The 21st Century
Center of Excellence (COE) Program

"Novel BMT Methods for Intractable Diseases: From Benching to Bedside"

October 27, 2007

at Osaka International Convention Center (12th floor)

(Organized by Prof. Susumu Ikehara)

Kansai Medical University
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8:55a.m.–9:00a.m. "Opening Remarks"

Toshio Yamashita
(President of Kansai Medical University)

Symposium

9:00a.m.–9:30a.m. Chairperson: Fumimaro Takaku
(President of Jichi Medical University)

"Stem Cells: Quo Vadis?"

Martin J. Murphy, Jr., Executive Editor
"Stem Cells" and "The Oncologist"

9:30a.m.–10:00a.m. Chairperson: Tatsuo Kinashi
(Professor, Kansai Medical University)

"A Hypothesis for an Embryonic Origin of Pluripotent OCT-4$^+$ Stem Cells in Adult Bone Marrow and Other Tissues"

Mariusz Z. Ratajczak, Professor
Stem Cell Institute at James Graham Brown Cancer Center
University of Louisville

10:00a.m.–10:20a.m. Chairperson: Mariusz Z. Ratajczak
(Professor, University of Louisville)

"Immunophenotype and Functional Characteristics of Human Primitive CD34-negative Hematopoietic Stem Cells: Significance of the Intra-Bone Marrow Injection"

Yoshiaki Sonoda, Professor
Department of Stem Cell Biology and Regenerative Medicine, Graduate School of Medical Science, Kansai Medical University

10:20a.m.–10:40a.m. Chairperson: Nader G. Abraham
(Professor, New York Medical College)

"In Vivo Bio-Imaging Using Photonic Rats – Fate of the Injected Bone Marrow-Derived Cell –"

Eiji Kobayashi, Professor
Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical University

10:40a.m.–11:00a.m. "Intermission"

11:00a.m.–11:30a.m. Chairperson: Seiji Ito
(Professor, Kansai Medical University)

"Bone Marrow Stem Cell Transplant into Intra-Bone Cavity Ameliorates Type II Diabetes: Role of Heme Oxygenase"

Nader G. Abraham, Professor
Director of Stem cells and Gene Therapy, Department of Medicine/Pharmacology, New York Medical College

11:30a.m.–11:50a.m. Chairperson: Junichi Fujisawa
(Professor, Kansai Medical University)

"Cell and Gene Therapy Using Mesenchymal Stem Cells (MSCs)"

Keiya Ozawa, Professor
Division of Hematology, Department of Medicine, Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical University

11:50a.m.–12:20p.m. Chairperson: Kazuichi Okazaki
(Professor, Kansai Medical University)

"Induction of Autoimmune Disease by Chemical Xenobiotic Exposure: the Knife Edge of Tolerance"

M. Eric Gershwin, Professor
Division of Rheumatology, Allergy and Clinical Immunology, University of California

12:20p.m.–1:30p.m. "Lunch"

1:30p.m.–2:00p.m. Chairperson: Hidehiko Saito
(Director of Nagoya Central Hospital)

"Will Hematopoietic Stem Cell Transplantation (HSCT) Cure Human Autoimmune Diseases?"

Alberto M. Marmont, Professor
Division of Hematology and Stem Cell Transplantation Center, Azienda Ospedaliera-Universitaria San Martino

2:00p.m.–2:30p.m. Chairperson: Toru Masaoka
(Adviser of Osaka Medical Center for Cancer and Cardiovascular Diseases, Hospital)

"Hematopoietic Stem Cell Transplantation for Autoimmune Diseases: What have We Learned?"

Richard K. Burt, Professor
Northwestern University Feinberg School of Medicine

2:30p.m.–3:00p.m. Chairperson: Shiro Fukuhara
(Professor, Kansai Medical University)

"Direct Intra-Bone Injection of Unrelated Cord Blood Cells Overcomes Delayed Engraftment or Graft Failure and Improves the Feasibility of Hematopoietic Transplantation in Adult Patients"

Francesco Frassoni, Head
Stem Cell Centre and Cellular Therapy, Ospedale San Martino in Genova

"Using Graft-vs-Tumor Effects to Treat Patients with Hematological Cancers"

Rainer Storb, Professor
Transplantation Biology Program Fred Hutchinson Cancer Research Center, University of Washington

3:30p.m.–4:00p.m. Chairperson: M. Eric Gershwin
(Professor, University of California)

"A Revolutionary BMT Method for Intractable Diseases"

Susumu Ikehara, Professor
First Department of Pathology, Center for Cancer Therapy, Kansai Medical University

4:00p.m.–5:00p.m. Discussion on "Future Directions"

Martin J. Murphy Jr., Executive Editor
"Stem Cells" and "The Oncologist"

6:00p.m. Till late "Party" in Rihga Royal Hotel

Stem Cells: *Quo Vadis*?

Martin J. Murphy, Jr.
Executive Editor, *Stem Cells*

Stem cells have long engendered controversy. In fact, until James Till and E.A. McCulloch's ground-breaking research in the 1960's, the very existence of "stem cells" was a matter of almost "religious belief" held by only a chosen few. The hematopoietic clonal nature of the spleen colony assay developed by Till and McCulloch laid that controversy to rest. But further controversy was not far off. Since 1998, when embryonic stem cells were isolated in a lab, questions over how — and whether — to use them have abounded. Through the use of research into embryonic stem-cell lines, the biology and etiology of disease is being explored, albeit with some research being hampered by governmental restrictions. Looking toward the future, stem cell research will be driven by age-old forces — the human imperative to understand disease and the drive to strive for longer, high-quality life. This research is and will be fostered by industry, in the near term into regenerative medicine, in which stem cells are focused on repairing or replacing diseased or defective tissues or organs. Regenerative medicine utilizes naturally occurring products in the body, such as proteins; native or engineered cells and tissues; embryonic stem cells, along with synthetic biomaterials. The promise of stem cells in regenerative medicine is a promise to find new therapeutic avenues for diseases and conditions that currently have limited or no treatment options.

Profile for Martin J. Murphy, Jr.

Dr. Martin J. Murphy, Jr. is Founding Chairman and Chief Executive Officer of *AlphaMed Consulting, Inc.*, a corporation that provides strategic support for academic cancer centers, and cancer drug development programs of global pharmaceutical and biotechnology companies.

He is Founding Executive Editor of the international, peer-reviewed journal, *The Oncologist*, which is read by more than 27,000 physicians entrusted with cancer patient care. He is Founder and Executive Editor of *Stem Cells*, a scientific journal that is distinguished by twenty-five years of publishing excellence in this fast-paced and promising research arena of stem cell biology and regenerative medicine.

Founder and former Chief Executive Officer of the *Hipple Cancer Research Center*, Dr. Murphy was professor of medicine and principal investigator of key anti-cancer drug development grants and research contracts, author of more than 150 peer-reviewed papers, editor of a score of books and chapters, and is a sought after speaker.

Dr. Murphy is a member of the Scientific Advisory Board of *Pappas Ventures*, which manages fledgling companies whose R&D efforts are on the cutting edge of science. He is a director of two biotech companies, *Jennerex* and *Aldagen*, and chairman of the Scientific Advisory Board of *Almac Diagnostics, Ltd.* He is a member of the board of trustees of the *American Cancer Society Foundation*, a charter member of *C-Change* (formerly, the *National Dialogue on Cancer*), co-chaired by former President George Bush and former First Lady Barbara Bush; United States Senator Dianne Feinstein serves as Vice Chair. Dr. Murphy is convener of the *CEO Roundtable on Cancer*, founded by Robert A. Ingram at the request of former President Bush. William C. Weldon is chairman of the *CEO Roundtable*, which numbers among its members the chief executives of some of America's greatest companies.

Dr. Murphy has been married for 42 years to Dr. Ann Murphy, president of AlphaMed Press. They have five children and eight grandchildren.

A Hypothesis for an Embryonic Origin of Pluripotent Oct-4$^+$ Stem Cells in Adult Bone Marrow and Other Tissues

Mariusz Z. Ratajczak, Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY 40202, USA.

Several lines of evidence support the hypothesis that pluripotent stem cells (PSC) are present in adult bone marrow (BM) and cord blood (CB). Further supporting this hypothesis is a report indicating the expression of typical PSC markers Oct-4 and Nanog (embryonic stem cells transcription factors) and SSEA (stage specific embryonic antigen) at the protein and/or mRNA level in BM- and CB-derived stem cells. Accordingly, these embryonic markers were demonstrated and described by our team in very small embryonic-like (VSEL) stem cells (*Leukemia 2006;20:857-869 & Leukemia 2007; 21: 297-303*) and by others in multipotent adult progenitor cells (MAPC), mesenchymal stem cells (MSC) and marrow-isolated adult multilineage inducible (MIAMI) cells. In addition to BM and CB, several groups have recently reported the presence of Oct-4$^+$ cells in epidermis, heart, pancreas, testis, and bronchial epithelium. Since SSEA, Oct-4 and Nanog are the markers characteristic for embryonic stem cells (ESC), epiblast stem cells (EPSC) and primordial germ cells (PGC), the presence of these cells in adult tissues supports the concept that adult tissues contain some population of PSC that is deposited in embryogenesis during early gastrulation. It is hypothesized that these cells could be direct descendants of the germ lineage. In order to pass genes on to the next generations, the germ lineage creates soma and thus becomes a "mother lineage" for all somatic cell lineages present in the adult body. It is also hypothesized that, as with PGC, PSC deposited in the developing tissues undergo erasure of their somatic imprint. This mechanism of erasure will protect the developing organism from the possibility of teratoma formation. However, it also affects some of the aspects of the "true pluripotentiality" of these cells (e.g., their potential to complete blastocyst development). It is postulated that these Oct-4$^+$ PSC play a role in steady-state conditions in tissue turnover (e.g., as a source of long-term hematopoiesis repopulating cells). Furthermore, during organ damage (e.g., heart infarct or stroke) these cells could be mobilized from the BM and perhaps other tissue-specific niches into peripheral blood, where they circulate in order to "home" to damaged organs and participate in their repair. On other hand they may be also a source of malignancies. Accordingly, if these cells i) do not erase somatic imprint, ii) go astray from the major migratory routes, iii) acquire critical mutations or iv) are mobilized at the wrong time into peripheral blood and are deposited in areas of chronic inflammation, they may contribute to the development of malignancies (e.g., teratomas, germinomas, pediatric sarcomas and other tumors respectively) instead of playing a role in regeneration.

Profile for Mariusz Z. Ratajczak

Ratajczak Mariusz, M.D., Ph.D., D.Sci. is a native of Poland, where he attended the Pomeranian Medical University. After graduating magna cum laude and obtaining his M.D. in 1981, he spent eight years in clinical training and research at the Center for Clinical and Experimental Medicine of the Polish Academy of Sciences in Warsaw, working with Dr. W. Wikotr-Jedrzejczak. In 1986 he obtained his Ph.D. in experimental hematology and in 1989 D.Sci. in clinical transplantology from the Polish Academy of Sciences. It was here that Dr. Ratajczak participated in the birth of the first successful clinical marrow transplantation program behind the "Iron Curtain". In 1990 he emigrated to the USA, where he become naturalized and began his work as a Postdoctoral Fellow at the University of Pennsylvania in Philadelphia, working in Dr. Alan Gewirtz's laboratory. In 1996 he obtained an independent faculty position at the University of Pennsylvania, and in 1998 he was promoted to Associate Professor. In 2001 he was recruited to the University of Louisville, Kentucky, as a Professor and Director of the Stem Cell Institute at James Graham Brown Cancer Center. For the past 25 years, he has worked to develop new concepts in hematopoietic transplantation biology. Studies include the development of novel strategies for the purging of hematopoietic transplants, protocols to accelerate platelet recovery after transplantation as well as novel strategies to enhance both mobilization and engraftment of hematopoietic stem cells. He pioneered studies into the role of innate immunity and complement cascade as a major modulator of the SDF-1-CXCR4 axis in the trafficking of stem cells, elucidated the molecular mechanisms responsible for metastasis of pediatric sarcomas to the bone marrow, and recently identified in adult bone marrow and cord blood a population of pluripotent very small embryonic-like stem cells. These cells, drawn from adult bone marrow, appear to mimic the ability of stem cells to multiply and develop into other kinds of cells. Dr. Ratajczak has published over 250 papers and book chapters, is a section editor in Leukemia, and editorial board member of Stem Cells, Experimental Hematology and Journal of Cellular and Molecular Medicine. His work is supported by NIH grants. Dr. Ratajczak has won numerous awards for his work, including the 2006 Annual Award from the Polish Foundation for Sciences (the highest scientific award in Poland), the Mosaic Award from the Jewish Family and Vocational Service, the Chad Kopple Spirit Award from the Leukemia & Lymphoma Society and, in 2006, was awarded the Stella and Henry Hoenig Endowed Chair from the University of Louisville. Throughout the past years, Dr. Ratajczak has trained many researchers in his laboratory. Several of them are today independent investigators involved in studying mechanisms that govern normal and malignant hematopoiesis.

Immunophenotype and Functional Characteristics of Human Primitive CD34-Negative Hematopoietic Stem Cells: Significance of the Intra-Bone Marrow Injection

Yoshiaki Sonoda, M.D., Ph.D.

Department of Stem Cell Biology and Regenerative Medicine,
Graduate School of Medical Science, Kansai Medical University, Osaka, Japan

Precise analysis of human CD34-negative ($CD34^-$) hematopoietic stem cells (HSCs) has been hindered by the lack of a simple and reliable assay system of these rare cells. Recently, we successfully identified primitive human cord blood (CB)-derived $CD34^-$ SCID-repopulating cells (SRCs) using the intra-bone marrow injection (IBMI) technique (Blood 101:2924,2003). $CD34^-$ cells did not show SRC activity by conventional tail vein injection, possibly due to their low levels of homing receptor expression and poor SDF-1/CXCR4-mediated homing abilities, while they clearly showed a high SRC activity by IBMI and sustained the long-term human cell repopulation in NOD/SCID mice. Moreover, these $CD34^-$SRCs showed slower in vivo differentiating and reconstituting kinetics than $CD34^+$ cells, suggesting their primitive nature of HSCs.

In contrast to murine $CD34^-$ KSL ($Kit^+Sca\text{-}1^+Lineage^-$) cells, human CB-derived Lin^-CD34^- cells did not express detectable levels of c-kit by flow cytometry. We investigated the function of flt3 in human CB-derived $CD34^-$ SRCs and both $CD34^+flt3^{+/-}$ cells showed SRC activity. In the $CD34^-$ cell fraction, only $CD34^-flt3^-$ cells showed distinct SRC activity by IBMI. In contrast, $CD34^+flt3^+$ cells showed a rather weak secondary repopulating activity, while $CD34^+flt3^-$ cells repopulated many more secondary recipient mice. However, $CD34^-flt3^-$ cells repopulated all the secondary recipients and the repopulating rate was much higher. Next, we cocultured $CD34^-flt3^-$ cells with the murine stromal cell line, HESS-5. After one week, significant numbers of $CD34^+flt3^{+/-}$ cells were generated and they showed distinct SRC activity. These results indicated that CB-derived $CD34^-flt3^-$ cells produced $CD34^+flt3^-$ as well as $CD34^+flt3^+$ SRCs *in vitro*. These results demonstrated that CB-drived $CD34^-$ SRCs, like murine $CD34^-$ KSL cells, do not express flt3. Based on these data, we propose that the immunophenotype of very primitive long-term repopulating human hematopoietic stem cells is $Lin^-CD34^-c\text{-}kit^-flt3^-$.

In order to further clarify the HSC characteristics of $CD34^-$ SRCs, we investigated the proliferative potential and redistribution kinetics of human CB-derived $CD34^-$ SRCs, and compare them with those of $CD34^+CD38^{+/-}$ SRCs using IBMI. These results indicated that $CD34^-$ SRC as well as $CD34^+CD38^{+/-}$ SRCs could actively migrate from the injected site to the other bones. However, the time of initiation of migration was different between $CD34^{+/-}$ SRCs. All these findings indicate that $CD34^-$ SRCs show different proliferative potential and redistribution kinetics, and suggest that our identified $CD34^-$ SRCs are a distinct class of primitive HSCs from $CD34^+CD38^{+/-}$ SRCs.

The unveiling of this novel class of primitive human $CD34^-$ SRCs by IBMI will provide a new concept of the hierarchy in the human HSC compartment, and has important implications for clinical HSC transplantation as well as basic research of HSCs.

Curriculum Vitae for Yoshiaki Sonoda

Name: Yoshiaki Sonoda, M.D., Ph.D.

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Education:

1975	M.D.	Faculty of Kyoto Prefectural University of Medicine
1984	Ph.D.	Doctor of Medical Science, Kyoto Prefectural University of Medicine

Professional Experience:

1975-1977	Intern, Kyoto Prefectural University of Medicine
1977-1978	Doctor of Internal Medicine, National Kobe Hospital
1978-1982	Clinical and Research Associate, Kyoto Prefectural University of Medicine
1982-1985	Head Physician of Internal Medicine, Gamochou Hospital
1985-1986	Senior Research Associate, Kyoto Prefectural University of Medicine
1986-1988	Postdoctoral Research Fellow (Prof. Makio Ogawa), Department of Experimental Hematology, Medical University of South Carolina
1988-1992	Assistant Professor, Department of Hygiene, Kyoto Prefectural University of Medicine
1992-2003	Associate Professor, Department of Hygiene, Kyoto Prefectural University of Medicine
2003-2004	Associate Professor, Department of Molecular-Targeting Cancer Prevention Kyoto Prefectural University of Medicine
2004-present	Professor and Chairman, Department of Hygiene, Kansai Medical University
2005-present	Professor of Department of Stem Cell Biology and Regenerative Medicine, Graduate School of Medical Science, Kansai Medical University
2007-present	Vice Director of Regeneration Research Center for Intractable Diseases, Kansai Medical University

Membership in Scientific Society:

Japanese Society of Hematology (Council member)
Japanese Society of Clinical Hematology (Secretary, Council member)
Japan Society for Hematopoietic Cell Transplantation (Director, Council member)
American Society of Hematology
International Society of Experimental Hematology
International Society of Hematology
International Society for Stem Cell Research

Membership in Journal Editorial Boards:

Haematologica (Journal of Hematology) (1999-2000)
Cytometry Research (2005-)

Dr. Yoshiaki Sonoda has served as chairman and professor of the Department of Stem Cell Biology and Regenerative Medicine at Kansai Medical University since September 2004. His expertise is in the fields of biology of hematopoietic stem cells and adult (somatic) stem cells, hematopoietic stem cell transplantation, and regenerative medicine. He has a long career as a hematologist, so he is interested in the translational research concerning hematopoietic stem cells. He has published approximately 70 peer-reviewed original articles in English, and 140 scientific publications in Japanese. He is the editor of three popular textbooks, in widespread use in Japan, on peripheral blood stem cell transplantation.

In Vivo Bio-Imaging Using Photonic Rats
—Fate of the Injected Bone Marrow-Derived Cells—

Eiji Kobayashi, M.D., PhD

Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke-shi, Tochigi 329-0498, Japan.

As a result of recent photonics technology, there have been dramatic advances in *in vivo* bio-imaging. And at the Center for Molecular Medicine, we have been developing engineered rats, such as GFP, RFP, LacZ, and Luciferase Tg animals, which provide a screening system for innovative cell therapy.

In this paper, we investigate bio-imaging for the *in vivo* assessment of injected bone marrow-derived cells (BMCs) or mesenchymal stem cells (MSCs). Luminescence imaging allows us to determine the fate of transplanted cells without sacrificing the animal, while fluorescence imaging allows us to see any changes in positive cells in the peripheral blood by flow cytometry. Histological samples also show X-gal-positive cells in the target tissue or organ. Considering the merits of the respective marker genes, we set up dual marker Tg rats, which have GFP plus luciferase, or LacZ plus luciferase. Using this system, we examined the following:

(1) Variations in the results of bone marrow transplantation between systemic vs. intra-bone marrow injection;
(2) The impact on the damaged liver of the repeated administration of BMCs;
(3) Differences in the efficacy of wound healing between fresh BMCs and established MSCs;
(4) Differences in the efficacy of MSCs between systemic vs. local injection for the damaged tissue.

Bio-imaging using dual-marker transgenic rats is a powerful tool in quantitative and morphological assessment. Immunological events such as rejection and graft-versus-host reaction, as well as the transdifferentiation of the transplanted BMCs, are able to be visualized.

Curriculum Vitae for Eiji Kobayashi

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Date of Birth: October 28, 1955

Professional Experience

2003-present	Director, Center for Experimental Medicine, Jichi Medical University, Japan
2001-present	Professor, Departments of Surgery and Pharmacology, Jichi Medical University, Japan
2001-present	Professor and Chairman, Division of Organ Replacement Research, and Animal Resource Project, Center for Molecular Medicine, Jichi Medical University, Japan
1995-2001	Associate Professor, Departments of Surgery and Clinical Pharmacology, Jichi Medical School, Japan
1992-1995	Senior Research Fellow at QIMR, University of Queensland, Australia
1991-1992	Teaching Fellow, Department of Surgery, Omiya Medical Center, Jichi Medical School, Japan
1989-1991	Research Fellow, Department for Medical Zoology and Immunology, Niigata University, Japan
1982-1989	Research Fellow, Department of Surgery, Faculty of Medicine, Niigata University, Japan

Education

1984-1989	Ph.D., Niigata University
1977-1982	M.D., Jichi Medical School

Bone Marrow Stem Cell Transplant into Intra-Bone Cavity Ameliorates Type II Diabetes: Role of Heme Oxygenase

Nader G. Abraham
Director of Stem cells and Gene Therapy
Department of Medicine /Pharmacology
New York Medical College, Valhalla, New York 10595

Abstract

Background: Bone marrow stem cells are the target of endogenous and exogenous cytokines and drugs that regulate redox signaling and superoxide/reactive oxygen species (ROS). Stem cell therapy has been used in various treatments for cardiovascular disease, but its success may be related to the levels of ROS. An increase in ROS can lead to unsuccessful transplant or stem cell failure. The de-regulation of ROS has been shown to affect the microenvironment, disturbing the capacity of hematopoietic stem cells (HSC) to self-renew and differentiate into endothelial progenitor cells (EPC). Upregulation of heme oxygenase (HO-1) decreases ROS, increases EPC function and EPC-eNOS, and restores vascular function, nitric oxide synthase and NO bioavailability in diabetic rats. The purpose of the present study was to compare the efficacy of intravenous (i.v.) injection and the administration into the bone marrow cavity (IBM-BMT) of bone marrow cells, including mesenchymal stem cells, on glucose levels and renal function and to determine the effect of HO-1 (BMC-HO-1) upregulation on BMT. Methods and Results: Bone marrow cells (BMC) or stem cells ($CD34^+$) from pre-diabetic OB mice at 4-5 weeks of age were harvested from the femur and were administered to diabetic OB mice (20 weeks old) either via i.v. or IBM-BMT at a dose of $5x10^7$ cells. CoPP (25 mg/kg), inducer of HO-1, was administered (i.p.) once a week before and after transplant up to 8 weeks. The i.v. administration of BMC or $CD34^+$ to diabetic mice did not increase BMC-HO-1 or urine creatinine, but modestly decreased glucose levels. Diabetic mice receiving IBM-BMT treatment showed a significant increase in BMC-HO-1, and decreases in both glucose and creatinine levels. Transplantation using the IBM-BMT method resulted in restoration of BMC-HO-1 levels in diabetic mice, which may be critical to maintenance of the proper microenvironment for stem cell differentiation and EPC function. The pharmacological induction of HO-1 by CoPP was associated with an increase in adiponectin, BMC-HO-1, EPC-eNOS and vascular function. The HO-1-mediated increase in bone marrow adiponectin may provide an environment similar to the "stromal niche" that is believed necessary for the regulation of HSC differentiation. Conclusion: These findings suggest that administration of bone marrow cells or stem cells alone via i.v. injection does not improve vascular function or improve diabetes. However, administration of BMC along with mesenchymel stem cells via IBM-BMT did prevent Type II diabetes, suggesting that Type II diabetes might be a result of stem cell disorders accelerated by hyperglycemia-mediated increases in ROS and decreases in HO-1 and eNOS. These results indicate that the combination of IBM-BMT plus the pharmacological induction of HO-1 results in decreased ROS and a successful stem cell transplant, and is effective in various cardiovascular diseases, including Type 2 diabetes.

Profile for Nader G. Abraham

Dr. Nader G. Abraham attended Mount Sinai School of Medicine, followed by postdoctoral training at The Rockefeller University, focusing on the role of heme oxygenase (HO) as a regulator of ROS and its impact on bone marrow stem cell function. This led to the elucidation of the role of ROS in stem cell differentiation and the finding that bone marrow stem cells act as a metabolic organ (Am J Med, 1982). Dr. Abraham's lab demonstrated that controlling ROS, via the induction of HO-1, increased stem cell longevity, as measured in LTBMC, and showed that the proper stromal microenvironment is essential for stem cell self-renewal and differentiation. These discoveries were published in a series of articles and books edited by Dr. Abraham in association with Drs. Medhi Tavassoli and Leo Sachs. Working in collaboration with Drs. Eugene Cronkite and Michael Freedman, Dr. Abraham showed that benzene inhalation damaged the stromal microenvironment, resulting in severe stem cell injury in LTMBC and causing leukemia in humans. These findings were instrumental in the removal of benzene and its derivatives from the U.S. market by the FDA. Dr. Abraham has also demonstrated that a proper stromal microenvironment is essential for promoting gene transfer into the $CD34^+$ subset. Consequently, many gene transfer protocols, including the NYMC IFN gene transfer protocol for the treatment of chronic myelogenous leukemia, have followed such a strategy. Dr. Abraham is leading his group in clinical trials using stem cells to treat heart failure and stem cells transduced with IFN gene to treat CML, and he believes that the use of whole bone marrow, not just $CD34^+$ cells, may be ideal.

Dr. Abraham is former Chairman of ISEH (1996) and has been Chairman of the International Symposium on the Molecular Biology of Hematopoiesis (MBH); Treatment of Leukemia and Cancer for the last 25 years. At the annual meeting in 1986, with Professors F. Takakau and D. Thomas as co-presidents of MBH, MBH hosted the 100th year celebration of NIH; the proceedings of the 100th year of NIH were published in Advances in Experimental Medicine and Biology in 1987. Dr. Abraham is among the vanguard of internationally recognized scientists and has received numerous awards including the RCDA from NIH; the Tinsley Harrison Award; the Dr. David M. Kovitz Lecturer Award, University of Calgary, Canada; Distinguished Professor Award, University of Saskatoon, Canada; the Dean's Distinguished Award for Stem Cell Research, University of Catania, Italy; Honored Professorship, Japanese Society for the Promotion of Science; Alma Mater Studiorum, Saecularia Nona Award, University of Bologna, Italy; and the Dean's Distinguished Award, NYMC, 2007. Currently, Dr. Abraham is Director of Stem Cell and Gene Therapy at NYMC. He is also a member of the NIH panels on Excellence in Gene Therapy and Excellence in Molecular Hematology, and is Chairman of the Renal Science Special Emphasis Panel. He serves as an external reviewer for the Austrian Research Council, the Global COE Program of Japan, and The Wellcome Trust. Dr. Abraham lectures extensively throughout the world and has authored/coauthored more than 300 scientific articles and books.

Cell and Gene Therapy Using Mesenchymal Stem Cells (MSCs)

Keiya Ozawa
Division of Hematology, Department of Medicine, and Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical University, Tochigi 329-0498, Japan

Mesenchymal stem cells (MSCs) attract a great deal of attention in the field of regenerative medicine, and are considered to be a promising platform for cell and gene therapy for a variety of diseases. The MSCs from bone marrow are capable of differentiating along multiple cell lineages, and have significant expansion capability in vitro. More importantly, MSCs are known to accumulate at the sites of inflammation and tumors as well as damaged tissues. First, in the field of hematopoietic stem cell transplantation, there are two applications of MSCs: 1) the improvement of stem cell engrafting and the acceleration of hematopoietic reconstitution based on the hematopoiesis-supporting ability, and 2) the treatment of severe GVHD (graft-versus-host disease) based on the immunomodulatory ability. The molecular mechanisms underlying these biological effects of MSCs remain obscure. Regarding the immunosuppressive ability, we found that Stat5 phosphorylation in T cells is suppressed in the presence of MSCs and that nitric oxide (NO) is involved in the suppression of both Stat5 phosphorylation and T cell proliferation. Furthermore, MSCs from inducible-NOS knockout mice had a reduced ability to suppress T cell proliferation. These results suggest that NO produced by MSCs is one of the major mediators of T cell suppression by MSCs. Recent clinical trials by other investigators suggest that MSC infusion is a promising treatment for severe steroid-refractory acute GVHD. Second, to assess the tumor tropism of MSCs, nude mice subcutaneously transplanted with 9L rat glioma cells or Rat-1 fibroblasts were subsequently injected with luciferase-expressing MSCs. An *in vivo* imaging analysis showed the significant accumulation of the MSCs at the 9L tumors but not at the injection site of Rat-1 fibroblasts. The findings suggest that MSCs can be utilized to target metastatic tumors and to deliver anti-cancer molecules locally. As the third application, MSCs may also be utilized as a cellular vehicle for protein-supplement gene therapy. When long-term transgene expression is needed, a therapeutic gene should be introduced with a minimal risk of insertional mutagenesis. To this end, site-specific integration into the AAVS1 locus on the chromosome 19 (19q13.4) by using the integration machinery of AAV (adeno-associated virus) would be particularly valuable. Cotransfection of MSCs with a plasmid harboring transgene flanked by AAV-ITRs (inverted terminal repeats) and an AAV-Rep plasmid caused preferential integration of the transgene into the AAVS1 locus. As described above, there will be wide-ranging applications of MSCs to frontier medical treatments in the near future.

Curriculum Vitae for Keiya Ozawa

Education:

1977	M.D.	Faculty of Medicine, University of Tokyo
1984	Ph.D.	Faculty of Medicine, University of Tokyo

Professional Training and Employment:

1980-1982	Research Associate, Department of Hemopoiesis, Institute of Hematology, Jichi Medical School, Tochigi
1984-1987	Research Associate, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo
1985-1987	Fogarty Fellow, Clinical Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland, U.S.A.
1987-1990	Assistant Professor, Department of Hematology-Oncology, The Institute of Medical Science, University of Tokyo, Tokyo
1990-1994	Associate Professor, Department of Hematology-Oncology, The Institute of Medical Science, University of Tokyo, Tokyo
1994	Professor, Department of Molecular Biology, Institute of Hematology, Jichi Medical School, Tochigi
1998-present	Professor and Chairman Division of Hematology, Department of Medicine Division of Cell Transplantation and Transfusion Division of Genetic Therapeutics, Center for Molecular Medicine *(formerly Department of Molecular Biology, Institute of Hematology)* Jichi Medical University, Tochigi Vice Director, Center for Molecular Medicine, Jichi Medical University

Memberships:

International Society of Hematology (Asian-Pacific Division)
International Society for Experimental Hematology
International Society for Cellular Therapy
International Society for Stem Cell Research
American Society of Hematology
American Society of Gene Therapy
European Society of Gene & Cell Therapy
American Society for Microbiology
American Association for the Advancement of Science
The New York Academy of Sciences
Japanese Society of Hematology
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Induction of Autoimmune Disease by Chemical Xenobiotic Exposure: the Knife Edge of Tolerance

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Abstract

The most difficult issue in autoimmune disease is the problem of causation. Although there has been considerable progress made on dissection of the effector mechanisms involved in immunopathology, there is still an enormous intellectual gap with respect to etiology. Clearly there is a genetic predisposition which involves a promiscuous host and often a family history of multiple autoimmune diseases. We submit that modification of autoantigens by chemical xenobiotic exposure can lead directly to loss of tolerance and induction of disease, and illustrate this paradigm by primary biliary cirrhosis (PBC). PBC is a progressive autoimmune liver disease with female predilection characterized by immune-mediated destruction of intrahepatic small bile ducts, leading to decreased bile secretion, fibrosis, and eventual liver failure.

PBC illustrates this enigma because of its high degree of concordance in identical twins, clinical uniformity and the presence of a highly specific serologic marker, AMA, which is directed at the E2 subunits of 2-oxo-acid dehydrogenase complexes. In addition, the MHC class I and class II-restricted T cell epitopes of liver-infiltrating, autoreactive T cells appear to localize to the same inner lipoyl domain of PDC-E2 as does the dominant autoreactive B cell epitope.

Following an *in vitro* screening procedure, we demonstrated that select chemical xenobiotics react as well or better than the native autoantigen to sera from patients with PBC. Importantly, immunization of mice, rabbits and guinea pigs with a chemical xenobiotic, 6-bromohexanoate, without the need for any autoantigen in the immunogen, led to loss of tolerance and a serologic profile identical to humans with PBC, with reactivity to the mitochondrial autoantigens. In addition, immunization of guinea pigs with 6-bromohexanoate led to a serologic and histological liver profile similar to human patients with PBC. The data from these studies have led to an assembly of a series of facts that serve as a foundation upon which we formulate a working model for the pathogenesis of human PBC, and which we believe is a paradigm for other human autoimmune diseases. These steps include: 1) a genetic predisposition to develop PBC; 2) an incredibly focused response of autoantibody; 3) autoreactive CD4+ and CD8+ T cell responses against PDC-E2; 4) a higher autoreactive PDC-E2-specific CD4+ T cell precursor frequency in PBC liver; and 5) a higher autoreactive PDC-E2-specific CD8+ T cell precursor frequency in the liver. Our finding that a chemically synthesized lipoate mimic in the form of 2-nonynoic acid reacts with a higher affinity than the parent lipoate in PBC and the ability of our xenobiotics coupled to BSA to break tolerance in experimental animals, further suggests that there are no selective host susceptibility factors in the bile duct target tissue. Rather, we propose that small bile ducts are not innocent victims; their unique apoptotic properties determine their targeting and their destruction.

This has extraordinary implication for tolerance. Tolerance is essentially the absence in the periphery of T and B cells with receptors of sufficient affinity to recognize/react with self, including the presence of so-called Tregs. If one or the other of these is "underdeveloped," the individual, whether a mouse or man, is on a knife edge of anti-self reactivity which a) might not ever happen; b) happens regularly in "highly" predisposed individuals when a native (unaltered) cell fragment "meets up" under immunogenic conditions with an undeleted anti-self lymphocyte, as in NOD or NZB mice; c) happens occasionally when a "moderately" predisposed individual is confronted with a near self-antigen under immunogenic conditions.

We submit that this is the first time that a chemical synthetic xenobiotic has been shown to break tolerance and not only induce native autoantigen specific immune responses, but in the appropriate genetically defined host, lead to pathology similar, if not identical, to human autoimmune PBC.

Profile for M. Eric Gershwin, M.D.

Eric Gershwin, M.D. is currently Distinguished Professor of Medicine as well as the Jack and Donald Chia Professor of Medicine. He is also Chief of the Division of Allergy and Clinical Immunology at the University of California School of Medicine in Davis. Dr. Gershwin graduated from Stanford Medical School in 1971 and subsequently trained in internal medicine and then immunology at Tufts University-New England Medical Center and the National Institutes of Health. He joined the UC Davis faculty in 1975 and has been Division Chief since 1982. Dr. Gershwin has been continuously funded by NIH since 1975 and currently has published more than 20 books, 600 experimental papers, and 200 book chapters or review articles. He is editor-in-chief of the Journal of Autoimmunity and also Clinical Reviews in Allergy and Immunology and on the editorial board of multiple other journals. His major contributions revolve around the theme of autoimmune disease. Dr. Gershwin was the first individual to clone an autoantigen, and identified the mitochondrial autoantigens of PBC in 1986; this antigen was identified as the E2 component of pyruvate dehydrogenase. Subsequently, his lab has focused entirely on PBC and his diagnostic reagents have become the standard throughout the world. More importantly, however, he has dissected the CD4, CD8 and B cell response in PBC and demonstrated that the autoepitope is nearly identical in each case. Further, his research has helped to explain why only small bile duct cells are involved, and this thesis has led to our understanding of the pathophysiology of biliary damage as an orchestrated response that begins with adaptive immunity and ends with innate immunity. He is listed in the top 1% of all cited authors in Pubmed in immunology and has published more original work on primary biliary cirrhosis than any other individual in the world. Finally, Dr. Gershwin has sat and chaired on committees for NIH, NSF, USDA, FTC and the FDA.

Will Hematopoietic Stem Cell Transplantation (HSCT) Cure Human Autoimmune Diseases?

Alberto M. Marmont

Experimental and clinical evidence has suggested that HSCT (allogeneic, autologous) might be curative in clinical severe autoimmune diseases (SADs). Animal experiments have shown that ADs can be both transferred and cured by means of allogeneic HSCT. The robust evidence that genetic predisposition is overwhelming in experimental ADs has suggested to Ikehara to consider them as polyclonal stem cell diseases.

Notwithstanding the extreme polygenicity of human ADs transplantation, results in coincidental diseases seemed to indicate that allogeneic HSCT might be curative for both diseases. However, because of its more convenient feasibility, autologous HSCT was first proposed for intractable SLE in 1993[1], and performed in 1996[2]. Since then over 1000 patients with different SADs have undergone autologous HSCT and have been registered worldwide (EBMT and IBMTR). The significant OS and EFS curves will be presented. Thirty-eight SAD patients have undergone autologous HSCT in Genoa, and four have undergone allogeneic HSCT. The autologous procedure's main achievements are a constantly decreasing TRM, a shift to predominantly lympholytic conditioning regimens, a dramatic disease-arresting effect, and the tendency to gradual, but generally drug-responsive, relapse. The drug-free post-transplant status appears more like a pipedream than sobering clinical reality, except for a minority of fortunate cases. A genuine post-transplant immune re-education has been proposed. Allogeneic HSCT has been considered as feasible and potentially one-shot curative because of its graft-vs-autoimmunity (GVA) effect, but some unexpected relapses in spite of full donor chimerism require further investigation[3], and indicate that the etiopathogenesis of ADs is more heterogeneous than in hematologic malignancies.

1. Marmont AM et al: Lupus 1993, 2: 151-6.
2. Marmont AM et al: Lupus 1997; 6: 545-8.
3. Burt RK et al: Arthritis Rheum 2006; 54: 3750-3760.
4. Marmont AM et al: Bone Marrow Transplant 2006; 38: 1-4.

Curriculum Vitae of Alberto Marmont Du Haut Champ

Alberto M. Marmont graduated as MD in 1942 in Genova, Italy. He became Libero Docente (Lecturing Professor) in Internal Medicine in 1951 and was confirmed in 1959. He became Libero Docente in Hematology in 1956. He was made Chief of Internal Medicine in the Sampierdarena General Hospital in 1961, and Chief of Hematology at S. Martino's Hospital in Genova in 1968. He retired in 1988, but is still active as Scientific Consultant to the Division of Hematology and Stem Cell Transplantation directed by Dr. Andrea Bacigalupo. He has been awarded the Gold Medal of the Italian Ministry of Health, and a *honoris causa* by the Pierre and Marie Curie University of Paris.

Alberto M. Marmont has authored over 700 scientific publications and seven monographs, including an *Atlas of Blood Cells* with D. Zucker-Franklyn and others, and the book *Stem Cell Therapy for Autoimmune Disease* in collaboration with Richard Burt. He proposed ASCT for clinical SLE in 1993, and with his co-workers performed the first one in Europe in 1996. He is Guest Editor with Riccardo Saccardi of a Special Issue of *Autoimmunity* dedicated to HSCT for severe autoimmune diseases. He is scheduled to give the 2008 EBMT Lecture, which will be entitled "Will hematopoietic stem cell transplantation cure human autoimmune diseases?" His former pupils Andrea Bacigalupo, Angelo Michele Carella and Francesco Frassoni have become leading clinical hematologists, transplanters and stem cell investigators.

Hematopoietic Stem Cell Transplantation for Autoimmune Diseases: What Have We Learned?

Richard K Burt MD

Autologous hematopoietic stem cell transplantation (HSCT) for autoimmune disorders began in the late 1990s using a cancer model of myeloablation and based on the assumption that treatment related mortality (TRM) would be < 2%. Unfortunately TRM in the European Bone Marrow Transplant (EBMT) registry is approximately 7%, and in a recent Fred Hutchinson Cancer Center report, the Center recorded a high TRM of 23%. This level of mortality is unacceptable for autologous HSCT of a non-malignant disorder or, for that matter, even a malignant disease. In contrast, at Northwestern University 180 patients have undergone autologous HSCT for autoimmune disorders with a TRM of 1.6%, all due to infection in patients on long-standing high-dose immune-suppressive therapy prior to HSCT. Experience from Northwestern University in several immune-mediated disorders will be presented to demonstrate that autologous HSCT for immune-mediated diseases should take into account the following recommendations:

1) HSCT should be non-myeloablative. The rationale of an autologous HSCT for immune-mediated disorders is to maximally suppress the immune system without irreversible ablation of the entire bone marrow compartment. Since hematopoietic recovery will occur without infusion of HSC, autologous HSC are given as a supportive blood product to shorten the duration of chemotherapy-induced neutropenia. Non-myeloablative HSCT is safer and may be as efficacious or more effective than myeloablative regimens.
2) Avoid conditioning agents that will further damage already injured organs. Unlike cancer in which visceral organ dysfunction is a contraindication for HSCT, in immune-mediated dysfunction, some organ damage is often the indication for HSCT.
3) Avoid conditioning regimen late toxicities. Immune-mediated diseases may, despite significant morbidity, have mortality rates similar to the general population and agents that cause late MDS/leukemia, such as total body irradiation, should be avoided.
4) Limit HSCT to the inflammatory stage of the disease. Burned out non-inflammatory stages of the disease will not respond.
5) Treating earlier in the disease course improves safety and may improve efficacy.
6) Do not select or lymphocyte deplete the graft. Manipulation or CD34 selection of the graft will increase infections but has not been demonstrated to improve efficacy and conceivably may even shorten remission duration.

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Profile for Richard Burt

RICHARD BURT MD was born in Billings, Montana where he grew up on a ranch. After graduating cum laude in chemistry and mathematics from the University of Missouri, he graduated cum laude from Saint Louis University School of Medicine. Internal medicine residency was at Baylor College of Medicine (Houston, Texas) where both he and his wife were selected as Chief Medical Residents. Dr Burt performed fellowships in both Hematology and Oncology at the National Institutes of Health (NCI and NHLBI) in Bethesda, Maryland before joining the faculty at Northwestern University School of Medicine in Chicago, Illinois. He has been at Northwestern University for the last 15 years where he pioneered the use of adult hematopoietic stem cells for autoimmune and immune mediated diseases. While at Northwestern University, Dr Burt was recognized with the Compassionate Care Physician of The Year award and for the last seven years has been Chief of the Division of Immunotherapy. Dr Burt applies stem cells across traditional divisional and departmental barriers to diverse immunologic disorders including: 1) neurologic diseases such as multiple sclerosis, chronic inflammatory demyelinating polyneuropathy, myasthenia gravis; 2) rheumatologic diseases such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, and polymyositis: 3) vasculitis such as neuro-vascular Behcets, neuro-vascular Sjogren's, andWegner's granulomatosis: 4) dermatologic disorders such as pemphigus, 5) ophthalmologic diseases such as autoimmune-related retinitis and optic neuritis (ARRON); 6) pulmonary diseases such as sarcoidosis, 7) endocrine disease such as type I diabetes, and 8) psychiatric diseases such as regressive autism. Dr Burt's division has performed more hematopoietic stem cell transplants for autoimmune disease (180 patients) than any center worldwide and has performed the first stem cell transplant in America or in the world for numerous autoimmune/immune mediated diseases. Dr Burt's publications have been presented on the floor of both the United States House of Representatives and United States Senate. Dr Burt's work has been reported in numerous lay publications including *The Wall Street Journal, New York Times, LA Times, Washington Post, Die Zeit magazine, People magazine, Readers Digest, Chicago Tribune,* and in numerous broadcasts including ABC News, NBC News, CBS News, Good Morning America, CNN, BBC, Good Morning Canada, and Australian Broadcasting Corporation. In 2006, the journal *Scientific American* honored Dr Burt has one of the 50 top policy makers in the world. Dr Burt's current clinical interests involve expanding autologous HSCT for autoimmune disorders in randomized controlled trials as well as initiating phase I allogeneic HSCT protocols for several immune-mediated diseases. His laboratory work focus is on application of embryonic stem cells to various animal models of tissue regeneration.

Direct Intra-Bone Injection of Unrelated Cord Blood Cells Overcomes Delayed Engraftment or Graft Failure and Improves The Feasibility of Hematopoietic Transplant in Adult Patients

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Background. Cord blood transplants (CBT) are associated with delayed or failed engraftment in a significant proportion of patients (pts). Two of our previous observations suggested (i) that, in the animal model, direct intra-bone (i.b.) injection improves seeding efficiency and (ii) that the delayed engraftment was not related to an insufficient number of hematopoietic stem cells but rather to their inability to differentiate and maturate. *Methods.* Unrelated CB cells (4/6 or 5/6 HLA antigen matched) were selected for 20 consecutive pts. Median transplant cell dose was 2.7 $x10^7$/kg (range 1.6-4.2). CB cells were concentrated in 4 syringes of 5-6 ml each and infused in the supero-posterior iliac crest (SPIC) under rapid general anesthesia (10 min. with propofol). Pts' median age was 38 years (18-63), 16 had acute leukemia, 2 chronic myeloid leukemia, 2 Hodgkin's disease. Sixteen pts had refractory or advanced disease, whereas 4 had high risk first remission leukemia. Most pts (n=16) were prepared with conventional CY-TBI.

Results. The infusion of cells i.b. in SPIC (some pts bilaterally; some monolaterally) was uneventful. Three pts were not evaluable because they died within day 10 of the transplant. All pts surviving more than 10 days engrafted (100%). Median for PMN engraftment (>0.5$x10^9$/l) was day 23 (14-40), whereas for platelets (>20$x10^9$/l) it was day 38 (range 22-60). Two pts relapsed and three died of infection. Twelve pts are alive and well in hematologic remission at a median follow up of 8 months (range 4-13). From day +30 full donor chimerism was documented in CD3, bone marrow and progenitor cells from both the injected and in un-injected SPIC documenting colonization and recovery of stem cell reservoir. From day +30, CFC progenitors had reached the lower values of the range of normal individuals in bilateral sites. Thus, direct injection of CB cells i.b. produces recirculation of stem cells to the whole hematopoietic system. Only 3 pts experienced acute GvHD (2 grade II and 1 grade I); 4 pts have moderate chronic GVHD. It is known that lymphocyte trafficking is one of the crucial factors in immunity. It is possible that two combined factors may contribute to the low incidence of acute GvHD: (i) Only a proportion of transplanted T cells will reach/circulate primarily in the lymphatic organs, where they would be immediately confronted with host antigen presenting cells as probably occurs after i.v. injection; (ii) intra-bone injected T cells come immediately in contact with mesenchymal stem cells (MSC) and osteoblasts, known to be potent immunosuppressants.

Conclusion. This study has shown that the intra-bone route of administration is responsible for robust hematologic recovery even when low numbers of HLA-mismatched CB cells are transplanted. This approach considerably expands the possibility to perform a cord blood transplant in adults minimizing the risk of graft failure. This may change our policy of hemopoietic cell transplants.

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Profile for Francesco Frassoni

Biographical Sketch

Head of the Stem Cell Centre e Cellular Therapy, Ospedale San Martino in Genova
Francesco Frassoni was born in Genova, August 13th, 1950

Education and Training
1976 Graduated in Medicine and Surgery at the University of Pavia Medical School "cum Laude".
1976-1981 Specialization in Internal Medicine (University of Pavia Medical School)
1981-1984 Specialization in Haematology (University of Genova Medical School)
1984-1988 Specialization in Oncology (University of Genova Medical School)

Professional Experience
- 1979-1980 Visiting Fellow in Paterson Laboratories, Christie Hospital and Holt Radium Institute (Manchester U.K.) (Director Prof. L.G. Lajtha) (ref 25)
- From 1981 Member of the Bone Marrow Transplant Team of the Department of Ematologia, Ospedale San Martino in Genova
- From January 1991: Secretary of Working Party for Acute Leukemia dell'European Group for Bone Marrow Transplantation (EBMT)
- From March 1998 to 2004: Chairman of the Working Party for Acute Leukemia dell'European Group for Bone Marrow Transplantation (EBMT)
- From April to July 2000: spent a period as visiting professor in Department of Human Genetics (director Prof. Lucio Luzzatto) at the Memorial Sloan Ketering Cancer Center in New York.
- Member of the ASH Program Committee 1998, 1999, 2000
- 1999 New Orleans, LA, 3-7 December, organizing and chairing the Educational Session "Hematological Disorders in the Developing World"

Research Experience

Research Activity in the Clinica Medica "A. Ferrata" University of Pavia
1976-1978: in the Physiopathology of Erythropoiesis Laboratory: research on myelofibrosis (ref 24).

Clinical research from the Bone Marrow Transplant Centre in Genova
From 1981 more than 1200 allografts and 600 autografts have been performed in the Bone Marrow Transplant Centre in Genova.

a) Relapse and chimerism after hematopoietic stem cell transplantation (HSCT).
b) Correlation between TBI dose and relapse.
c) Introduction of the concept of "hemopoietic competition" between normal and leukemic hematopoiesis after HSCT.
d) Development of the project of Ph-negative cell mobilization and transplant in chronic myeloid leukemia.
e) First clinical report on the activity of the infusion of expanded mesenchymal stem cells with hematopoietic cells to induce tolerance i.e. reduction of graft-versus-host disease.
f) Hematopoiesis after transplant: low reconstitution of hematopoietic reservoir.
g) Role of $CD34^+$ cells in myelofibrosis (ref 10). i) Attempt to quantify normal and leukemia "stem cells" in CML. Identification that leukemic (Ph-positive) "stem cells" are fewer than normal "stem cells" in patients with CML in chronic phase. ii) Role of NK in allograft.
h) Observation that the delay in hematopoietic recovery after cord blood transplant is not related to an insufficient number of stem cells but to a slow rate of maturation of their progeny.
i) Intrabone injection of hematopoietic cells to overcome the problem of low seeding efficiency.
k) Stem cells in neurological diseases: use of mesenchymal cells in experimental autoimmune encephalistis.

Research Activity as Secretary and Chairman of Acute Leukemia Working Party of European Group for Bone Marrow Transplantation.

a) Long term assessment of the impact of hematopoietic stem cell transplant in patients with leukaemia.
b) Centre Effect in outcome in hematopoietic stem cell transplant.
c) The role of cell dose in the outcome of hematopoietic stem cell transplant.
d) Comparison of the outcome of hematopoietic stem cell transplant with unrelated adult source of hematopoietic cells and cord blood.

Using Graft-vs-Tumor Effects to Treat Patients with Hematological Cancers

Rainer Storb, MD
Fred Hutchinson Cancer Research Center

Conventional allogeneic hematopoietic cell transplantation (HCT) relies on high doses of systemic chemoradiation both to eradicate cancer and to achieve host immunosuppression for graft acceptance. The allograft then serves to rescue patients from chemoradiation-induced marrow aplasia and contributes graft-vs-tumor effects of uncertain magnitude in patients with hematologic malignancies. Treatment related toxicities have limited conventional HCT to younger, medically fit patients with therapies administered on specialized hospital wards. Given that median ages of most patients with myeloid leukemias, myelodysplasias and B cell malignancies range from 65-70 years, most of them have been excluded from transplantation due to age restrictions. In an attempt to address this limitation, we developed a nonmyeloablative transplant approach in which the burden of tumor eradication has been shifted from high-dose cytotoxic agents to the graft-vs-tumor effects. In studies using a large random-bred canine model, we replaced the customary intensive conditioning regimens used for host immunosuppression in HCT, and instead optimized immunosuppression with a combination of mycophenolate mofetil (28 days) and cyclosporine (35-56 days) given for a short period of time posttransplantation. This proved to not only control serious graft-vs-host disease (GVHD) but, as importantly, reduced the magnitude of the host-vs-graft reaction. The conditioning regimen consists of a small and non-marrow ablative dose of 2 Gy of total body irradiation (TBI). Clinical results in more than 1000 patients have shown this nonmyeloablative regimen to be safe and minimally toxic. Side effects seen with conventional HCT, such as alopecia, gastrointestinal toxicities, pancytopenia, and transfusion dependency, were not encountered. Consequently, 51% of eligible patients have been treated entirely in the ambulatory clinics, with others having only relatively short hospitalizations. Most HLA-matched related recipients had stable engraftment despite the use of only 2 Gy TBI conditioning. An initially observed 18% graft rejection rate was significantly reduced to 3% by the addition of three doses of fludarabine immediately preceding TBI. The use of fludarabine has also allowed for sustained engraftment with HLA-identical unrelated HCT. Approximately half of the patients experienced acute GVHD, though mostly of moderate severity. For recipients of unrelated grafts, we extended the period of postgrafting immunosuppression to a minimum of 84 days after transplant in order to reduce the risk of GVHD. The optimal duration of immunosuppression still remains to be determined. Initial mixed donor-host hematopoietic chimerism was unstable in most patients, progressing to full-donor chimerism. Impressive antitumor responses have been seen with complete remissions in 66% of patients who had measurable disease before transplant. Attainment of complete remission is accomplished over time, often months, and in some cases, exceeds 1 year. The durability of these remissions is still unknown, the longest follow-up currently being 7½ years.

In the future, allogeneic nonmyeloablative HCT is likely to become a more powerful and precise therapy. Increased understanding of tissue-specific polymorphic minor histocompatibility antigens might lead to the development of vaccines that could be used to direct the donor cytotoxic T cells toward tumor targets rather than the typical tissues involved in GVHD. Targets include hematopoietic antigens present on both malignant and nonmalignant marrow cells.

Profile for Rainer Storb

Rainer Storb, MD, is a native of Germany, where he attended the University of Freiburg Medical School. After graduation, he spent two years doing clinical training in Essen and Munich, and then three years doing research in Paris on a NATO Science Fellowship, working with Drs. Najean, Bernard and Bessis. In 1965, Dr. Storb traveled to Seattle on a Fulbright Fellowship and began work in the Division of Hematology at the University of Washington with Dr. E. Donnall Thomas. It was here that Dr. Storb participated in the birth of the Seattle marrow transplantation program. For the past 41 years, he has worked to develop new concepts in transplantation biology and apply them to patients. Studies include the demonstration of peripheral blood stem cells for allogeneic transplantation in the 1960s and 1970s, the importance of *in vitro* histocompatibility typing for outcome of related and unrelated transplants in the 1960s and 1970s, the definition of immunologic recovery after marrow transplantation, the development of conditioning programs for transplantation, uncovering the nature of graft-host tolerance, developing strategies of treating and preventing graft-vs-host disease, and studies on hematopoietic engraftment. Many transplantation protocols currently in use have been directly extrapolated from his studies. His work applied to patients with aplastic anemia has defined and improved treatments and increased the long-term survival of this patient group to greater than 90 percent. His current studies to develop protocols for establishing chimeric grafts use transplant regimens that have little toxicity and allow for the treatment of genetic and malignant diseases in both old and young patients in the outpatient setting. In these transplants, cures of malignancy are achieved through an allogeneic graft-vs-tumor effect rather than through the high-dose cytotoxic radiochemotherapy previously used. Dr. Storb has won numerous awards for his work, including the Alexander von Humboldt Award, the Joseph Steiner Award, the Gustav Carus Prize of the German Academy of Natural Sciences, the Meyenburg Prize, the Henry M. Stratton Medal and the E. Donnall Thomas Prize from the American Society of Hematology, the Joseph H. Burchenal Clinical Research Award from the American Association for Cancer Research, the Don Metcalf Lecture Award from the International Society of Experimental Hematology, and the Jacqueline Seroussi Memorial Foundation for Cancer Research Award. Throughout the years, Dr. Storb has trained over 130 researchers in his laboratory. These researchers are now raising the standard of hematopoietic cell transplantation biology research throughout the world.

A Revolutionary BMT Method for Intractable Diseases

Susumu Ikehara, M.D., Ph.D.

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We have recently established an innovative BMT method for the treatment of intractable diseases. The method includes the perfusion method (PM) for the collection of bone marrow cells (BMCs)[1] and intra-bone marrow (IBM)-BMT for the direct injection of collected whole BMCs into the bone marrow cavity.[2] The PM, in comparison with the conventional aspiration method, can minimize the contamination of BMCs with T cells from the peripheral blood. Therefore, without removing T cells, no graft-versus-host disease (GvHD) develops in the case of the PM. Since BMCs collected by the PM contain not only hemopoietic stem cells (HSCs) but also mesenchymal stem cells (MSCs), the injection of whole BMCs (including both cells) directly into the bone marrow cavity (IBM-BMT) facilitates the engraftment of donor hemopoietic cells. In organ allografts with IBM-BMT, no graft failure occurs even if the radiation dose is reduced.[3],[4] In addition, IBM-BMT is applicable to regeneration therapy and various age-associated diseases such as osteoporosis, since it can efficiently recruit donor-derived normal MSCs.[5]

Finally, we have shown that IBM-BMT in conjunction with donor lymphocyte infusion (DLI) can prevent GvHD[6] but suppress tumor growth.[7]

To confirm that this method (PM plus IBM-BMT) is safe and effective before applying it to humans, we have carried out extensive monkey experiments over a period of more than 5 years. We were able to confirm its safety and efficacy using more than 100 cynomolgus monkeys, [8] and therefore applied it to a patient with β-thalassemia major, since only a very small number of patients with β-thalassemia major survive to adulthood because of the side effects of blood transfusion.

The transplantation (PM plus IBM-BMT) was successfully carried out: On day 55, the WBC count reached 8,600/μL, and 98% of the peripheral blood cells were donor-derived. Although the patient regrettably died of asphyxia resulting from sticky sputum, there was no evidence of infection or GvHD.[9]

We are now in the process of establishing an exact protocol (including conditioning regimens) for clinical applications using cynomolgus monkeys (manuscript in preparation). We have also started a "Phase I Study" to confirm the safety of the PM using poor mobilizer patients in malignant lymphomas (manuscript in preparation).

We hope that these experiences will help make our new strategy more readily available for the treatment of patients with various intractable diseases.

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Profile for Susumu Ikehara

Dr. Ikehara received his M.D. in 1967 and his Ph.D. in medicine in 1976 from Kyoto University School of Medicine. He worked for the Memorial Sloan-Kettering Cancer Center in New York in 1978 to 1981.

Dr. Susumu Ikehara is a Professor in the First Department of Pathology, Kansai Medical University, Osaka, Japan. He is also the Director of the Center for Cancer Therapy in Kansai Medical University. He has authored over 350 peer-reviewed scientific articles, which have been published in journals such as Nature, J.Exp.Med., PNAS, Blood, Stem Cells, etc. Dr. Ikehara is on the editorial board of Stem Cells, J. Autoimmunity, Autoimmunity, etc. Current research funding is in excess of one million US dollars per year, the majority of which is from the 21st Century COE (Centre of Excellence) Program, supported by the Japan Society for the Promotion of Science (JSPS).

Dr. Susumu Ikehara is primarily responsible for bringing to light the role of allogeneic bone marrow transplantation (BMT) in the treatment of autoimmune diseases as early as 1985.

The collective impact of Dr. Ikehara's research on the use of allogeneic BMT to treat various autoimmune diseases opens new avenues for curing many diseases. In addition, he has found that autoimmune diseases can be induced in normal mice by the transplantation of partially purified hemopoietic stem cells (HSCs) from autoimmune-prone mice, which indicates that autoimmune diseases are stem cell disorders.

Further, his initial studies with chimeric-resistant MRL/1pr mice led Dr. Ikehara's group to discover that the recruitment of stromal cells, including mesenchymal stem cells (MSCs), is essential for successful allogeneic BMT. These studies cover a broad range of areas but specifically address the ideal strategies to recruit donor-derived stromal cells and perform intra-bone marrow-BMT (IBM-BMT), i.e., the use of whole bone marrow cells (HSCs and MSCs). Dr. Ikehara's group developed a new method (perfusion method: PM) to harvest bone marrow cells without T-cell contamination (i.e., minimizing contamination to below 10%) using cynomolgus monkeys. This new strategy ("PM" + "IBM-BMT") allows not only the use of HLA-mismatched donor marrow but is also applicable in the treatment of numerous intractable diseases, including mesenchymal stem cell disorders such as osteoporosis and emphysema.

Finally, rapid developments in the use of "PM" + "IBM-BMT" herald a revolution in transplantation and regenerative therapy, which would not have been possible without the conceptual and experimental infrastructure provided by Dr. Ikehara's group.