent mortality and morbidity responses have been defined and documented. The volume of data collected is enormous, especially from the "Adult Health Study" (AHS) cohort, whose members have undergone biennial health examinations with a high participation rate and have donated blood samples over a period of five decades. The strength of the studies has been based on the fact that the cohorts are quite large, the radiation doses for individuals cover a wide range, and the doses have been reconstructed using a sophisticated and recently upgraded dosimetry system, called DS02.[7,8]

The RERF biosample repository includes a large number of autopsy specimens, frozen blood fractions (plasma and sera), and frozen, immortalized, viable lymphocytes. While some of the biosamples have been used in a number of studies, RERF has been very cautious in utilizing this valuable resource since scientific technologies for analyses of such biosamples have been developing rapidly and improving their capabilities, speed, sensitivities, accuracy, and efficiency. While in the past RERF has probably been judged to be prudent in not consuming the precious and finite resource, especially given their uniqueness and potential value, two recent reports (by the RERF Senior Review Panel and the RERF Scientific Council) have encouraged RERF scientists to begin to apply the newest and most appropriate technologies to address some important scientific questions. In order to make the optimum and most efficacious analyses, the scientists were encouraged to collaborate with the best scientists from Japan and other countries and to conduct the experiments in order to take advantage of the expertise and the expensive equipment and special facilities required for implementing the latest scientific methodologies. In summary, RERF's scientists were uniquely poised to respond to the NIAID RFP and have developed a work plan in collaboration with a carefully selected team composed of scientific collaborators from institutions in the U.S. and Japan to rigorously answer the most critical questions related to radiation and immunological aging.

B. Summary of ABCC-RERF Immunological Findings

The major causes of death within the LSS cohort have been analyzed by producing estimates of the excess relative risk (ERR) expressed per radiation dose (Gy) for the years from 1950 to 2002. Early risk estimates were found to be elevated for leukemia and later for solid cancers, but in recent years a number of non-cancer diseases also appear to be elevated in the radiation-exposed A-bomb survivors. One plausible premise to interpret those findings is that a

better understanding of the defects in an individual's immunological system over time and with prior radiation exposure is critical to human health from at least two standpoints: first, it will provide a solid, scientifically-grounded basis for risk assessment, regardless of the disease outcome of interest; and second, it will open opportunities for prophylaxis, prevention, and treatment of the resulting disorders. More than 60 years after the A-bombings, significant radiation-related alterations in the immune system have been observed in the survivors. To address the question of how exposure to A-bomb radiation has affected the human immune system and caused diseases, we have hypothesized that A-bomb radiation has accelerated immunological aging.

Immunological aging found in A-bomb survivors is mainly characterized by (i) a decrease in thymic T-cell production; (ii) frequent clonal expansion of memory cell populations; (iii) dysregulated autoimmune responses; and (iv) enhanced inflammatory responses. It should be possible to apply the newly available biomedical technologies (i.e., so-called "omics," such as immunogenomics, immunomics, proteomics, metabolomics, etc.) to probe RERF's unique databases and stored biosamples to examine the nature and degree of age/radiation exposure-associated decrements in the immune system. We also hypothesize that individual immunogenetic factors may be involved in determining individual susceptibility to radiation-associated diseases. Therefore, RERF's testing of various immunological parameters in A-bomb survivors with long-term follow-up could be used to investigate links between immunological phenotypes, genotypes, and disease outcomes. Such an immunogenetic approach should provide new clues for identifying groups at high risk of radiation-associated diseases and assess the gene-radiation interaction in disease development. Past and ongoing studies at ABCC-RERF have provided several types of assessments that are expected to have relevance to immunosenescence.

1. Assessments of blood lymphocytes of A-bomb survivors

Studies have shown significant radiation effects on T-cell functions (Table 1). By contrast, no significant long-term effects of A-bomb irradiation on blood NK cells, monocytes, or granulocytes, in terms of specific functional endpoints (e.g., K562 cell lysis, phagocytosis, *in vitro* migration, etc.), have been noted.[14] However, a cautionary note is warranted, in that an integrated and systematic examination of the survivor's innate immune system (as well as its interaction with adaptive immunity) has yet to be conducted.

2. Assessments of lymphocyte subsets of A-bomb survivors

Studies have indicated: (i) significant effects of A-bomb irradiation on T- and B-lymphocyte subpopulations (Table 2); (ii) radiation dose-dependent decreases in the proportion of CD4 T cells, especially the naïve CD4 T cells, as well as dose-dependent increases in the proportion of memory CD 8 T cells; (iii) dose-dependent increases in the proportion of B cells; (iv) significantly higher percentages of $CD4^-$ $CD8^-$ TCR α/β T cells in survivors who had been exposed to radiation doses greater than 1.5 Gy[15]; and (v) no significant radiation effect on $CD3^+$ TCR γ/δ T cells, $CD3^+$ $CD56^+$ T cells, $CD4^+/CD28^-$ T cells, $CD8^+/CD28^-$ T cells, the Th1/Th2 cell ratio, or $CD16^+$ NK cells. A dose-dependent increase in the proportion of $CD4^+/CD25^+/CD127^-$ regulatory T cells has recently been observed.[16]

3. Radiation-associated persistent inflammation and infection-related factors in the survivors

Previous findings include: (i) increased plasma levels of selected immunoglobulins such as IgM, IgG, and IgA with increasing radiation dose[17]; (ii) increased prevalence of high-titered antibody responses to the early antigen of Epstein-Barr (EB) virus among A-bomb survivors, compared with non-exposed individuals[18]; (iii) dose-dependent decreases in plasma levels of antibody to *Chlamydia pneumonia* among A-bomb survivors, compared with non-exposed individuals[19]; (iv) radiation dose-dependent elevations of plasma inflammatory cytokine levels for the Th2-related cytokines, interleukin-6 (IL-6) and IL-10, as well as the Th1-related

Table 1. Radiation-related alterations in cellular immune functions among A-bomb survivors

Cell type	Function	Radiation-related alteration	Study period and number of study subjects (N)	Reference
T cells	PHA response	Decrease	1974–77 (683)	9
	MLR	Decrease	1984–85 (139)	10
	IL-2 production	Decrease	1988–92 (410)	11
	Sag response	Decrease	1992–95 (723)	12
NK cells	K562 cell lysis	NS	1983–86 (1,316)	13

PHA = Phytohemagglutinin; MLR = Mixed lymphocyte reaction; Sag = Super antigen; NK = Natural killer; NS = No significant alteration

Table 2. A-bomb radiation-associated alterations of lymphocyte subsets

Lymphocyte subset	Effects			Study period (d)	Ref
	Gender (a)	Age (b)	Radiation (c)		
T cells					
CD4 Total	F>M (5%)	Decrease (5%)	Decrease (2%)	1992–95 (723)	12
Naïve					
$CD45RA^+$	F>M (3%)	Decrease (8%)	Decrease (5%)	1992–95 (723)	12
$CD45RO^-/CD62L^+$	NS	Decrease (25%)	Decrease (9%)	2000–03 (533)	24
Memory					
$CD45RA^-$	F>M (8%)	NS	NS	1992–95 (723)	12
$CD45RO^+/CD62L^+$	F>M (10%)	Decrease (11%)	NS	2000–03 (533)	24
$CD45RO^+/CD62L^-$	F>M (7%)	Increase (8%)	NS	2000–03 (533)	24
CD8 Total	NS	NS	NS	1992–95 (723)	12
Naïve					
$CD45RO^-/CD62L^+$	F>M (19%)	Decrease (35%)	Decrease (8%)	2000–03 (533)	24
Memory					
$CD45RO^+/CD62L^+$	NS	NS	Increase (12%)	2000–03 (533)	24
$CD45RO^+/CD62L^-$	M>F (12%)	Increase (6%)^{sug}	Increase (8%)	2000–03 (533)	26
B cells	F>M (5%)	Decrease (7%)	Increase (8%)	1988–92 (411)	25
NK cells	M>F (20%)	Increase (21%)	NS	1988–92 (411)	25

(a) Percents in parentheses represent change between genders
(b) Percents in parentheses represent change per 10-year period of aging
(c) Percents in parentheses represent change estimated per Gray (Gy)
(d) Numbers in parentheses represent number of subjects
Note: All percent changes are significant ($p < 0.05$) unless designated as NS = Non-significant ($p > 0.1$) or sug = Suggestive significance ($0.05 < p < 0.1$)

cytokines, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)[17,20]; and (v) radiation dose-dependent elevation of inflammatory markers, i.e., plasma C-reactive protein (CRP) levels[20,21] and erythrocyte sedimentation rate (ESR).[22] The results are consistent findings throughout several surveys conducted over the past decade, and gave rise to the "bottomline" conclusion that "past radiation exposure accelerated age-dependent increase of the known inflammatory markers." [20,23]

4. Assessments of the T-cell receptor (TCR) repertoire

Results have shown: (i) no radiation-specific alteration in the TCR Vβ family profile of either naïve or memory CD4 T cells[24]; (ii) decreased T-cell proliferative responses to superantigen staphylococcal enterotoxins almost regardless of TCR Vβ usages[12]; (iii) memory CD4 T-cell TCR Vβ family expression levels significantly diverged from the population average for control subjects, especially in individuals who had been exposed to higher radiation doses and were 20 years of age and older at the time of the bombing[24]; and (iv) larger differences in the TCR Vβ repertoires between naïve and memory CD4 T-cell pools in heavily exposed survivors than in unexposed controls.[24]

5. Studies on memory T cells

Results revealed: (i) co-stimulatory functions of CD43 in human memory CD4 T cells[26]; (ii) identification of functionally distinct $CD4^+/CD45RO^+$ human memory T-cell subsets by using a monoclonal antibody to CD43 with novel lineage specificity for the analyses[27]; (iii) dose-dependent increases in the proportions of hypofunctional (CD43 intermediate) and anergic (CD43 low) memory CD4 T-cell subsets in the peripheral blood of A-bomb survivors[28]; (iv) identification of clonal chromosome aberrations of memory T-cell origin, indicating frequent clonal expansion of a subset of memory T-cell populations in A-bomb survivors[29]; and (v) markedly uneven distributions of naïve and memory T cells in the CD4 and CD8 T-cell populations derived from single stem cells in A-bomb survivors, suggesting an infrequent entry of naïve T cells into memory T-cell pools in adulthood among the radiation-exposed.[30]

6. Immunogenome studies

Experimental results demonstrated: (i) an increased prevalence of type-2 diabetes in heavily exposed individuals with particular class II *MHC DQA1* and *DRB1* alleles, compared to non-exposed individuals[31]; and (ii) *NKG2D* haplotype associations with natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence in a non-exposed

population.[32]

7. TCR mutation studies

Studies served to establish novel assay systems to detect mutant CD4 T cells that lack expression of TCR/CD3 complexes in humans[33] and mice.[34] With those assays, the following observations were obtained: (i) significant increase in the mutation fractions (MF) of *TCR*-mutant cells with age in healthy donors,[35] despite no significant radiation dose-dependent effect on the *TCR* MF in A-bomb survivors; (ii) significant increase of *TCR* MF in patients who had been treated with Thorotrast,[36] patients with thyroid disease who had been treated with the iodine radioisotope, I^{131},[35] and patients with ataxia telangiectasia[33]; (iii) two-fold higher *TCR* MF in the *p53* null and *p53* hemizygous mice than those in similarly irradiated *p53* wild-type mice[37]; (iv) significant strain difference in the sensitivity to radiation-induced *TCR* MF among normal mice, and the dose response for BALB/c mice was significantly higher than that for C57BL/6 or C3H/He mice two weeks after X-irradiation[34]; and (v) *TCR* mutations might be used as a marker of *in vivo* resting CD4 T cells, since it was suggested that the mutant cells exhibit blunted proliferation in response to antigen stimulation due to their lack of TCR/CD3 signaling (Note: a long-term kinetics study on survival of radiation-induced *TCR* mutants among $CD4^+$ $CD45RA^-$ memory and $CD4^+$ $CD45RA^+$ naïve T-cell fractions in radiotherapy patients showed that the life span of mature CD4 T cells was two to three years in the absence of TCR/CD3 expression, i.e., in the resting stage, regardless of its memory or naïve phenotype[38]).

8. Studies on hematopoietic stem cells (HSC)

Experimental results suggested: (i) a dose-dependent increase in the absolute number of circulating $CD34^+$ cells in female survivors (unpublished observation), but this pilot study requires a more thorough follow-up using a refined assay; (ii) decades-long persistence of radiation-induced somatic mutations in HSC, as indicated by the dose-dependent increase in glycophorin A (*GPA*) mutant fractions of circulating erythrocytes[39]; (iii) the presence of an *HPRT* mutation that had possibly originated from a mutation in a single stem cell of one male survivor[40]; (iv) the presence of a clonal chromosome aberration that had originated from a single stem cell aberration of one female survivor[41]; (v) a developmental arrest of human dendritic cells at the $CD34^-/CD4^+/HLA\text{-}DR^+$ stage in the bone marrow of NOD/SCID-human chimeric mice[42]; and (vi) an increased radiosensitivity of $CD34^+/CD38^-$ cells to induced apoptosis, along with increased production of intracellular O_2- and decreased intracellular pH, compared to $CD34^+/CD38^+$ and $CD34^-/CD38^+$ cells.[43]

9. Evaluations of processes related to NK-mediated cancer immunosurveillance

Results of experiments suggested: (i) an inverse relationship between individual natural cytotoxic activity of peripheral blood lymphocytes and cancer incidence among a non-exposed population[44]; and (ii) an NK-mediated elimination of mutant lymphocytes that have lost expression of major histocompability complex (MHC) class I molecules.[45,46] Haplotypes of *NKG2D* receptor gene were identified as a potent genetic factor of individual natural cytotoxic activity.[47] Current studies include examining the relationship between *NKG2D* haplotypes and the consequence (persistent infection or clearance) of viral hepatitis in A-bomb survivors.

10. Evaluations of T-cell homeostasis and immunological aging

Studies are ongoing that include: (i) enumeration of CD4 and CD8 T lymphocytes that contain T-cell receptor-rearrangement excision circles (TREC), in order to address the question as to whether or not prior A-bomb irradiation impaired thymic T-cell production; (ii) determining lengths of telomere repeats in naïve and memory CD4 T cells, CD8 T cells, and granulocytes in order to test the hypothesis that T cells, especially memory T cells, had divided more frequently during the initial recovery phase following A-bomb irradiation; and (iii) analyzing the aging profiles in lymphocyte subsets (e.g., decreased proportion of naïve T cells, increased proportion of particular types of T cells, etc.) in longitudinal samples by flow cytometry in order to provide evidence for the concept that A-bomb irradiation accelerated T-cell immunological aging as suggested by RERF's longitudinal follow-up data of individuals.

11. Characterization of persistent inflammation in terms of disease outcome

Ongoing experiments include: (i) assays of serum inflammatory cytokine levels in relation to age and radiation dose; and (ii) estimations of net oxidative stress in the survivors by testing production of reactive oxygen species (ROS) in blood cells and plasma. Serum levels of ROS metabolites revealed a significant radiation-dose dependence (unpublished data).

12. Attempts to characterize the immunogenetic background of the survivors

We are continuing to examine the wide and varied response of the immune system of A-bomb survivors and the equally-varied disease outcomes. Studies involve: (i) identifying gene polymorphisms responsible for the individually-differing immune and somatic-mutability responses to prior radiation exposure among the survivors (such gene polymorphisms may further relate to individual susceptibil-

ity to radiation-associated diseases); and (ii) genome association studies relative to specific radiogenic disease outcomes, e.g., differing risks of radiation-associated stomach cancer by *IL-10* haplotypes (unpublished data) and radiation-associated lung cancer risks by *EGFR* genotypes.[48]

13. Radiation-induced genomic instability and long-term responses of the lymphohematopoietic system of the survivors

In collaboration with the Cytogenetics Laboratory in the Department of Genetics at RERF, we are investigating the change in chromosome aberration frequencies as a consequence of clonal propagation of T cells *in vivo* or *in vitro*. So far no clear evidence has indicated that genomic instability persists for a long period in peripheral T-cell populations in A-bomb survivors.[49] The following studies are underway: (i) determining the frequencies of micronucleated reticulocytes within blood of A-bomb survivors; (ii) conducting comparative *in vitro* and *in vivo* analyses of genetic damage and inflammatory biomarkers, including measures of net oxidative stress, in blood cells of survivors (the latter is based on the hypothesis that radiation-induced genomic instability may partly be involved in long-lasting inflammation); and finally (iii) examining associated measures of radiation-associated genomic instability and cancer.

Results of the previously completed and ongoing studies indicate that RERF's scientists have considerable data and information relative to the immunological status of the radiation-exposed A-bomb survivors. The observations have provided input to the development of the hypotheses that will be tested in RERF's proposed work plan and experimental designs that were submitted to NIAID in February, 2009. After extensive NIAID review, including reviews by external scientists, the RERF proposal was accepted and a contract to perform the experimental work for a period of five years was signed by RERF and NIAID effective September 30, 2009.

C. Studies to be Conducted by RERF and by Collaborating Subcontractors

Four major statements of work or projects are identified in the NIAID contract to address key questions related to critical elements of immunosenescence.

1. Project 1: Effects of ionizing radiation exposure and aging on hematopoietic stem cells (HSC)

This study will examine the effects of ionizing radiation exposure and aging on blood-derived HSC including the determination of numerical and functional changes in the circulating HSC pool in relation to age and radiation dose. HSC provide the regulated lifelong supply of leukocyte progenitors able to differentiate into an array of specialized immune cells. HSC are speculated to diminish in number and decline in capacity to self-renew with physiological aging. The primary question addressed by this project is whether the prior A-bomb irradiation accelerated this process, and if so, to what extent, and by what process(es) (e.g., increasing the accumulation of oxidative damage to DNA or by shortening telomeric terminals of chromosomes).

Self-renewal and differentiation potentials will be evaluated along with measurements of cell-cycle and redox status and genomic damage in HSC, and other parameters such as micronuclei frequencies in reticulocytes, the *GPA* mutant fraction in erythrocytes, and telomere length in granulocytes. The project will also include experiments that will assess the human HSC ability to reconstitute the immune system using a humanized mouse model. Experiments will analyze HSC from mice following natural aging and/or radiation exposure and will determine effects of irradiation and aging on hematopoietic microenvironments.

2. Project 2: Effects of ionizing radiation exposure and aging on dendritic cells (DC) and their precursors

In a similar manner to the first project that focuses on HSC, this study will examine effects of ionizing radiation exposure and aging on DC and their precursors. DC are crucial in triggering primary immune responses against pathogens, but also in the control of adaptive immunity, and may therefore be involved in impaired T-cell responsiveness and impaired homeostasis with aging in irradiated individuals. The project will address the question as to whether or not prior A-bomb irradiation affects cell-mediated immunity and interferes with the ability of effector T lymphocytes to modulate an adaptive immune response by impacting DC.

Experiments will determine numerical changes in circulating (cDC) and plasmacytoid (pDC) populations, as well as those in blood T-helper and regulatory-T (Treg) subsets in relation to age and radiation dose. Changes in the functional and differentiation status of circulating cDC and pDC populations will also be ascertained relative to age and radiation dose, along with their activation status (Toll-like receptor [TLR] expression, HLA-DR, co-stimulatory molecules, etc.), and production of cytokines before and after TLR triggering. Radiobiological analyses of human DC differentiation and function will also be investigated *in vitro* following *in vitro* irradiation. Finally, to explore mechanisms more thoroughly, radiobiological analyses of mouse DC differentiation and function *in vitro* and *in vivo* will be conducted.

3. Project 3: Effects of ionizing radiation exposure and aging on vaccination responses and investigation of methods to augment immune responses

Vaccination is perhaps the most important measure for prevention of infections, particularly in the elderly. A primary question is whether or not prior A-bomb irradiation in combination with physiological aging significantly alters the effectiveness of vaccination. Project 3 will evaluate the effects of ionizing radiation exposure and aging on vaccination effectiveness in terms of host immune responses to influenza vaccination in A-bomb survivors who have elected to be immunized in response to annual public health guidelines. Antibody titers and *in vitro* re-stimulation of cytokine responses will be measured along with plasma levels of cytokines and inflammation-related proteins. T-cell responses to influenza vaccination, lymphocyte subsets, and intracellular activation markers (cytokine production, transcription factors, etc.) will be measured along with cytokines in culture supernatant of T-cell stimulation. Mechanistic studies will also be conducted *in vivo* in mouse models.

4. Project 4: Development of an integrated scoring system for human immune competence as it relates to age and ionizing radiation

The goal of this project is to develop an integrated scoring system for human immune competence as it relates to age and past ionizing radiation exposure using cross-sectional data (single measurements on individual subjects). Development of such an integrated scoring system for immunological capacity and competence would provide a valuable clinical tool for evaluating the health status of A-bomb survivors and people who were exposed to radiation in general. The primary question is whether a clinically-relevant, statistically-based model can be developed using the various parameters and biomarkers of radiation exposure and immunosenescence. A computational scoring system predictive of exposure levels will be developed and validated along with the identification and validation of new biomarkers and measurements. Approximately 500 different immune biomarkers will be incorporated to predict immunological impacts of irradiation in an aging population. The construction of a comprehensive immune scoring system will make use of the unique RERF longitudinal database (repeated measurements on individual subjects). RERF scientists will attempt to complete the longitudinal database of study subjects, including the genetic factors, and incorporate the data of newly-developed biomarkers with use of protein chip arrays and/or multiplex immunoassays. Associated measurements of telomere lengths in blood cells will also be incorporated. By linking with the RERF follow-up data, the prediction model will be expanded to include disease outcomes.

In order to successfully accomplish the studies in the four areas summarized above, RERF has assembled a team of collaborators in laboratories in Japan and the U.S. Those scientific collaborators have essential expertise and/or methodological systems in place to complement the research capabilities of RERF and critically contribute to the successful performance of the four projects described above.

The prime contractor for the project is RERF and the Foundation's scientists will conduct research on immunosenescence and ionizing radiation as specified in the four tasks/projects. Experiments are designed to determine the contribution of the immune system to disease development in those exposed to A-bomb radiation. The research will focus on the nature of the combined actions of prior irradiation and aging and its impact on immunosenescence. Research proposed will examine the relationships between immune dysfunction and a persistent inflammatory state, and the resulting diseases and infections that may occur as a consequence of immunosenescence in the A-bomb survivors. RERF scientists will rely heavily on the appropriate use of the existing cohorts and a wide-range of reconstructed radiation dose estimates for individual cohort members, including appropriate control (no significant A-bomb radiation exposure) populations. They will also rely on the appropriate use of the unique and valuable resource of biological specimens that have been contributed by A-bomb survivors at biennial clinical examinations, beginning in 1969. Animal models will also be used in order to supplement, detail, and refine proposed descriptive and mechanistic studies on radiation/age-related immunosenescence using biospecimens from the A-bomb survivor cohorts.

A major component of RERF scientists will be from the Department of Radiobiology/Molecular Epidemiology (RME) in Hiroshima, but scientists from other RERF departments will be involved in the projects. The former Chief of RME, Kei Nakachi, is the responsible Principal Investigator (PI) for the overall contract. Yoichiro Kusunoki, the current Acting Chief of RME, along with Tomonori Hayashi, current Chief of the Immunology Laboratory, will serve as the co-PIs on the study. Dr. Kusunoki will serve as the 'responsible scientist' for Projects 1 and 2, while Dr. Hayashi will serve as the 'responsible scientist' for Projects 3 and 4. The Associate Chief of Research (Evan Douple) and the Associate Chief of Secretariat (Douglas Solvie) will serve as the administrative and financial managers, respectively, of the overall study.

Before signing a contract with NIAID and before beginning the project, RERF senior leadership made presentations at meetings of the Local Liaison Councils in Hiroshima and in Nagasaki to inform those stakeholders and to address the questions they had in order to create a climate of transparency and

understanding, especially amongst the A-bomb survivors, cooperating physicians, and community members. Although as many experiments as possible will be performed by RERF scientists within RERF's laboratories, a team of five key scientists from the U.S. and four key scientists from Japan has been selected on the rationale that the members' expertise and experience are complementary to that of RERF scientists and/or they have special equipment and established methodologies that are not available in the RERF laboratories. In addition, three U.S. and two Japanese scientists have been recruited to serve as key 'subject matter experts' or consultants on the project. The following list indicates the key co-investigators and their institutions.

Susan M. Geyer, Ph.D., Senior Biostatistical Scientist, Center for Biostatistics, Ohio State University

Yoko Hirabayashi, M.D., Section Chief and Senior Scientist, Division of Cellular and Molecular Toxicology, National Center for Biological Safety and Research, Japan National Institute of Health Sciences

Kayo Inaba, Ph.D., Professor, Laboratory of Immunobiology, Department of Animal Development and Physiology, Graduate School of Biostudies, Kyoto University

Tohru Inoue, M.D., Ph.D., Consultant, ToxSafety Consultations (ToxSCO), and Visiting Scientist, National Center for Biological Safety and Research, Japan National Institute of Health Sciences

Atsushi Iwama, M.D., Professor, Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University

Shigeo Koyasu, Ph.D., Professor and Chairman, Department of Microbiology and Immunology, Keio University School of Medicine

Nancy R. Manley, Ph.D., Chair of Developmental Biology, and Professor, Department of Genetics, University of Georgia

Donna Murasko, Ph.D., Professor of Bioscience and Dean, College of Arts and Sciences, Drexel University

Janko Nikolich-Zugich, M.D., Ph.D., Chairman, Department of Immunology, and Co-Director, Arizona Center on Aging, Arizona University College of Medicine

Thomas M. Seed, Ph.D., Principal Consultant, Tech Micro Services

Gregory D. Sempowski, Ph.D., Duke Human Vaccine Institute, Associate Professor, Department of Medicine and Pathology, Duke University

Marcel R. M. van den Brink, M.D., Ph.D., Professor of Immunology and Head, Division of Hematologic Oncology, Memorial Sloan-Kettering Cancer Center

Nan-ping Weng, M.D., Ph.D., Senior Investigator and Chief, Lymphocyte Differentiation Section, Laboratory of Immunology, U.S. National Institute on Aging

Koji Yasutomo, M.D., Ph.D., Professor and Chairman, Department of Immunology and Parasitology, Institute of Health Biosciences, University of Tokushima

As a result of the funding provided by NIAID, and the assembled team of collaborators who will be providing special analyses and complementary experimentation with carefully-monitored use of biosamples and protection of personal information, it is anticipated that RERF scientists will be able to take its research to a new level of excellence and honor their pledge to apply the latest and best scientific methodologies to the data and biosamples that the A-bomb survivors have entrusted to RERF. It is expected that the efforts of the collaborating scientists will be synergistic and the project is expected to contribute important information to basic science, the A-bomb survivors, and mankind as to whether radiation exposure has an important role in modifying the process of immunosenescence and whether that role explains in part the health effects that have been observed in the studies of ABCC and RERF.

(This new study is supported by Contract No. HHSN272200900059C with the U.S. National Institute of Allergy and Infectious Diseases.)

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Highlights of RERF Departments: Radiation Research Activities in the Department of Clinical Studies

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Abstract

The Departments of Clinical Studies in Hiroshima and Nagasaki are the key departments for examining A-bomb survivors in a clinical setting. Those examinations, based on the RERF platform protocol called the Adult Health Study (AHS), have been conducted over a period of more than 50 years. They enable RERF scientists to determine radiation risks for diseases that cannot be documented adequately through the larger mortality study called the Life Span Study (LSS). For example, the clinical studies include the effects of radiation on the risk of non-fatal heart disease or stroke, high blood pressure, various benign tumors, and other adverse health effects. Since it is well known that both lifestyle factors and genetic factors, in addition to radiation exposure, contribute to the development of disease, it is necessary to further analyze the lifestyle-related factors, including smoking and dietary habits, biological characteristics, psychological factors, and genetic background. Studies using stored biological samples help clarify the joint effects of radiation and other environmental and genetic factors. AHS subjects have provided biological samples at biennial examinations, repeated samples that are rarely available elsewhere in the world and that allow us to observe changes over time.

Another important cohort is the F_1 Clinical Study (FOCS) in which approximately 12,000 offspring of A-bomb survivors, have participated in a health examination to evaluate the possible association between parental radiation exposure dose and the prevalence of various common diseases in the offspring (such as heart disease, stroke, hypertension, and diabetes). That group is still young—their average age is only about 50 years—and thus relatively little disease has developed. We are making plans for a continued clinical follow-up of the offspring of the A-bomb survivors.

Additional studies of the department are summarized in this article including special clinical studies, special cancer studies, immunological studies, sera collection, cataract studies, and studies of senile dementia. We will conclude with a discussion of our department's future plans.

Adult Health Study (AHS)

The AHS was initiated to provide the first scientific information on the long-term clinical health consequences of A-bomb radiation. Biennial comprehensive medical examinations began in 1958 with a targeted population of about 20,000 survivors and controls in the contact areas of Hiroshima and Nagasaki. In 1978, the AHS sample was enriched with about 2,400 additional higher-dose subjects and all available (~1,000) persons who were exposed *in utero*. Those exams are in their 26th cycle. During the 25th cycle (July 2006–June 2008), a total of 3,609 individuals were examined, representing approximately 70% of the AHS cohort still living in the contact areas of interest.

The purpose of the studies has been to determine (a) the types of diseases, and (b) the physiological or biochemical abnormalities that may have occurred, as a consequence of previous exposure to ionizing radiation, and to correlate this information with other life experiences and modes and patterns of death. The AHS clinical examination functions as a principal source of biological materials for a wide variety of other special studies. The AHS has greatly increased in importance in recent years as a result of the accumulation of an enormous body of data from the serial medical examinations. Particularly noteworthy is the accumulating evidence of the radiation dose-related increase in non-cancer disease morbidity, such as cardiovascular disease, hyperparathyroidism, thyroid diseases, uterine myoma, chronic liver disease, and cataracts. Those important, largely unexpected relationships could never be properly studied using death certificate data alone. Another unexpected finding made largely retrospectively is that radiation is associated with premature menopause, and this, in turn, may result in the earlier onset of other conditions, such as an increase in cholesterol levels and cardiovascular disease. Given the advancing age of the survivors, the time for such studies is limited, making it imperative that research opportunities involving the individuals be considered in a timely manner.

The Departments of Clinical Studies in Hiroshima and Nagasaki follow exactly the same procedures for

clinical examination and laboratory testing (history taking, physical examination, electrocardiography, chest X ray, abdominal ultrasonography, and hematological and biochemical examination) as well as computer data entry for AHS participants based on the AHS platform research protocol (RP), allowing analysis of combined AHS cohort data in the two cities. There are 6 research scientists, 6 nurses, and 26 clinical staff in Hiroshima, and the corresponding numbers in Nagasaki are 4, 4, and 18, respectively.

Previous RERF studies demonstrated that younger survivors showed higher radiation risk for cancer and certain non-cancer diseases (benign thyroid tumors, hyperparathyroidism, hepatitis B virus (HBV) infection, and myocardial infarction) compared with those exposed in adulthood or when elderly. Consequently, we are adding 1,900 survivors who were exposed before the age of 10 years to the AHS population to investigate their radiation risk of non-cancer diseases, which will substantially improve the precision of risk estimates for this younger age group. In October 2007 we started to examine as a pilot study an augmented sample of younger cohort subjects and, in November 2008, RERF began a full-scale study that will include biennial examinations of the younger cohort from July 2010.

Other recently initiated radiation-related studies include studies of liver stiffness, chronic kidney dysfunction/disease and the risk of cardiovascular disease, and pre-clinical measurements of atherosclerosis. Recent reports of new findings include radiation and lifetime risk of stroke, radiation and dementia, metabolic cardiovascular risk factors and subclinical hypothyroidism, genotypes associated with diffuse-type noncardia gastric cancer, and associations of biological age and mortality.

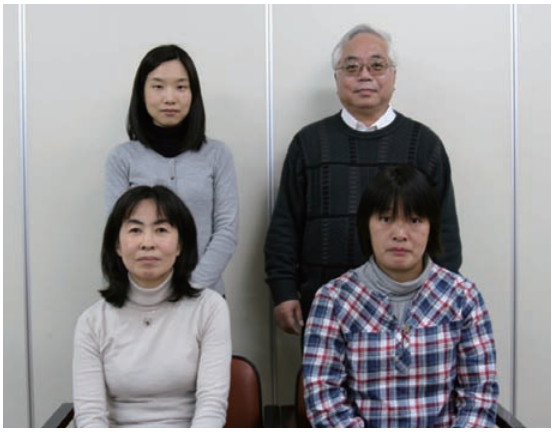
F_1 Clinical Study (FOCS)

The FOCS is intended to assess the possible genetic effects and associated long-term health consequences among children of Japanese parents who were acutely exposed to ionizing radiation from the A-bomb detonations over Hiroshima and Nagasaki. The hypothesis behind the FOCS is that ionizing radiation may induce genetic mutations in germ cells that cause heritable diseases, in particular, adult-onset multi-factorial diseases including diabetes, essential hypertension, coronary heart disease, stroke, etc. The study has three major objectives: (1) to investigate the effects of parental exposure to A-bomb radiation on the development of multifactorial diseases among the children of A-bomb survivors, (2) to preserve blood samples for future molecular biological studies, and (3) to contribute to the health and welfare of the F_1 population via health examinations, and health guidance, based on measurements reflecting clinical or preclinical changes related to multi-factorial diseases.

The FOCS examinations began in January 2002 and were completed in September 2006. RERF conducted several trans-generational studies in the past, and is conducting the mortality follow-up study of A-bomb survivors' children and the genetic analyses of 1,000 family trios (father, mother, and child). However, owing to the young age of the F_1 group (mean age of 48 years), most of the disease experience is yet to occur, so we are converting the sample to a cohort for a prospective follow-up, and plans for the F_1 clinical follow-up study have almost been completed.

A total of 11,951 participated in the health examinations during the study period from 2002 to 2006. The clinical assessment of nearly 12,000 offspring of A-bomb survivors provided no evidence for an increased prevalence of adult-onset multi-

Research Scientists of Hiroshima Clinical Studies (first row from left) Michiko Yamada, Saeko Fujiwara (Department Chief), Kazuo Neriishi, (second row from left) Ikuno Takahashi, Waka Ohishi, Yoshimi Tatsukawa



Research Scientists of Nagasaki Clinical Studies (first row from left) Ayumi Hida, Nobuko Sera, (second row from left) Misa Imaizumi, Masazumi Akahoshi (Department Chief)

Table 1. Adjusted prevalence odds ratios for multifactorial disease among offspring in relation to parental radiation dose

	Odds ratio at 1 Gy (95% confidence interval)*		
	All participants	Male offspring	Female offspring
Father's dose	0.91 (0.81–1.01)	0.76 (0.65–0.89)	1.04 (0.90–1.21)
Mother's dose	0.98 (0.86–1.10)	0.97 (0.81–1.17)	0.98 (0.83–1.16)
Sum of both doses	0.94 (0.86–1.02)	0.85 (0.75–0.96)	1.02 (0.91–1.13)

* Odds ratios were adjusted for categories of age, sex, city, body mass index, parental history of multi-factorial disease, female menopause, smoking, alcohol intake, and occupation.

factorial diseases in relation to parental radiation exposure (see Table 1).[1]

Analyses of genetic effects on individual multifactorial diseases such as hypertension, hypercholesterolemia, and diabetes are ongoing with the collaboration of the Departments of Statistics, Epidemiology, and Genetics. RERF has an F_1 Clinical Follow-up Study Research Group that has been discussing the research plan details for a long-term health study of F_1.

Special Clinical Studies

In addition to collaborating on the main "platform" research protocols, the two clinical departments in Hiroshima and Nagasaki also actively collaborate, with departmental staff members serving as primary investigators, on 9 additional Special Clinical Studies. Further, Nagasaki Clinical Studies is working independently on five Special Clinical Studies while the Hiroshima department is working independently on three additional clinical studies. Some examples are illustrated as follows.

Cardiovascular diseases

1. A study of arteriosclerosis as one of the potential mechanisms by which radiation may promote cardiovascular disease is underway. Past studies have reported a significant association between radiation exposure and atherosclerotic disease mortality/morbidity among A-bomb survivors. Atherosclerosis conceptually has two aspects: atherosis (fatty degeneration) and sclerosis (arterial stiffness). In this cross-sectional study, we are evaluating the associations of radiation and arterial stiffness taking into account correlations among stiffness indices (brachial-ankle pulse wave velocity [baPWV], augmentation index [AI]), atheromatous disease indices/risk factors (ankle-brachial blood pressure index [ABI], carotid artery intima-media wall thickness [IMT], aortic calcification, and left ventricular hypertrophy), and atherosclerosis risk factors (Framingham risk scores), both in Hiroshima and in Nagasaki. The protocol was approved at the end of 2009, and we started to measure the indices among AHS subjects in April 2010.
2. A study is evaluating a set of underlying genetic factors associated with survival among A-bomb survivors who probably suffered from radiation injuries, burns, and early infections, in order to determine if these factors also increased the risk of chronic inflammation and myocardial infarction. Subjects are all the 1,100 individuals who participated in the first cycle of the AHS clinical examinations and who were exposed when young (<30 years of age at the time of the bombings) to at least 1 Gy of radiation, and 1,100 of their sex-, age-, and city-matched <5 mGy exposed controls. We are testing known genetic polymorphisms within an array of genes recognized to be responsive to external stress based on the hypotheses that (a) genetic polymorphisms control the magnitude of inflammatory responses to many lifestyle-associated stresses; and that (b) these same polymorphisms serve as risk factors for cardiovascular disease. Such an effect could have led to a "population bias" in cardiovascular disease risk, especially in the early decades after the A-bomb exposure. Specific genetic polymorphic loci will be examined, including *LTA* single nucleotide polymorphisms (SNP), representative of the haplotype-block encompassing the *LTA*, *NFKBIL1*, and *BAT1* genes, and a *TLR2* deletional polymorphism in exon 1. A total of 2,274 study subjects were selected for this study, and stored blood samples of 1,928 subjects from whom we have obtained an informed consent for genetic studies are being used.
3. A study started in February 2010 is examining the association between chronic kidney disease (CKD) and cardiovascular disease (CVD) in A-bomb survivors since CKD has recently been recognized as a risk factor for CVD. CKD and CVD share many common risk factors such as obesity, insulin resistance, impaired glucose tolerance, hypertension, dyslipidemia, and nephritis. This study will examine whether CKD is related to radiation dose, and whether CKD might serve as a mediating variable in the association of radiation with CVD. The prevalence

of cases of CKD diagnosed during the four-year baseline period (1988–1991) and incident cases of CKD diagnosed during the 15-year follow-up period of 1992–2006 will be ascertained in the AHS cohort. Investigators will also identify both prevalent and incident cases of CVD during the above-mentioned periods, respectively. CVD will include coronary heart disease (CHD) and stroke. Based on the data, the investigators will determine whether the effects of A-bomb radiation exposure can be observed for several endpoints, with adjustment for other risk factors.

Liver stiffness

In order to determine whether A-bomb radiation exposure has increased liver stiffness, which is a marker of liver fibrosis severity, a study measured liver stiffness using an elastometer in examinations of Hiroshima A-bomb survivors. The purpose of the study is to examine the relationship between liver stiffness and radiation dose in order to determine whether radiation exposure is involved in increases of chronic hepatitis and liver cirrhosis. Additionally, we will examine whether increased liver fibrosis is involved, through insulin resistance, in the development of atherosclerotic diseases, in order to elucidate mechanisms of radiation effects underlying these diseases. During two examination cycles (four years), we will measure liver stiffness as a marker of liver fibrosis severity with an elastometer and measure blood cytokines related to chronic inflammation and insulin resistance for about 3,800 AHS participants (including the expanded group of younger survivors) in Hiroshima. The research protocol was approved in October, 2008, and measurements of liver stiffness and blood cytokines for younger A-bomb survivors have been conducted since November 2008. During the period of November 2008 to March 2010, we measured liver stiffness with the elastometer for 1,579 survivors. We also measured blood cytokine levels related to chronic inflammation and/or insulin resistance such as TNF-α, IL-6, IP-10, MCP-1, PAI-1, leptin, resistin, IGF-1, and IGFBP-3 for 1,451 survivors.

Thyroid diseases

The objective of the current study is to investigate whether there are positive associations between radiation dose and thyroid disease. RERF scientists found that malignant thyroid tumor, benign nodules, and cysts were increased with radiation dose and the relationships were significantly higher in those exposed at younger ages. On the other hand, autoimmune hypothyroidism and Graves' disease were not associated with radiation dose.[2] No significant dose responses for thyroid diseases were observed among those exposed *in utero*, although the risk estimates were similar to those with juvenile exposure and the null results may reflect limited statistical power (see Table 2).[3] Cancers were more frequently detected in subjects with solid thyroid nodules than in nodule-free controls[4] suggesting that a thyroid nodule is a risk factor for subsequent thyroid cancer. We are now conducting thyroid examinations in the newly expanded cohort of AHS subjects to study the effects of low-dose radiation on thyroid diseases exposed at younger ages.

Special Cancer Studies

Inflammation

1. The objective of one study is to investigate the relationship between inflammatory biomarkers and cancer incidence in AHS participants. Laboratory and epidemiological studies have reported a relationship between inflammation and cancer. Because A-bomb survivors have radiation dose-dependent increases of inflammatory biomarkers, we are investigating the relationship between the biomarkers and cancer incidence among 12,870 AHS participants followed from 1965 to 1999. We examine white blood cell (WBC) counts (measured since 1958), erythrocyte sedimentation rate (since 1958), alpha 1 and alpha 2 globulin (since 1985), and sialic acid (1988–1992) as parameters in relation to cancer incidence data for 1965–1999 from the Hiroshima and Nagasaki tumor registries. A manuscript on longitudinal trends in leukocyte counts is in press.[5] A joint model to estimate causal associations among radiation, inflammation, and solid cancer incidence was specified and analyzed. Three potential types of intermediate variables are being considered: (1) average leukocyte count, (2) longitudinal trends of leukocyte count, and (3) a latent inflammatory factor estimated using multiple indicators. A preliminary result of a joint model using persons' average WBC counts as a mediating factor shows that there is

Table 2. Odds ratios of all solid thyroid nodules by mode of exposure

	No. (%)	Odds ratio at 1 Gy (95% confidence interval)	*P*
In utero exposure	35/319 (11.0)	2.91 (0.53, 12.18)	0.20
Childhood exposure	63/437 (14.4)	2.65 (1.96, 3.65)	<0.001
Combined*	98/756 (13.0)	2.66 (1.97, 3.63)	<0.001

* Combined analysis of *in utero* exposure and childhood exposure.

a significant causal association of radiation, average WBC count, and solid cancer incidence. The proportion of the mediating effect is about 8% of the total radiation effect on cancer.

2. The primary goal of a case-control study of atrophic gastritis and gastric cancer using frozen sera and genomic DNA is to determine whether the radiation-exposure dependence of gastric cancers seen in the A-bomb survivors is related to chronic tissue inflammation associated with *H. pylori* infection. Specific aims include establishing (1) new biomarkers for pathogenic *H. pylori* and related chronic gastritis; and (2) the genetic factors controlling the host's inflammatory response to bacterial infections. Results have indicated that *H. pylori* infection, chronic gastritis, and smoking are all independent predictors of gastric cancer. In terms of radiation-dose dependency, higher relative risks were noted with the diffuse type of gastric cancers, whereas much lower risks were noted with the intestinal type of gastric cancers, after adjusting for the risk factors.[6] The *LTA 252* genotype is associated with noncardia gastric cancer of the diffuse type in Japan, and the genotype was an effect modifier for radiation dose[7] (Table 3). We are also analyzing the relationship between gastric cancer and chronic gastritis in relation to radiation exposure. Radiation risk was significant only for people without chronic gastritis in developing diffuse type non-cardiac gastric cancers. To confirm the results, a research protocol on model building for joint effects of radiation and radiation related intermediate risk factors in nested case-control studies has been prepared by colleagues in the Department of Statistics.

Breast and endometrial cancer

The purpose of this nested case-control study of breast and endometrial cancer etiology is to characterize the joint effects of radiation and serum-based indicators of hormonal status, oxidative stress, and phytoestrogen consumption. The research protocol had examined a series of hormone-related serum measurements, including total estradiol (E2), free E2, testosterone, sex hormone binding globulin (SHBG), progesterone, insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and prolactin. The study also included genistein measurements to index phytoestrogen consumption, and biomarkers indicative of antioxidant availability and iron-mediated oxidative stress, D-Rom and ferritin, respectively. This is one of the first studies to simultaneously analyze such a wide variety of blood serum components in relation to breast or endometrial cancer, and is one of the few to utilize blood samples collected up to 30 years before cancer diagnosis. Controls were selected by counter-matching on radiation dose so as to increase the statistical power to detect radiation effects. All the laboratory measurements have been completed on the 243 breast cancer cases and 486 matched controls. Preliminary results revealed a significant radiation dose-dependent increase in estrogen and testosterone levels among postmenopausal women. Appropriate analytical methods for the joint relationship of radiation and hormones upon cancer risk will be developed, with which the data will be analyzed.

Liver cancer

1. The primary objective of one study is to investigate the relationship between radiation exposure and the risk of hepatocellular carcinoma (HCC) among A-bomb survivors after taking into account hepatitis virus infection. Our working hypothesis is that radiation exposure accelerated HCC occurrence in the early stage of liver fibrosis after hepatitis C virus (HCV) infection. The study includes evaluations of the interactions between (a) the initial dose of radiation; (b) the status of hepatitis virus infection; and (c) the severity of liver fibrosis, along with other potential risk factors to be evaluated in terms of the etiology of HCC. For this purpose, among cases and controls, selected biomarkers of evolving liver disease are being assayed, including hepatitis virus markers, and fibrosis markers. Currently, there are stored sera from some 224 HCC

Table 3. Interaction among three risk factors: radiation risk for non-cardia gastric cancer is restricted to subjects with LTA 252G-carriage and smoking status

Risk categories	Relative risk	95% confidence interval	*P*
Radiation dose (1 Gy)	0.8	0.5–1.2	0.3
Radiation dose (1 Gy) for current smoker with LTA 252G-carriage	1.3	0.6–1.9	0.4
Radiation dose (1 Gy) for noncurrent smoker with LTA 252AA	2.0	0.6–3.4	0.2
Radiation dose (1 Gy) for noncurrent smoker with LTA 252G-carriage	3.8	1.7–5.9	0.009

(*Helicobacter* 2009; 14:571–9)

cases and three controls per case matched on age, sex, city, and time of sera storage, and countermatched on radiation exposure. HBV and HCV infection, alcohol consumption, and body mass index (BMI) of >25 kg/m^2 (Obesity) 10 years before HCC diagnosis were independent risk factors that contributed to increased HCC risk. HBV and HCV infection and obesity remained independent risk factors after adjusting for severity of liver fibrosis.[8] We estimated relative risks (RR) of HCC for HBV or HCV infection and excess relative risk (ERR) of HCC for liver dose of radiation. After adjusting for alcohol consumption and BMI, the ERR per Gy (ERR/Gy) of radiation exposure for HCC, was 0.55 ($P = 0.003$), while the RRs for HBV or HCV infection were 61 ($P < 0.001$) and 80 ($P < 0.001$), respectively. Those estimates changed little when radiation and viral effects were fit jointly. The ERR/Gy of radiation exposure for non-B, non-C HCC was 1.15 ($P = 0.026$) with adjustment for alcohol consumption or BMI. The results indicated that HBV and HCV infection and radiation exposure are associated independently with increased risk of HCC, and that radiation exposure is a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption or BMI.

2. The hypothesis behind a nested case-control study is that chronic inflammation due to radiation exposure may be involved in the development of HCC through insulin resistance. The objective of the study is to examine the contribution of insulin resistance to HCC risk, taking into account radiation exposure, hepatitis virus infection, lifestyle-related factors, and severity of liver fibrosis. This research protocol is an addendum to a study of the effects of radiation exposure, hepatitis virus infection, and lifestyle-related factors on the risk for development of HCC. Furthermore, the ongoing analyses suggest that radiation exposure, alcohol consumption, and BMI are all independently associated with increased risk of non-B, non-C HCC. This research protocol was approved in April, 2009 and we have started measurements of blood cytokines using stored sera obtained from HCC cases and controls of this nested case-control study. Of 1,372 samples, we have measured blood cytokine levels such as TNF-α, IL-6, MCP-1, leptin, and resistin, with the enzyme-linked immunosorbent assay (ELISA) method or the multiplex Luminex method, for 1,146 samples.
3. A third study of liver disease is studying the relationship between radiation dose and infection by HBV and HCV. The hypothesis behind this study is that ionizing radiation may increase the incidence of HCC either by increasing the rates of HBV or HCV infection or by facilitating disease progression after hepatitis virus infection. The purposes of this study are to investigate the associations of radiation with (a) rates of HCV infection, (b) HBV activity (hepatitis B e-antigen: HBeAg) in HBV carriers, and (c) HBeAg and hepatitis B surface antigen (HBsAg) seroconversion rates among HBV carriers. We have assessed the effects of immunogenetic background (*HLA-DRB1* and/or *NKG2D* polymorphisms) and radiation dose on the course following HCV infection in collaboration with the Department of Radiobiology/Molecular Epidemiology. Preliminary analyses suggested that *HLA-DRB1* and/or *NKG2D* polymorphisms affect the clearance and/or persistence following HCV infection, and that an *NKG2D*-mediated immune response is involved in the mechanisms underlying HCV clearance.

Immunological Studies

Members of the Department of Clinical Studies have a long-standing collaboration with RERF scientists who have been measuring immunological markers and factors in the blood samples provided by the AHS participants. The results of the studies suggest that radiation in many ways mimics the natural immunosenescence associated with aging populations. As a result of these studies, RERF was able to obtain significant funding to conduct four major projects that are described in page 13 of this issue of *Update*. The RPs associated with the new projects include a study of the response of AHS participants to influenza vaccination which will be conducted in collaboration with members of the Department of Clinical Studies.

Cataract and Other Opthalmologic Studies

1. There are several ophthalmologic studies of the A-bomb survivors. The prevalence of radiation cataracts is being evaluated within two study subject groups, namely (a) a select group of survivors who were relatively young (13 years old or younger) at the time of the atomic bombing, but had not been previously given ophthalmic examinations; and (b) a larger group that had been evaluated previously, but by older methods. For both groups, dose-response analyses were conducted for posterior subcapsular axial opacities (Figure) and polychromatic changes and for peripheral opacities using a standard grading system, while adjusting for a variety of potential confounding factors. A total of 883 persons underwent ophthalmologic examinations in Hiroshima and Nagasaki, from which three papers were published.[9–11] An analysis of the prevalence of severe cataract cases with surgical lens removal was also published (see the Fig-

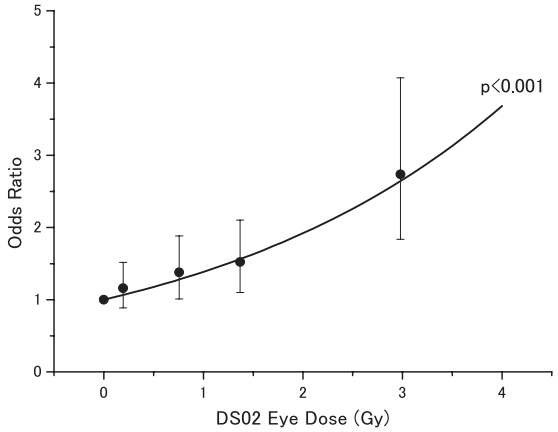


Figure. The main-effect model dose-response curve in regression analysis after adjusting for city, sex, age at the time of the bombings, and diabetes mellitus in A-bomb survivors who had undergone lens removal surgery (odds ratio at 1 Gy, 1.39; confidence interval, 1.24–1.55).

ure).[12] Stored lens images collected during 2000–2002 were used to conduct a re-evaluation with the Merriam-Focht cataract scoring method. The preliminary results of an opacity re-evaluation with the Merriam-Focht method indicate that the dose responses for the two major research cohorts, A-bomb survivors and Chernobyl clean-up workers, are almost identical.

2. A second project is investigating storage conditions for cataract tissue of A-bomb survivors and a collection and storage program. The goal of the project is to confirm the adequacy of a storage method for cataract tissues of AHS participants who undergo a cataract operation, and to collect and store the tissue for future analyses. The stored cataract tissues are expected to contribute significantly to future research on radiation-induced cataract. When enough numbers of tissues are collected, a new research protocol for biological studies will be prepared. Based on the established method, an actual program of collection and storage of lens tissue started on January 2009 in Hiroshima and 20 samples have been collected and stored as of March 2010. The same program was more recently begun in Nagasaki.
3. A study was designed to investigate on a quantitative basis the opacity of eye lenses and retinal arteriolosclerosis of the offspring of A-bomb survivors, and to look into the relationship of this value with several risk factors. We will also study whether or not the incidence of age-related cataracts and congenital cataracts increases due to A-bomb exposure in the parents. The digital images were computerized and stored. Cataract is a multi-factorial disease that stems from a combination of environmental and genetic factors. Animal experiments have suggested the possibility of an increase in the incidence of congenital cataracts in relation to radiation exposure. However, since the expected number of cases is small, we will study it qualitatively rather than quantitatively. The study is quantitatively investigating lens opacity and retinal arteriolosclerosis in the offspring of A-bomb survivors who are at least 50 years of age at the time of examination. It is examining the association of lens opacity with both multi-factorial diseases and parental radiation doses. The examinations were completed in September 2006. We will conduct further analyses to look into the relationship of the cataracts with several risk factors.
4. An association between ataxia telangiectasia mutated (*ATM*) gene and lens radiosensitivity has been well documented in experimental animals.[13,14] Since A-bomb survivors demonstrated a significant dose-response relationship in prevalence of cataract surgery with A-bomb radiation, and since genotyping data on *ATM* and other genes are available from another study at RERF, a protocol aims to investigate an association between polymorphisms of *ATM* and other genes and the dose-dependent prevalence of cataract surgery in A-bomb survivors. Among 5,126 AHS participants who underwent medical examination during 2000–2001, those who have information on polymorphisms of *ATM* and other genes in the study and who agreed to use the information for the study were selected. In 5,126 AHS participants, there were 645 persons with cataract surgery. The dataset of polymorphisms of *ATM* and other genes will be obtained from the Department of Radiobiology/Molecular Epidemiology and merged with the cataract surgery data. The prevalence of cataract surgery will be compared by radiation dose and polymorphisms of *ATM* and other genes. Two types of analysis are envisioned. One would be based on individual candidate SNPs and the other on haplotypes.
5. A final ophthalmologic study proposes to investigate additional questions, specifically (a) whether or not radiation-induced cataracts progress with time and (b) whether or not there is a dose response when cataracts are assessed by a radiation-specific classification system (the Merriam-Focht method). Ten years will have passed since the conduct of the previous study; stored digital lens images obtained in that study will be compared with those obtained in the proposed study. Ophthalmological examinations on AHS participants will be conducted by ophthalmologists. The methods will be essentially identical to those of the previous study. More than 700 AHS subjects under age 13 who participated in the previous study, and an addi-

tional 300 or more eligible new participants not previously examined, are expected to visit RERF during 2010–2012. The statistical power of that study is calculated as more than 90% for the radiation-associated prevalence of cataract cases.

6. A Radiation Cataractogenesis Workshop was held in March 2009 at RERF. The results from RERF, together with other data presented at the workshop, have provided strong evidence of a lower threshold for the radiation dose response than had been assumed previously. The workshop summary, including our current results, and another review of the RERF and other epidemiologic cataract findings have been published.[15,16]

Age-related Disease Study (Senile Dementia)

RERF scientists are examining the effects of radiation exposure on cognitive function, the prevalence and incidence of dementia, and other age-related physiologic variables such as reaction time at older ages among adult survivors in the AHS. A wide spectrum of radiation effects on the central nervous system have been well documented in RERF studies, especially for individuals who were exposed prenatally or during childhood. This study investigates the hypothesis that the effects of ionizing radiation on the mature central nervous system may be manifested as accelerated neurological aging. Study subjects were survivors exposed at ≥13 years of age. We evaluated cognitive performance for about 3,113 subjects in Hiroshima and Nagasaki with the Cognitive Abilities Screening Instrument (CASI) during the period 1992–1998. The prevalence of dementia and its subtypes was assessed among 2,648 Hiroshima AHS subjects aged 60 years or older at baseline examination (1992–1996). Dementia prevalence was observed, and 2,286 dementia-free subjects were followed up to assess dementia incidence. Manuscripts regarding radiation effects and other risk factors on dementia incidence have been published[17,18] (Table 4). Although no association was found between previous radiation exposure and cognitive impairment and/or development of dementia among survivors exposed at ≥13 years of age, survivors exposed <13 years of age may well be more vulnerable. We are considering potential instruments for assessing cognitive and other psychoneurological function among the younger and *in utero* survivors.

Table 4. Radiation effects on dementia incidence (Results of Poisson regression analysis)

	Hazard ratio	95% CI	*p*-value
All dementia			
dose 1	0.82	0.59–1.14	0.238
dose 2	0.94	0.65–1.33	>0.5
Probable AD			
dose 1	0.64	0.37–1.09	0.105
dose 2	0.94	0.54–1.62	>0.5
Possible AD			
dose 1	0.88	0.45–1.09	>0.5
dose 2	0.87	0.40–1.81	>0.5
Probable VaD			
dose 1	0.84	0.37–1.84	>0.5
dose 2	0.77	0.32–1.77	>0.5

Dose 1: 5–499 mGy group vs <5 mGy group
Dose 2: ≥500 mGy group vs <5 mGy group
CI: confidence interval
AD: Alzheimer's disease, VaD: Vascular dementia
Model is adjusted for age, $(age)^2$, education, BMI, smoking, drinking, menopausal age, and history of hypertension, diabetes, and stroke.

Biological Sample Collection

The AHS and its biennial examinations have provided extensive biological specimens and information concerning lifestyle or other potential risk factors for many fields of study, including cytology, genetics, immunology, radiobiology, and medical biodosimetry. The biological specimens collected are used for clinical determinations and stored for future studies. Research protocols using stored specimens are being conducted to evaluate possible interactions between radiation and infectious agents or hormones and cancer risk, and phenotypic and genetic factors associated with inflammation and myocardial infarction. Tissue collection and storage of surgically-removed cataract tissue was begun in 2009. Sera have been collected and stored from the AHS participants during examinations since 1969. A Microsoft Access™-based user-friendly inventory of stored sera has been established in collaboration with the Department of Information Technology. A digital recording and alarm system for biological samples in deep freezers was installed to safeguard the precious samples. Examples of clinical studies using stored sera include the evaluation of interactions between radiation and infectious agents or hormones in the development of HCC, gastric, and breast cancer.

Summary of Recent Findings

In summary, highlights of the Clinical Studies Department's more recent findings include:

- The prevalence of cataract increased with radiation dose with a dose-effect threshold that was much lower than that previously believed,
- A significant linear radiation dose response for thyroid nodules exists in A-bomb survivors, but there is no significant radiation dose response for autoimmune thyroid disease nor for thyroid antibodies,
- The dose response to radiation for thyroid nodules or autoimmune thyroid diseases among those exposed *in utero* was not statistically

significant, but the risk estimate for solid thyroid nodules was similar for those exposed *in utero* and those exposed in childhood,
- The dose-response relationship for the incidence of hypertension, hypercholesterolemia, or cardiovascular disease among survivors exposed *in utero* was not statistically significant,[19] and
- No significant dose-response relationship was observed for Sjögren's syndrome[20] prevalence or for dementia incidence.

Future Studies

AHS

During the next year or two, RERF's Clinical Studies scientists plan to publish several papers pertaining to radiation effects on: sub-types and long-term risk of stroke, incidence of cataract extractions, and prevalence of glaucoma. Modifiers of radiation risk are also being analyzed: radiation, serum sex hormones and breast cancer; radiation, chronic atrophic gastritis, and gastric cancer; and radiation, hepatitis virus infection, and liver cancer. As a follow-up to some of the recent findings, new studies have just begun or will soon begin based on the highlights from recent findings in the study of the AHS. Some examples include two new studies related to cardiovascular disease entitled "Association between chronic kidney disease and cardiovascular disease among atomic bomb survivors" and "Study of arteriosclerosis in the Adult Health Study population (Part 1. Physiological indices of arteriosclerosis)." Three new RPs on radiation ocular effects, a follow-up of early-to-moderate cataracts to document cataract progression, a study to evaluate retinal arteriolosclerosis as an early marker for cardiovascular disease risk and glaucoma, and a study on ATM and cataract, have recently been approved by RERF.

Non-cancer diseases and conditions that have so far shown a positive relationship to radiation exposure are thyroid disease, hyperparathyroidism, uterine myoma, cataracts, chronic liver diseases, HBV carrier status, and cardiovasculoar diseases. The scientists in the Department of Clinical Studies plan to continue to clarify the etiological nature of those diseases and conditions and to estimate the radiation component among other risk factors. Some examples of projected studies include:

1. ***Mechanisms of radiation effects at low/moderate doses on CVD***: various mechanistic or pathway studies are proposed that will be prioritized after discussion in the RERF CVD Working Group.
2. ***Effect modification or mediating variables for radiation effects on stroke incidence***: analyses of a number of potential risk factors such as body mass index, cholesterol, blood pressure, smoking, etc., for overall stroke and subtypes of stroke in relation to radiation dose.
3. ***Radiation and arteriosclerosis***: study of the physiological indices of atherosclerosis and acceleration of arterial stiffness as sub-clinical measures of arteriosclerosis, as well as studies of the cytokine network related to arteriosclerosis and the relationship between markers of factors related to inflammation and differentiation in development of arteriosclerosis in relation to radiation dose.
4. ***Chronic kidney disease***: examination of whether CKD incidence is related to radiation dose and whether CKD radiation damage is a mediator of CVD risk in A-bomb survivors.
5. ***Valvular disease among A-bomb survivors***: examination of radiation dose on incidence of valvular heart disease in the AHS cohort.
6. ***Reanalysis of stored digital photos of cataract***: using the classic Merriam-Focht radiation-specific scoring system to compare risk of cataract in the AHS with other populations exposed to radiation.
7. ***Radiosensitivity differences for cataract surgery***: investigation of the association between polymorphisms of ATM and other genes on the dose-dependent prevalence of cataract surgery.
8. ***Additional ophthalmologic follow-up***: to answer questions as to whether radiation-induced cataracts progress with time and whether there is a dose response when assessed by a radiation-specific classification system (the Merriam-Focht method).
9. ***Neurocognitive function among in utero-exposed or in early childhood***: use of current diagnostic medical technologies to evaluate neurocognitive function in those exposed in utero or <13 years of age.
10. ***Radiation and liver disease***: to investigate whether radiation affected the progression of liver fibrosis and/or development of HCC by analyzing the relationship between liver stiffness and blood cytokines and to analyze whether blood cytokines are related to chronic inflammation and insulin resistance.
11. ***Radiation and diabetes mellitus***: using a standardized criteria for diabetes in both Hiroshima and Nagasaki and measuring several biomarkers of insulin resistance among younger survivors.
12. ***Rheumatoid arthritis and Sjögren's syndrome among A-bomb survivors***: a comprehensive autoimmune disease study in Hiroshima and Nagasaki using a highly specific measure of rheumatoid arthritis and Sjögren's syndrome to examine the possible association between autoimmune disease and A-bomb radiation.

We have added survivors who were exposed at ages 0–9 years at the time of the bomb to the AHS population in order to evaluate risk better among those who were exposed to radiation when young, a group believed to be especially radiosensitive. Full-scale clinical examinations were begun in November 2008, and we expect to examine approximately 1,900 subjects as of June 30, 2010. Repeated biennial examinations of those subjects, in the same manner as the original AHS subjects, will begin in 2010.

F_1 Clinical Study

RERF scientists are continuing to analyze the effects of parental exposure to A-bomb radiation on the development of individual multi-factorial diseases, such as hypertension, diabetes, hypercholesterolemia, cataract, and physiological conditions. The initial clinical F_1 study of about 12,000 was on subjects whose average age was only 48 years, far too young for us to draw definitive conclusions as to whether the radiation exposure of their parents may have impacted the development of multi-factorial diseases among them. Since the hereditary risk of radiation exposure upon adult-onset cancer or multi-factorial diseases is so important an issue and there are virtually no other human data to address it, it is essential that the F_1 cohort follow-up be continued for another 30–40 years to evaluate lifetime risk. We have completed research plans of a continued clinical follow-up. The early assessments that were conducted already provide a useful baseline for assessing future disease incidence.

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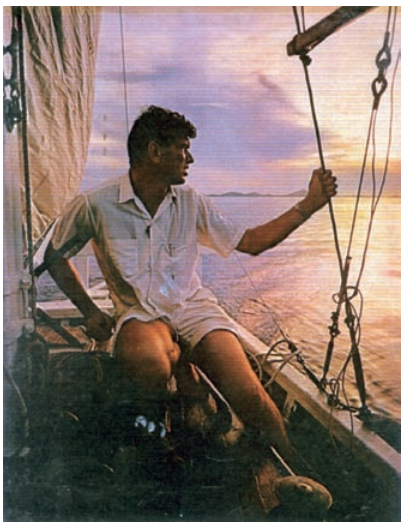
An Intriguing Story of Mystery and Humanitarian Service

Evan B. Douple, Associate Chief of Research

An interesting email was forwarded to me by an early ABCC scientist and long-time supporter of ABCC-RERF, Aaron Bertrand "Randy" Brill (ABCC 1957–1959) who is currently a Research Professor of Radiology and Radiological Sciences at Vanderbilt University Medical Center in Nashville, Tennessee. Randy had been contacted by a cancer researcher and professor in a Belgium medical university, Jan Bernheim, who, along with his daughter who is a filmmaker, was interested in writing a biography of a physician named Alphonse Van Schoote (AVS). Dr. Van Schoote (or AVS) was an M.D. and Lieutenant in the Medical Corps of the Belgium United Nations Command during the Korean War in 1953–1954. After his discharge from the military in 1954, there is a gap in the knowledge about AVS's life, but he eventually continued his medical training in pathology in the U.S. universities at Columbia, Duke, and M.D. Anderson (University of Texas). AVS' medical colleagues indicated that he developed an interest in the human health consequences of exposure to atomic explosions and that the interest took him to Micronesia where from 1961 until 1969 he was a public-health physician in the Truk Archipelago, not far from Bikini and Eniwetok atolls that were near nuclear testing sites in the Marshall Islands. He was respected for his dedicated assistance to the health needs of the natives whom he visited by sailing large distances in his small boat. He became widely known for his humanitarian services and was called the "atoll doctor." In 1969, as he was apparently sailing to replenish his medical supplies, AVS was caught in a storm and disappeared at sea. Attached is one of the last photos that apparently are available of AVS kindly provided by professor Bernheim.



The "Atoll Doctor" Alphonse Van Schoote in his boat (courtesy of Jan Bernheim)

So professor Bernheim was interested in trying to fill in the gaps after AVS left Korea. He knew that AVS's family had received an AVS letter from Nagasaki in 1953. Could AVS's interest in the health effects of victims of nuclear bombings have developed from a visit to ABCC? RERF's Associate Chief of Secretariat, Douglas Solvie, went to the ABCC personnel files and sure enough—11 documents, including handwritten letters from AVS—confirmed that AVS had visited ABCC. Frank H. Connell, ABCC Director, Nagasaki, introduced him to the ABCC Director in Hiroshima, Robert H. Holmes. AVS asked whether he could study with ABCC doctors in Hiroshima after his discharge from the army in Korea in October, 1954, and he indicated he would be willing to work for only meals and a room. Director Holmes accepted AVS's request and indicated he would be "working in hematology and parasitology" and "Dr. Dobos [Emeric I. Dobos, M.D.; ABCC Chief, Laboratories and Chief, Microbiology Department, 1954–1956] has recommended you highly, and you will be working with him."

AVS's ABCC termination clearance form shows he was at ABCC for only a few months, but his letters indicate that he was "highly impressed by the high level of the ABCC." I would like to think that his favorable impressions of the work of ABCC and his short exposure to ABCC contributed to his dedicated interest in helping victims exposed to radiation and influenced his humanitarian service to the island peoples in the Trust Territories. If anyone reading this has memories of AVS or additional information, his only surviving heir, a brother, and professor Bernheim, would appreciate hearing from you.

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In Memoriam

Donald G. MacPhee, Ph.D.
(9 April 1942–16 September 2009)

RERF employees were saddened to learn that Donald G. MacPhee, a former RERF Chief of Radiobiology (21 September 1999–30 June 2002), died at the age of 67 in Australia, 16 September 2009, with his wife (Valerie) and children (Cait and Murdoch) by his side. Dr. MacPhee had decided to stop treatment for lymphoma and he will always be admired for his positive outlook on life and his valiant struggles with a number of health challenges. Born in Inverness, Scotland and a citizen of the UK and Australia, Dr. MacPhee was recruited to RERF by the National Academy of Sciences. He will be remembered as a kind man and a popular department chief who set high professional standards and encouraged scientists to work hard and to excel. After stepping down as department chief on 30 June 2002, he continued to serve RERF as Research Advisor until 30 September 2004. Dr. MacPhee's research interests included the heritability of induced epigenetic events and he encouraged RERF's scientists to explore the field of epigenetics that is currently receiving considerable attention. He was especially remembered in March when RERF hosted an International Epigenetics Workshop following the Scientific Council Meeting.

Dr. Donald G. MacPhee in summer of 2001

Research Protocols Approved in October 2009–March 2010

RP 6-09 Evaluation of the Nonmelanoma Skin Cancer Risk among Heterozygotes Bearing a Founder Mutation Allele Unique to a Japanese Population at *Xeroderma Pigmentosum Group A (XPA)* Gene

Hirai Y, Nakamura N, Noda A, Cullings HM, Ozasa K, Tokuoka S, Yonehara S, Fujihara M, Moriwaki S, Nishigori C, Mabuchi K, Kraemer KH, Land CE, Kodama Y

The frequency of patients (homozygotes) with cancer-prone recessive hereditary disorders, such as xeroderma pigmentosum (XP), is usually low, at several cases per 100,000 people, but carriers (heterozygotes) are not rare, with a frequency close to the order of one percent. However, there is little data regarding cancer risk in the carriers of these heterozygotes, as they are generally difficult to identify. But, the examination of carriers of an inactive mutant allele is most appropriate as a model in establishing a cancer risk of mutation heterozygotes, on the assumption that the cellular functions decreased to about 50% among the relevant carriers. The aim of this research protocol is to evaluate the cancer risk of heterozygotes by comparing the frequency of carriers bearing a founder mutation in the *XPA* gene in nonmelanoma skin cancer patients to control population, by taking advantage of the fact that a founder mutation at the *XPA* gene (inactive mutation) exists at a relatively high frequency among the Japanese population and can be found easily using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Based on the frequency from the pilot study, we propose to conduct screening of 1,000 nonmelanoma skin cancer cases and compare the frequency in a control population (estimated from our previous results plus 500 new cases in Hiroshima to be studied in this RP). This research protocol is suitable for a model in establishing a cancer risk of heterozygous carriers of mutations at genes related to cancer-prone recessive hereditary disorders.

RP 7-09 Study of Arteriosclerosis in the Adult Health Study Population (Part 1. Physiological Indices of Arteriosclerosis)

Takahashi I, Hida A, Akahoshi M, Kohata M, Yamada M, Hsu WL, Misumi M, Takahashi T, Kihara Y, Matsumoto M, Fujiwara S

Past studies have reported significant association between radiation exposure and atherosclerotic disease mortality/morbidity among A-bomb survivors. Atherosclerosis comprises two aspects: atherosis (fatty degeneration) and sclerosis (acceleration of arterial stiffness), both of which are semi-independently related to atherosclerotic disease. In this study, measures pertaining to atheroma include the ankle-brachial index (ABI), toe-brachial index (TBI), and intima-media thickness (IMT), while those used for arterial stiffness include the augmentation index (AI) and brachial-ankle pulse wave velocity (baPWV). Although acceleration of arterial stiffness might be caused by structural changes in arterial walls, the association of radiation and radiation-induced injury and repair of arterial walls has not been fully investigated.

In this study, we hypothesize that radiation exposure is a significant risk factor for arterial stiffing. We propose to conduct a cross-sectional study to obtain measurements of atherosclerosis and arterial stiffness. The study cohort will comprise about 4,000 Adult Health Study (AHS) subjects, including the expanded AHS group of younger subjects. The associations of radiation and the aforementioned arteriosclerosis indices will be analyzed using the generalized estimating equations (GEE) model to account for correlation among the indices, with due consideration paid to atheromatous disease indices (ABI/TBI, IMT, etc.) and arteriosclerosis risk factors (Framingham risk scores; FRS).

RP 1-10 Estimation of Genetic Effects of Radiation in Male Germ Cells of Mice: Study for Assessment of High-density Microarray CGH Platform

Asakawa J, Kodaira M, Cullings HM, Shimada Y, Nakamura N

Planning a full-scale genetic study in the offspring of A-bomb survivors is a difficult task because, compared with animal studies that used large radiation doses, A-bomb survivors received much smaller doses on the average, and radiation sensitivity of human germ cells is not well understood. On the other hand, animal data are not free from problems either; they are mostly limited to Russell's 7 loci and practically no information exists on the whole genome.

Recently, high-density (HD) microarray slides have become commercially available, which enables us to conduct high-resolution comparative genomic hybridization (CGH) studies. This HD-array allows us to examine copy number changes (deletions and/or amplifications) at every 1 kb throughout the whole genomes between two DNA samples from the same species (examination of a total of 2.1 million DNA loci).

In the proposed study, we will first conduct a model experiment to estimate the detection rate and the size of the smallest copy number variation (CNV) by examining the polymerase chain reaction (PCR)-confirmed CNVs existing in two different mouse genomes. Following the characterization of the methods, we will undertake a small-scale screening

study of 30 DNA samples from F_1 mice derived from irradiated spermatogonial cells and 30 samples from control F_1 mice to obtain crude information on the spontaneous as well as radiation-induced mutation rates that are essential for planning a larger scale study for determining the dose response. Finally, it is crucially important to know if radiation exposure may induce not only deletions but also amplifications in this animal model study.

RP 2-10 Evaluation of Retinal Arteriolosclerosis and Age-related Macular Degeneration Using Stored Retinal Images with Standardized Measurements in Relation to Glaucoma Development in Atomic Bomb Survivors and to Association with Aortic Arteriosclerosis (Addendum to RP 1-05)

Neriishi K, Yanagi M, Kawasaki R, Takahashi I, Nakashima E, Hsu WL, Yokoyama T, Takamatsu M, Kinoshita H, Tsuiki E, Uematsu M, Kumagami T, Kiuchi Y, Kitaoka T, Fujiwara S, Hida A, Akahoshi M

The preliminary analysis of glaucoma study (RP 1-05) indicated that normal tension glaucoma prevalence is significantly associated with A-bomb radiation and retinal arteriolosclerosis is reportedly associated with normal tension glaucoma as a causal factor. We plan an evaluation based on standardized retinal measurements of stored retina images. We will investigate if retinal arteriolosclerosis is involved as an intermediate risk factor in radiation-associated glaucoma. This study should provide evidence regarding a possible mechanism for radiation-associated glaucoma.

A second endpoint that may be related to retinal arteriolosclerosis and age-related macular degeneration is aortic arteriosclerosis. Using stored retina images and standardized measurements, we also plan to evaluate retinal arteriolosclerosis and age-related macular degeneration in order to investigate their associations with aortic arteriosclerosis.

RP 3-10 Ophthalmologic Follow-up Study in Atomic Bomb Survivors (Addendum to RP 3-00)

Neriishi K, Yokoyama T, Takamatsu M, Kumagami T, Uematsu M, Tsuiki E, Minamoto A, Kiuchi Y, Kitaoka T, Nakashima E, Hida A, Fujiwara S, Akahoshi M

This addendum proposes to investigate several unanswered questions in a previous ophthalmologic study conducted from 2000 to 2002 based on RP 3-00. The ophthalmologic study of 837 A-bomb survivors based on RP 3-00 had revealed a statistically significant dose response with posterior subcapsular and cortical cataracts, and suggested a lower threshold although not significant. However, several important research questions still remain unanswered. This addendum proposes to investigate those questions.

Ophthalmological examinations on the Adult Health Study participants will be conducted by ophthalmologists. The methods will be essentially identical to those of the previous study in order to investigate whether there are changes from the previous findings after nine years. In particular, (1) subjects will be those who were age 13 or less at the time of the bombs, (2) the Lens Opacity Classification System II will be used for grading, (3) a variety of potentially confounding factors will be incorporated in the analysis, and (4) digital computer images of lens and retina will be stored. For a population-based cross-sectional study, more than 1,000 participants, including more than 700 subjects who participated in the previous longitudinal study, are expected to visit RERF for examination during 2009–2011. The power of this study is calculated as more than 90% for the radiation-associated prevalence of cataract cases.

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(Japanese): the original article is in Japanese.

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