

The 21st Century Center of Excellence (COE) Program

“New Strategies for Treatment of Intractable Diseases”

**Satellite Symposium of the International Symposium,
15th Molecular Biology of Hematopoiesis (MBH)**

August 9, 2004

The Westin Awaji Island Resort & Conference Center

(Organized by Prof. Susumu Ikehara)

Kansai Medical University

8:25a.m.-8:30a.m.

“Opening Remarks”

Koshiro Hioki
(President of Kansai Medical University)

Special Lecture

Chairperson: Fumimaro Takaku
(President of Jichi Medical School)

8:30a.m.-9:15a.m.

**“HIV in the 3rd Decade: Some Lessons from
Past Experiences and Future Prospects”**

Robert C. Gallo
Professor and Director
Institute of Human Virology
University of Maryland Baltimore
725 W. Lombard Street, Suite S307,
Baltimore, MD, USA

Symposium

Chairperson: Jun-ichi Fujisawa
(Professor of Department of Microbiology)

9:15a.m.-9:45a.m.

**“Molecular Targets & Molecular Treatments: The
Promise of Anticancer Drug Development”**

Martin J. Murphy, Jr.
Executive Editor
STEM CELLS, AlphaMed Press
One Prestige Place, Suite 290
Miamisburg, Ohio 45342-3758, USA

9:45a.m.-10:15a.m. **“pDC/IPC Development and Biology”**

Yong-Jun Liu
Chair & Professor, Department of Immunology
Director, Center for Cancer Immunology Research
South Campus Research Building, 3.1035, USA

10:15a.m.-10:30a.m.

“Intermission”

Chairperson: Kazuichi Okazaki
(Professor of Third Department of Internal Medicine)

10:30a.m.-11:00a.m. **“Progress in Defining the Role of RSV in Allergy and Asthma: from Clinical Observations to Animal Models”**

Laurel J. Gershwin

Professor of Immunology
Dept. of Pathology, Microbiology, & Immunology
School of Veterinary Medicine
University of California,
Davis, CA 95616, USA

11:00a.m.-11:30a.m. **“AAV (Adeno-Associated Virus) Vectors and Their Application to Gene Therapy”**

Keiya Ozawa

Professor and Chairman
Division of Hematology, Department of Medicine
Division of Cell Transplantation and Transfusion
Division of Genetic Therapeutics,
Center for Molecular Medicine
Jichi Medical School,
3311-1 Yakushiji, Minamikawachi-machi
Kawachi-gun, Tochigi 329-0498, Japan

11:30a.m.-12:00p.m. **“The Molecular Basis of the Autoimmune Disease, Primary Biliary Cirrhosis”**

M. Eric Gershwin

The Jack and Donald Chia Professor of Medicine
Division of Rheumatology
Allergy-Clinical Immunology
University of California
One Shields Avenue, TB192
Davis, CA 95616, USA

12:00p.m.-1:00p.m.

“Lunch”

Chairperson: Seiji Ito
(Professor of Department of Medical Chemistry)

1:00p.m.-1:30p.m. “Advances on the Use of Stem Cells for Type I Diabetes: Restoration of Pancreatic Function and Antioxidant Genes”

Nader G. Abraham
Professor
Director of Gene Therapy
Department of Pharmacology
New York Medical College
Valhalla, New York 10595, USA

1:30p.m.-2:00p.m. “Strategies for Enhanced Regeneration of Bone Marrow by Stem Cell Transplantation”

IL-Hoan Oh
Director, Associate Professor
Cell & Gene Therapy Institute
Catholic University of Korea, School of Medicine
505, Banpo-Dong, Seocho-ku, Seoul, Korea

Chairperson: Yasuo Kamiyama
(Professor of Department of Surgery)

2:00p.m.-2:30p.m. “Transplantation of Circulating Stem Cells in the Heart in Severe Cardiac Failure”

Philippe RG. Henon
ARHT-Hôpital du Hasenrain
87 Avenue d'Aitkirch
Mulhouse, 68100, France

2:30p.m.-3:00p.m. “Advances in Stem Cell Transplantation for Lymphoma: Role of Monoclonal Antibodies”

Issa Khouri
Professor
M.D. Anderson Cancer Center
Department of Blood and Marrow Transplantation
1515 Holcombe Blvd, Unit 423,
Houston, TX, 77030, USA

3:00p.m.-3:15p.m. **“Intermission”**

Chairperson: Shirou Fukuhara
(Professor of First Department of Internal Medicine)

3:15p.m.-3:45p.m. **“Allogeneic Blood and Embryonic Stem Cell Transplantation for Autoimmune Diseases”**

Richard Burt

Chief, Division of Immunotherapy and
Autoimmune Disease (DIAD)
Northwestern University Medical Center
250 East Superior,
Wesley Pavilion,
Room 162, Chicago, III,
USA 60611

3:45p.m.-4:15p.m. **“Allogeneic Bone Marrow Transplantation from HLA-Mismatched Mothers”**

Chunfu Li

Professor of Department of Paediatrics
Nanfang Hospital
The First Military Medical University
Guangzhou 510516, China

Chairperson: Chiyoko Inagaki
(Professor of Department of Pharmacology)

4:15p.m.-4:45p.m. **“Identification of a Novel Class of CD34-Negative Hematopoietic Stem Cells Using the Intra-Bone Marrow Injection”**

Yoshiaki Sonoda

Associate Professor
Department of Molecular-
Targeting Cancer Prevention
Graduate School of Medical Science
Kyoto Prefectural University of Medicine,
465 Kajii-cho, Hirokojiagaru, Kawaramachi,
Kamigyo-ku, Kyoto City,
Kyoto 602-8566, Japan

4:45p.m.-5:15p.m.

“Perfusion Method” + “Intra-Bone Marrow – BMT” as a New Strategy for Treatment of Various Intractable Diseases, Possibly in Humans”

Susumu Ikehara

Professor of First Department of Pathology
Director of Transplantation Center
Director of Regeneration Research Center for Intractable Diseases
Director of Center for Cancer Therapy
Kansai Medical University,
10-15 Fumizono-cho, Moriguchi City,
Osaka 570-8506, Japan

5:30p.m. till late

“Party”

"HIV in the 3rd Decade: Some Lessons from Past Experiences, and Future Prospects"

Satellite Symposium of the 13th International Symposium on Molecular Biology of Hematopoiesis

**Awaji Island, Japan
August 9, 2004**

**Robert C. Gallo, MD
Director
Institute of Human Virology
University of Maryland Baltimore**

By the late 1970s a consensus emerged in the medical-scientific community that epidemics were over, that viruses were not causes of any human cancers, and that retroviruses of humans did not exist. These ideas were shattered by the early 1980s when human retroviruses were discovered, some of them (HTLV-1) shown to cause unusual forms of leukemia, other viruses shown to cause about 20% of all human cancer, and another retrovirus (HIV) shown to cause the most serious epidemic of the century (AIDS) and one of the worst in medical history. On the other hand, fortunately, just before AIDS was first recognized, the technology to grow human T-cells with Il-2 and very sensitive assays for retroviruses had been developed. Both technologies were critical to the discoveries of human retroviruses.

It is now over 20 years since the first discovery of human retroviruses (HTLVs, 1980), the first description of AIDS (1981), and the first isolate of HIV (1983), and the demonstration that HIV was the cause of AIDS (1984). During this period enormous progress has been made in basic studies of human retroviruses, including the elucidation of their replication cycles and much of their pathogenic mechanisms.

Both HTLV and HIV have multiple redundant mechanisms for their survival. For HTLV it is in the several mechanisms which promote growth of provirus-containing cells. This appears to be necessary because HTLVs replicate inefficiently. In contrast, HIV has evolved multiple redundant mechanisms for maintaining virion numbers and survival. It is by these redundant mechanisms, evolved for their survival, that diseases occur. These diseases include the occasional T-cell leukemia of HTLV and the common immune deficiency by HIV (see Gallo R. Human Retroviruses after 20 years: Perspective from the past and Prospects for their future control. *Immunol Rev* 185; 236-262, 2002).

Probably we now know as much about HIV as we do about any virus. Similarly, we may know as much about AIDS as, and possibly more than, we do about any other disease. However, at the onset of the AIDS epidemic HIV presented unique challenges. Unlike past viral epidemics or the recent SARS epidemic, AIDS became manifest only after some 5-15 years from the time of infection. Moreover, by the time clinical AIDS developed, the patient had multiple microbial infections. These two features combined to make the demonstration of the HIV cause of AIDS a formidable challenge. Essential to success was the development of a sensitive, specific, and simple blood test which provided overwhelming evidence that HIV was the cause of AIDS. The blood test was also quickly adopted to screen the supply of blood for medicinal purposes, and soon (by early 1985) made the blood supply safe for most industrial nations. This became the field's first practical advance. A second practical advance also began in the mid-1980s with the first anti-HIV therapy (AZT) and culminated in the mid-1990s with combination of inhibitors of HIV enzymes (RT and protease) in a 3- to 4-drug cocktail, which provided a dramatic clinical achievement and for the first time in medicine enforced the notion that persisting viruses can be chemically attacked.

Thus, for some, HIV no longer produces a predictable death. Rather, with therapy, HIV is often a chronic disease with far less morbidity. Furthermore, pediatric AIDS in the industrial world is almost over. However, the good news is quickly tempered by: (1) the emergence of HIV drug-resistant mutants; (2) the toxicity of drugs when used and needed for years and probably for life; (3) the rise in cancer incidence in infected persons; (4) the increasing epidemic in some populations in Western nations; (5) the increasing numbers of doubly infected people with TB or HCV and HIV, and their worse prognosis; (6) the still considerable uncertainty of the future of the epidemic; (7) the lack of preventive vaccine; and (8) lastly, and most important, the dramatic epidemic in parts of the world that provide an enormous financial and infrastructure challenge.

I will use this presentation to: (1) summarize some of HIV's pathogenic mechanisms; (2) discuss some of the new therapeutic approaches; and (3) outline a path to a preventive vaccine.

BIOGRAPHICAL SKETCH

Robert C. Gallo, M.D.

Since 1996, Dr. Robert C. Gallo has been Director of the Institute of Human Virology at the University of Maryland, Baltimore. Previously (for 30 years) he was at the National Cancer Institute in Bethesda, MD. Dr. Gallo's career-long interest has followed one theme: the study of the basic biology of human blood cells, their normal and abnormal growth, and the causes of abnormal growth whether excessive, e.g., leukemias or insufficient, e.g., immune deficiencies.

Dr. Gallo and his co-workers opened and pioneered the field of human retrovirology when, in 1980, they discovered the first human retrovirus (HTLV-1), and with others showed it was a cause of a particular form of human leukemia. (This was the first, and to date, the only known human leukemia virus and one of the few known viruses shown to cause a human cancer). A year later he and his group discovered the second known human retrovirus (HTLV-2). Dr. Gallo and his colleagues also independently discovered HIV (the 3rd known human retrovirus), and provided the first results to show that HIV was the cause of AIDS. They also developed the lifesaving HIV blood test.

The discoveries of human retroviruses, including HIV, were to a great extent dependent on being able to grow human T-cells (lymphocytes) in the laboratory, and this was achieved by the use of a growth factor called Interleukin-2 or IL-2. Dr. Gallo and his co-workers discovered Interleukin-2 in 1976, thus setting the stage for all groups to culture human T-cells. Today IL-2 is used not only in laboratory experiments, but also in some therapies for cancer and AIDS.

Main Recognition

Dr. Gallo has been awarded 19 honorary doctorates from universities in the United States, Sweden, Italy, Israel, Peru, Belgium and Argentina. He is a member of numerous professional and honorary societies including the National Academy of Sciences, the Institute of Medicine of the U.S. National Academy of Sciences, the Royal Society of Medicine (Glasgow, Scotland), and the Royal Society of Medicine (Brussels, Belgium) among several others.

He has received numerous major scientific honors and awards, for example uniquely the U.S. Albert Lasker Prize award twice (1982, 1986), and most recently the World Health Award from President Gorbachev in Vienna in November 2001. He was the most cited scientist in the world 1980-1990, according to the Institute for Scientific Information (*Science* July 27, 1990, p. 358)

Molecular Targets & Molecular Treatments: The promise of Anticancer Drug Development

Martin J. Murphy

During the past decade, several molecules that contribute to proliferation, invasion, and metastasis of cancer cells have been identified. Members of the EGFR superfamily are overexpressed in many tumors and are associated with poor prognosis. Therefore, they have become an important target for novel anticancer therapies, especially in the treatment of lung cancer, where new and less toxic approaches are desperately required.

The results of gefitinib, an ^{Iressa} epidermal growth factor receptor inhibitor (EGFR) in non-small cell lung cancer have been very instructive. Single agent response rates in heavily pretreated patients are in the 12 to 18% range. As the majority of these patients express the EGFR, it has been concluded that EGFR expression does not correlate with response. The modest results in these clinical trials have fueled skepticism regarding the utility of molecularly targeted agents. However, it has always been clear that there is a subset of lung cancer patients who responded dramatically to gefitinib and these were predominantly non-smoking women with bronchoalveolar tumors. Two recent reports have shown that this subset of patients has EGFR mutations that render the EGFR hypersensitive to ligand and to gefitinib. Thus, this represents an example where the target expression is high, but frequency of activation is low. However, this subgroup of patients has an extremely high response rate and demonstrates that, in this subgroup of patients, the tumors are dependent on the activity of the mutated EGFR. In both the preceding examples, careful evaluations of subsets of responding patients can yield important insights into disease pathogenesis and the mechanism of response to an agent.

The implications of the results with imatinib in GIST and gefitinib in patients with EGFR mutations are extremely important. It would be hard to argue that these are simple \pm cancers. GISTs are extremely heterogeneous tumors, often with complex karyotypes, and responses to multi-agent chemotherapy are observed in less than 5% of patients. Although it could be argued that tumors arising in non-smokers may be less complex than those occurring in smokers, all of the patients enrolled in the gefitinib clinical trials had treatment refractory disease. Thus, the implications of these results are that even advanced solid tumors have Achilles \perp heels and that developing agents that modulate these targets is an extremely effective therapeutic strategy. Of course, it could now be said that this represents only 10% of lung cancer. However, the issue then becomes defining similar Achilles \perp heels in the other 90% of lung cancers as well as other tumors.

It is expected that EGFR-directed therapies will be established as effective novel treatments for patients with lung cancer and other malignant diseases once we understand how best to use them. For example, it has been postulated that, in the elderly or those with poor performance status, anti-EGFR monotherapy may be equally efficacious and better tolerated than conventional treatments. Clearly, considerable research is still required, but the wealth of knowledge gained from these early biological therapy trials cannot be understated, and these studies offer hope for new and effective therapies in the future.

Dr. 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 21,000 physicians entrusted with cancer patient care. He is Founder and Executive Editor of *Stem Cells*, a scientific journal that is distinguished by more than two decades of publishing excellence in this fast-paced and promising research arena of stem cell biology. Both journals are available online (www.TheOncologist.com and www.StemCells.com).

Founder and former Chief Executive Officer of the Hipple Cancer Research Center, Dr. Murphy was professor of medicine and an NIH principal investigator of \$21 million of 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 A.M. Pappas & Associates, which manages fledgling companies whose R&D efforts are on the cutting edge of science. He is a director of Momentum Bio Ventures, Inc., a corporate advisory and investment firm focusing on identifying opportunities, evaluating technology, building companies, providing seed and later stage capital to foster the growth of life science companies. He is chairman of the Scientific Advisory Board of Arragen, Ltd., a biotech company developing anti-cancer drugs in Belfast, Northern Ireland. He is a member of the board of trustees of the American Cancer Society Foundation, a national director of the American Cancer Society, Inc., 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 and chaired by Robert A. Ingram at the request of the former President Bush. The CEO Roundtable numbers among its members the chief executives of more than 40 of America's greatest companies.

pDC/IPC Development and Biology.

Yong-Jun Liu, Yui-Hsu Wang and Tomoki Ito, Department of Immunology and CCIR, The University of Texas, MD Anderson Cancer Center, Houston, TX 77030.
E-mail: yjliu@mdanderson.org

Type 1 interferon-(α , β , ω)-producing cells (IPCs), also known as plasmacytoid dendritic cell precursors (pDCs), represent 0.2-0.8% of peripheral blood mononuclear cells in both humans and mice. PDCs/IPCs represent a separate hematopoietic lineage, which appear to be more close to B lineage than myeloid lineage. They are continuously produced from hematopoietic stem cells within the bone marrow, and then released into the peripheral blood stream. Unlike myeloid cells that enter the secondary lymphoid nodes from afferent lymphatics, PDCs/IPCs enter the lymph nodes through HEV (similar to the T and B lymphocytes) and then colonize the T cell-rich areas. In the steady state, pDCs/IPCs appear to play a critical role in maintaining peripheral immune tolerance, since the depletion of pDC/IPCs leads to asthmatic immune reactions to harmless inhaled antigens. This may be due to the ability of resting pDC/IPCs to prime naïve T cells to produce IL-10. Unlike monocytes and myeloid DCs, which preferentially express surface pattern recognition receptors for bacteria products (TLR2, 4, 5, 6), pDC/IPCs only express TLR7 and TLR9 within the endosomal compartment, which appears to be specifically designed to recognize single-stranded viral RNA or double stranded viral DNA. Upon microbial infection, in particular viral infection, pDC/IPCs rapidly produce a large amount of type 1 IFN, which not only has a direct inhibitory effect on viral replication, but also directly contributes to the activation of NK cells, B cells, T cells and myeloid DCs, leading to the induction and expansion of an anti-viral immune response. The ability of activated pDC/IPC to activate immature myeloid DCs through type-1 IFN appears to be critical for the induction of a productive adaptive T cell-mediated anti-viral immune response. After producing a huge amount of type 1 IFNs, pDC/IPCs rapidly differentiate into mature DCs through an autocrine mechanism mediated by type 1 IFNs and TNF- α . PDC/IPC-derived DCs appear to have a unique ability to prime both CD4+ T cells and CD8+ T cells to produce IL-10, which may contribute to the contraction of an anti-viral immune response at a later stage.

Yong-Jun Liu, MD, PhD
Chair, Department of Immunology
Director, Center for Cancer Immunology Research

Dr. Yong-Jun Liu has served as Chair of Immunology and Director of the Center for Cancer Immunology Research at the University of Texas M. D. Anderson Cancer Center since October 2002. Prior to 2002, Dr. Liu was a Senior Staff Scientist for DNAX Research Institute of Molecular and Cellular Biology, Inc., from 1997-2002. He was a Maitre de Recherche [H1] and a Charge de Recherche in the Laboratory for Immunological Research at Schering-Plough from 1991-1997. Dr. Liu was a Research Fellow in the Department of Immunology at the University of Birmingham School of Medicine in the United Kingdom from 1989-1991. He was also a Research Associate in the Department of Cell Biology at Norman Bethune University School of Medicine in China from 1984-1985. He received his MD in 1984 at Norman Bethune University, China and his PhD in 1989 at the Birmingham University School of Medicine in the United Kingdom.

Positions and Employment

- | | |
|--------------|--|
| 1989-1991 | Research Fellow, Department of Immunology, University of Birmingham School of Medicine, United Kingdom |
| 1991-1994 | Charge de Recherche, Laboratory for Immunological Research, ScheringPlough, Dardilly, France |
| 1994-1997 | Maitre de Recherche, Laboratory for Immunological Research, Schering-Plough, Dardilly, France |
| 1997-2002 | Senior Staff Scientist, DNAX Research Institute of Molecular and Cellular Biology, Inc. |
| 2002-present | Professor and Chairman, Department of Immunology, The University of Texas MD Anderson Cancer Center, Houston, TX |
| 2002-present | Director, Center for Cancer Immunology Research, The University of Texas MD Anderson Cancer Center, Houston, TX |

Other Experience and Professional Memberships

- | | |
|-----------|---|
| 1986-1991 | British Society for Immunology |
| 1994-1995 | American Association for the Advancement of Science |
| 1996- | New York Academy of Sciences |
| 1999 | American Association for Immunology |

Honors

- | | |
|------|---|
| 1987 | British Overseas Student Award |
| 1999 | Scientist selected by Chronological Record of Progress in Oncology over the last Millennium for recognition of purification of cells from human blood producing large quantities of interferon. Edited by H.S.J. Lee. |

Progress in defining the role of RSV in allergy and asthma: from clinical observations to animal models

Laurel J Gershwin, D.V.M., Ph.D.

Department of Pathology, Microbiology, & Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616; Phone: (530) 752-6643; FAX: (530) 752-3349; e-mail: ljgershwin@ucdavis.edu

Respiratory syncytial virus (RSV), an RNA virus in the family Paramyxoviridae, causes respiratory disease in humans. The impact of RSV on human health is demonstrated annually when infants are admitted to the hospital in large numbers. Nearly every child will have been infected with RSV by the age of three years. While the disease is most severe in young infants and elderly people, it can re-infect adults causing mild upper respiratory tract disease throughout life. In addition, there is growing evidence that RSV infection may also predispose some children to the development of asthma. This is based on the observation that children who wheeze with RSV-induced bronchiolitis are more likely to develop into allergic asthmatics. Recent studies describe attempts to create an RSV-induced asthma model in mice and other species; these have shown some degree of success. Such reports of case studies and animal models have suggested a wide range of factors possibly contributing to RSV induced asthma; these include timing of RSV infection with respect to allergen exposure, prior allergic sensitization, environmental conditions, exposure to endotoxin, and the genetic background of the person or animal. Unlike many other viruses, RSV's mechanism for entry into susceptible cells has not fully been elucidated. In a recent review, RSV was said to bind toll-like receptor 4 (TLR-4) prior to cell entry. TLR-4, CX3R1 (fractalkine receptor), sodium heparin, and caveolin have been suggested as potential cellular receptors that are important for viral entry. TLR-4 binding to RSV does occur but is apparently not required for infection to be established. Previous work has demonstrated that RSV viral titers in the lungs of TLR-4-deficient C57BL10/ScCr mice were increased and IL-6 levels were suppressed. They also found that CD14 is necessary in conjunction with TLR-4 for stimulation of IL-6, TNF α , IL-8, and IL-1 β production. The results of this study infer that the initial immune response to RSV may be instigated by TLR-4 binding to RSV. The influence of RSV infection on allergic sensitization and the influence of allergic sensitization on RSV bronchiolitis have also been examined using relevant studies. While the effects of LPS and allergen exposure on pathogenesis of RSV infection have been well studied in mouse models, the cellular and molecular events that determine the ultimate outcome of the immune responses remain to be fully elucidated. Indeed, mechanisms that favor the role of RSV in enhancing allergic sensitization are very likely not the same as those that facilitate asthma exacerbation when the allergic asthmatic child is infected with RSV. Further studies in other appropriate model systems such as the rhesus monkey/RSV and bovine calf/BRSV species may help to elucidate the complex interaction between virus, host, and allergen. In this presentation, I will focus on the pathogenic basis and relationship between RSV infection and development of asthma.

BIOGRAPHY

Laurel J. Gershwin, DVM, Ph.D.

Professor of Immunology

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EDUCATION:

COLLEGE: University of California, Davis
B.S., 1969 Veterinary Science, with honors

VETERINARY SCHOOL: University of California, Davis, D.V.M., 1971

INTERNSHIP: Angell Memorial Animal Hospital (Small Animal Medicine and Surgery),
Boston, Massachusetts, Certificate, 1972

GRADUATE STUDY: University of California, Davis, Ph.D., September, 1979

PROFESSIONAL EXPERIENCE:

1994 - present: Professor of Immunology, Department of Pathology, Microbiology, and Immunology,
University of California, Davis.

1991 - 1994: Chairperson, Department of Veterinary Microbiology and Immunology, Director of
Clinical Immunology and Virology Laboratory, Veterinary Medical Teaching Hospital, School
of Veterinary Medicine, University of California, Davis, California.

1988 - 1991: Associate Professor of Immunology, and Chairperson, Department of Veterinary
Microbiology & Immunology, and Director of Clinical Immunology and Virology Laboratory,
Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California,
Davis.

1985 - 1987: Associate Professor of Immunology. Department of Veterinary Microbiology and
Immunology, School of Veterinary Medicine, University of California, Davis.

1985 - 1986: Honorary Senior Lecturer. LaTrobe University, Melbourne, Australia

1979 - 1985: Assistant Professor of Immunology. Department of Veterinary Microbiology and
Immunology, School of Veterinary Medicine, University of California, Davis.

Dr. Laurel Gershwin has published approximately 100 experimental papers, reviews and book chapters. She is author of a popular textbook, in widespread usage, on veterinary immunology. Her expertise has been in the role of respiratory syncytial virus and air pollution in reducing allergic responses. She has served as Chairperson of the Department of Veterinary Immunology at UC Davis, is past President of the American Association of Veterinary Immunology, and member of the board of the Research Council of the American Veterinary Medical Association.

AAV (adeno-associated virus) vectors and their application to gene therapy

Keiyo Ozawa, M.D., Ph.D.

Division of Hematology, Department of Medicine; Division of Cell Transplantation and Transfusion; and Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical School, Tochigi 329-0498, Japan

AAV vectors are considered to be promising gene-delivery vehicles for gene therapy, because they are derived from non-pathogenic virus, efficiently transduce non-dividing cells, and cause long-term gene expression. Appropriate AAV serotypes are utilized depending on the type of target cells; e.g., an AAV1 vector is most suitable for muscles, and neurons are efficiently transduced with AAV2 and AAV5 vectors. As for muscle-mediated gene therapy, the therapeutic effects of AAV vectors expressing interleukin-10 (AAV-IL-10) were investigated. As a result, intramuscular administration of AAV-IL-10 into apolipoprotein E-deficient mice inhibited atherogenesis through anti-inflammatory and cholesterol-lowering effects. Protective effects on the arterial damage such as hypertensive arteriosclerosis were also examined using the stroke-prone spontaneously hypertensive rats (SHR-SP). Systemic IL-10 expression caused a decrease in blood pressure, proteinuria, and stroke-episode, resulting in increased survival rate. Histological examination revealed that arteriosclerotic changes were almost completely abolished in the brain and the kidney. In addition, intramuscular injection of AAV-IL-10 was effective in suppressing angiogenesis, tumor growth, and peritoneal dissemination of VEGF-producing ovarian cancer cells (SHIN-3) *in vivo*, resulting in improved survival of tumor-bearing mice. AAV vectors are also appropriate for gene therapy of neurological disorders. Parkinson's disease (PD) is a progressive neurodegenerative disorder that predominantly affects dopaminergic neurons in the substantia nigra. There are two major approaches to gene therapy of PD; i.e., 1) intrastriatal expression of dopamine (DA)-synthesizing enzyme genes, and 2) neuroprotection using GDNF gene to prevent the disease progression. As for the initial step of clinical application, AADC (aromatic L-amino acid decarboxylase; the enzyme converting L-DOPA to DA) gene transfer in combination with oral administration of L-DOPA would be appropriate, since DA production can be regulated by adjusting the dose of L-DOPA. The efficacy of this therapeutic strategy was demonstrated in the preclinical studies using MPTP-induced parkinsonian monkeys. Taken together, these studies indicate that AAV vectors would be valuable in clinical gene therapy for many chronic diseases in the near future.

CURRICULUM VITAE

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Jichi Medical School
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Kawachi-gun, Tochigi 329-0498, Japan

Education:

1977 M.D. Faculty of Medicine, University of Tokyo
1984 Ph.D. (Doctor of Medical Science), Faculty of Medicine, University of Tokyo

Professional Training and Employment:

1977-1979 Resident in Internal Medicine, Tokyo University Hospital, Tokyo
1979-1984 Clinical and Research Fellow, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo
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 School, Tochigi

Appointments:

1990-1992 Editorial Board, International Journal of Hematology
1993-2000 Editorial Board, Japanese Journal of Clinical Oncology
2000- Associate Editor, Japanese Journal of Clinical Oncology
1996-2002 Editorial Board, Japanese Journal of Cancer Research
1998-2000 Editorial Board, Experimental Hematology
2000- Editorial Board, Journal of Gene Medicine
2001- Associate Editor, Current Gene Therapy

The molecular basis of primary biliary cirrhosis: From induction through destruction

Zhe-Xiong Lian¹, Susumu Ikehara², M. Eric Gershwin¹

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PBC is an autoimmune disease of the liver characterized by the presence of antimitochondrial autoantibodies and intrahepatic bile duct destruction. Elevated serum IgM is a prominent serological and diagnostic feature of PBC but the mechanism that gives rise to hyper-IgM is unknown. We hypothesize that the sustained elevated IgM reflects the existence of a population of hyper-responsive B-cells that secrete IgM in response to stimuli. The studies conducted herein address this issue by determining the percentage of intracellular IgM⁺ B cells, expression of TLR9, and levels of IgM synthesized by PBMC, from PBC patients and controls cultured *in vitro* with CpG ODN. Our data demonstrate that CpG-B but not CpG-A increases the frequency of B cells that synthesize intracellular IgM in the PBMC in PBC, but not controls, with a peak at day 4 after CpG stimulation. The increase in intracellular IgM⁺ B cells is the result of an increase in the relative expression of TLR9, and the production of IgM is primarily from memory B cells and requires the help of other cell lineages. This work is the first to demonstrate a role for bacterial CpG DNA in the induction of hyper-IgM in the autoimmune disease, PBC. IgM responses play an important role in innate immunity by providing a first line of host defense against infectious agents through agglutination and complement activation. IgM is known to enhance antigen-driven IgG responses and functions as an important link between innate and acquired immunity, as well as being involved in regulating IgG autoantibody production. While the precise mechanism of immunologic self tolerance remains to be defined, it has been known for some time that certain antigens, in particular those with repeated sequences, often non-proteinaceous in nature, appear to induce polyclonal B cell activation accompanied by the synthesis of IgM with no detectable memory B cell generation. Examples include bacterial DNA sequences with unmethylated CpG motifs. Such substances are said to possess pathogen-associated molecular patterns (PAMPs), and these have been reported to have immunostimulatory effects in humans. A number of studies have suggested a bacterial etiology in PBC, although the precise mechanisms by which such bacteria lead to PBC remains to be defined. Thus, we hypothesize that hyper-IgM in PBC could be a reflection of a history of bacterial infection leading to a chronic polyclonal innate immune response to bacteria in patients with PBC and, in particular, responsiveness to PAMPs associated with this chronic exposure. In this presentation, we will focus on the relationship between immune dysfunction that leads to elevated IgM, as well as the production of antimitochondrial antibodies in such patients.

BIOGRAPHY

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- COLLEGE:** Syracuse University, Syracuse, New York
A.B., 1966 (summa cum laude), Phi Beta Kappa, Pi Mu Epsilon
(mathematics)
- MEDICAL SCHOOL:** Stanford University, Stanford, California
M.D., 1971 (Alpha Omega Alpha)
- GRADUATE SCHOOL:** Centre for Astrophysics and Supercomputing, Melbourne, Australia
M.S., 2002 (Astronomy and Astrophysics)
- PROFESSIONAL EXPERIENCE:**
- 2003-present Distinguished Professor of Medicine, Division of Rheumatology/Allergy and Clinical Immunology, University of California Davis, Davis, California
- 1994-present The Jack and Donald Chia Professor of Medicine, Division of Rheumatology/Allergy and Clinical Immunology, University of California Davis, Davis, California
- 1989-2001 Chairperson, Graduate Group in Immunology, University of California Davis, Davis, California
- 1985-1986 Guggenheim Fellowship and Visiting Scientist, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.
- 1982-present Chief, Division of Rheumatology/Allergy and Clinical Immunology, University of California School of Medicine, Davis, California.
- 1981-present Professor of Medicine (Rheumatology and Allergy), University of California School of Medicine, Davis, California.
- 1977-1981 Associate Professor of Medicine (Rheumatology and Allergy), University of California School of Medicine, Davis, California.
- 1975-1977 Assistant Professor of Medicine (Rheumatology and Allergy), University of California School of Medicine, Davis, California.
- 1973-1975 Clinical Associate, Immunology, National Institutes of Health, Bethesda, Maryland.
- 1971-1973 Internship, Residency: Tufts-New England Medical Center, Boston, Massachusetts.

Dr. Gershwin graduated from Stanford Medical School and interned at Tufts-New England Medical Center. He trained in immunology at the National Institutes of Health and joined the faculty of the University of California at Davis in 1975. Currently, he is the Jack and Donald Chia Professor of Medicine and Chief of the Division of Clinical Immunology. Dr. Gershwin has authored over 500 experimental papers and more than 20 books. Dr. Gershwin was the first scientist to clone the gene that produces the autoantigen involved in the anti-mitochondrial antibody test for patients with primary biliary cirrhosis. Dr. Gershwin has traveled frequently to Japan and has trained more than 25 Japanese postdoctoral scholars since 1980

Advances on the use of stem cells for Type I diabetes: Restoration of pancreatic function and antioxidant genes

Prof. Dr. Nader G. Abraham,
Director of Gene Therapy,
New York Medical College, Valhalla, NY
and
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Type I diabetes mellitus is caused by an autoimmune destruction of the insulin-producing beta cells. Diabetes has been linked to a decrease in circulating stem cells and an over-production of oxidants, which contribute to destruction of the pancreas beta cells as well as a decrease in heme oxygenase-1 (HO-1) gene expression. Decreased HO-1 is associated with an increase in oxidative stress and endothelial cell detachment in both human and animal Type 1 diabetes. Human HO-1 gene transfer has been shown to enhance angiogenesis and pancreatic blood vessel formation, and human HO-1 gene transfer into diabetic animals has been shown to decrease endothelial cell detachment. HO-1 is an immune modulator, which increases the levels of interleukins, including IL-10, IL-6 and IL-1. It has been shown that bone marrow stem cells can prevent diabetic complications and restore pancreatic function (Ikehara et al., Proc. Natl. Acad. Science 1985;82:7743 and 1990;87:8341). We used an animal model, the nonobese diabetic (NOD) mouse, in this study of Type 1 diabetes. NOD are characterized by development of autoimmune disease as a result of a striking infiltration of T-cells into the pancreatic islets and destruction of β -cells, and were used to assess the levels of HO-1 gene expression. We assessed the effect of stem cell transplant on the expression of HO-1, and immune and pancreatic function. When NOD mice (6 months old) were irradiated and reconstituted with bone marrow cells from young BALB/c mice (<2 months old), the NOD mice exhibited neither insulinitis nor overt diabetes, and displayed normal T- and β -cell functions. The newly developed T-cells in the allogenic bone marrow cell recipients were tolerant to cells with both donor- and host-type major histocompatibility complex determinants. Three months after bone marrow transplant, NOD mice showed the same glucose tolerance test results as BALB/c mice. HO activity in aorta and renal tissue in nontransplanted NOD mice decreased compared to NOD transplant mice. Aortic HO activity was 0.38 ± 0.11 and 0.67 ± 0.14 nmol bilirubin/mg/hr in nontransplanted and NOD transplanted mice, respectively. The increase in HO activity was accompanied by elevation of HO-1 protein ($n=5$, $p<0.05$) and was associated with a significant decrease in superoxide anion (O_2^-). Aortic O_2^- in nontransplanted NOD mice was 2.81 ± 0.37 $\mu\text{mol/mg}$ protein compared to 1.16 ± 0.37 $\mu\text{mol/mg}$ protein in NOD mice three months after transplantation. Since upregulation of HO-1 prevents endothelial cell death *in vitro* and *in vivo* and increases insulin levels, we believe that one mechanism by which bone marrow transplant prevents vascular complications in diabetes is by restoration of the levels of antioxidants and cytokines. These results demonstrate that stem cells transplant restores the antioxidant gene, HO-1, and pancreatic function. These results provide a novel strategy to support the concept of using stem cells to cure Type 1 diabetes.

Prof. Dr Nader G Abraham
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CITIZENSHIP: United States

PERSONAL STATUS: Married with two children

EDUCATION

1975-1976 Post-Doctoral Fellow
The Rockefeller University, New York, NY
1972-1975 Mount Sinai School of Medicine, New York, NY
Thesis: Regulation of Mitochondrial Poly-ribosomal Protein Synthesis
by Nuclear Initiation Factors.

ACADEMIC APPOINTMENTS

1997-Present Director of Gene Therapy
Professor of Medicine and Pharmacology
New York Medical College, Valhalla, NY
1994-present Visiting Professor
The Rockefeller University, New York, NY

HONORS AND AWARDS

Recipient of more than 12 international awards for Stem cells and heme oxygenase research

MEMBER OF PROFESSIONAL SOCIETIES AND EDITOR OF BOOKS

Member of many societies and associate editor for many journals as well as Ad Hoc reviewer of Journal of Biological Chemistry, Science, FASEB, American Journal of Physiology, Journal of Pharmacology and Experimental Therapeutics, Experimental Eye Research

CURRENT RESEARCH SUPPORT: Three NIH grants

SPECIAL PARTICIPATION AND INVITATIONS

- ◆ Chairman, Cyclosporin and Renal Toxicity; XIth International Congress of Nephrology, Tokyo, Japan, 1990
- ◆ Chairman, Growth Factor Session; International Society of Experimental Hematology, Dusseldorf, Germany, 1995
- ◆ Chairman, 25th Annual Meeting of International Society for Experimental Hematology, Sheraton New York, New York; August 23-27, 1996
- ◆ Chairman of Molecular Diagnosis Session, 10th Symposium on Autologous Peripheral Blood Stem Cell Transplantation, location, September 5, 1999.
- ◆ Chairman of Gene Therapy Session in The International Society for Experimental Hematology, location, August 1999.

SELECTED PUBLICATIONS (Totaling 245 in peer reviewed journals and 22 invited book chapters)

- 1- Chertkov, J.L., Jiang, S., Lutton, J.D., Harrison, J., Preti, R., Levere, R.D. and Abraham, N.G. The hematopoietic stromal microenvironment promotes retrovirus-mediated gene transfer into hematopoietic stem cells. Stem Cells 11:161-175, 1993.
- 2- NG Abraham, Ikehara, S , Stem cells for prevention of endothelial cell detachment and diabetes. American Heart Association, 2004

More papers can be screened on Pub Med.

A Novel strategy for enhanced regeneration of bone marrow **Il-Hoan Oh, MD, Ph.D**

**Director, Catholic High-Performance Cell Therapy Center, The
Catholic University of Korea**

Transplantation of hematopoietic stem cells (HSCs) and subsequent reconstitution of bone marrow is an important treatment modality for many diseases, including leukemia, autoimmune diseases and congenital genetic diseases. The development of strategies to enhance the repopulation of bone marrow has therefore been of major interest in these fields.

Here, we present two strategies for enhanced repopulation of transplanted HSCs, one increasing graft input amount by transplanting multi-donor-derived HSCs, and the other increasing the output of transplanted HSCs.

As a first strategy, the feasibility that two allogeneic umbilical cord bloods (UCB) could be mixed and transplanted into the same recipients was studied by experimentally transplanting two UCBs into NOD/SCID mice and subsequently examining donor origins with donor-specific PCR-SSOP or real time quantitative PCR on short tandem repeats (STR). When two units of UCB were mixed and transplanted as total nucleated cells, cells from one donor predominated over the other regardless of HLA matching status, and no additive engraftment from the two grafts was seen, as compared to single unit transplantation control groups. However, depletion of lineage-positive cells before grafting resulted in alleviation of one-donor predominance, indicating immunological competition between the grafts. Importantly, cotransplantation of culture-expanded mesenchymal stromal cells obtained from a 3rd party could alleviate one-donor predominance without the need for lineage depletion of the grafts. Furthermore, MSC-mediated alleviation of donor predominance was well correlated to a corresponding increase in the overall engraftment from mixed UCB transplantation, suggesting potential benefits in clinical transplantation.

The other strategy to increase repopulation of bone marrow is to enhance the regenerative potential of transplanted HSCs. We previously showed that expression of dominant negative STAT3 (dnSTAT3) in murine fetal liver cells could selectively suppress their repopulating activity, but not with wild-type STAT3 (Oncogene 21; 4778, 2002). However, constitutive activation of STAT3 in murine bone marrow cells could enhance the regenerative capacity up to 1 year after transplantation. Interestingly, STAT3-activated HSCs, while exhibiting higher regenerative potential, did not override normal physiological feedback mechanisms, nor showed any sign of leukemic transformation. Our results suggest that the STAT3 signal may be an important parameter for the extent of in-vivo amplification of HSCs. Further studies on in-vivo self-renewal of HSCs will facilitate development of more efficient cell therapeutic strategies.

Curriculum Vitae

Il-Hoan Oh, MD, Ph.D

1986: M.D, The Catholic University of Korea, School of Medicine

1987: Internship in St. Mary's Hospital

1997: Ph.D, Fels Institute of Molecular Biology and Cancer Research, Temple University, USA

1998-2000: Post-doctoral Fellow & NCIC Fellow, Terry Fox Lab, Canada

2001- present:

Associate Professor, Dept. of Cellular Medicine, The Catholic University of Korea.

Director, Cell & Gene Therapy Institute,

Director, Catholic High Performance Cell Therapy Center

Committee

International Member, American Society of Hematology

Consultant, Cellomics, Korean Research Institute of Biotechnology

Planning member, Korea 21century Frontier Project, Stem Cell Science Area

Consultant, Cell Therapy Evaluation Committee, Korean Food and Drug Agency (KFDA)

Transplantation of circulating stem cells in the heart in severe cardiac failure

Ph. Hénon – Institut de Recherche en Hématologie et Transplantation and Université de Haute-Alsace, Mulhouse, France

There is a growing and tremendous interest for the new concept of regenerative medicine to cure patients with intractable diseases due to particular types of cells not functioning correctly. Using embryonic stem cells in this way is not realistic in the immediate future, mainly because of important technical reasons associated with “hot” ethical problems. On the contrary, new knowledge recently acquired regarding adult stem cells might prompt their use for regeneration of wounded or degenerative tissues. Among them, hematopoietic stem cells (HSCs), easily available, have been studied and used in clinics exclusively for hematopoietic transplantation for a very long time. It now appears that HSCs could also participate in the regeneration of other tissues, particularly cardiac tissue. Several groups, mainly from Germany and Japan, have recently conducted different phase-I clinical studies in which autologous bone marrow (BM) mononuclear cells (MNCs) were reinjected either directly in the ischemic area or intra-coronary in patients with severe post-infarct cardiac failure, resulting in a significant improvement of myocardium viability and/or reperfusion. However, besides “true” HSCs, BM-MNCs represent a mixture of mesenchymal progenitor cells, angioblasts, and maybe other progenitor cells, which makes it impossible to identify the type(s) of cells potentially responsible for improvement. Moreover, the obstructed coronary artery was always re-permeabilized, which biases the evaluation of posttransplant myocardial reperfusion. We have personally chosen another original approach using mobilized and purified circulating CD34⁺ cells. We and others have indeed demonstrated that mobilized CD34⁺ cells can in fact be subdivided into various subsets: of course, the most important ($\approx 75\%$) is the truly hematopoietic subset (CD34⁺/133⁺), of which a CD38⁻ part is probably close to the very primitive HSC. But other smaller subsets are immunophenotypically characterized either as mature (CD34⁺/VEGFR-2⁺) or immature (CD34⁺/133⁺/VEGFR-2⁺) endothelial progenitor cells – thus potentially capable of neoangiogenesis -, or as muscle progenitors (Desmin⁺) and even more as cardiomyocytes (Troponin-T⁺). In a phase-I trial benefiting of the approval of the regional ethical committee, patients suffering post-infarct cardiac failure are selected according to the following criteria: left ventricular ejection-fraction (LVEF) $\leq 35\%$; distinct area of left ventricular-wall akinesis determined by PetScan; candidates for coronary artery by-pass grafting (CABG), **but without any re-permeabilization of the coronary artery involved in the infarction**; age ≤ 70 y. After a 6-day mobilization by G-CSF, circulating CD34⁺ cells are collected, then purified by immunomagnetism and immediately reinjected at d+7 during CABG, all around and within the infarcted area. The first evaluable patients well tolerated cell mobilization – and collection phases, as well as operative and post-operative periods. Three patients have presently a follow-up ≥ 1 year post-transplantation. Two show a striking gain in LVEF (14 and 20% respectively) with an important improvement in myocardium viability, reperfusion and contractility, and finally in exercise capacity (from class IV to class I in New-York Association functional class). Although very encouraging, these results have to be confirmed in further patients.

CURRICULUM VITAE

Philippe Hénon

1. ACADEMIC QUALIFICATIONS

- *Medical studies*

- Higher Medical Doctoral Thesis, School of Medicine of the University of Paris (France) 1968
- Certified in Hematology, Paris (France) 1970

- *Academic positions*

- U.E.R. of Hematology (Director: Pr. Jean Bernard) of the Medical University Saint-Louis Lariboisière, Paris (France) :
 - Senior Lecturer: 1970-1974
 - Assistant Professor: 1974-1978
- Director of the Educational Program on Hematopoiesis and Cellular Therapy at the Faculty of Sciences and Technologies, University of Haute Alsace, Mulhouse, France
- Professor at the University of Haute-Alsace
- Visiting Professor at the Institute of Medical Sciences at University of Lincoln, UK, 2004-2007

2. PROFESSIONAL EXPERIENCE

- Internship, "Hôpitaux de Paris" (France) 1965-1968
- Medical Assistant, Hematology Laboratory, Hôpital Avicenne, Assistance Publique Paris, (France) 1968-1969
- Head of the cytology laboratory in the Blood Transfusion Center of Versailles, France - 1969
- "Assistant des Hôpitaux de Paris", Hôpital Lariboisière, Department of Hematology, (Director : Prof.J.Caen) Paris (France) 1970-1974
- Assistant-Director of the Department of Hematology , Hôpital Lariboisière (Director: Pr.J.Caen) Paris, France - 1974-1978
- Head of the Department of Hematology, Mulhouse Hospitals, Mulhouse, France, since 1979
- Founder and Director of the "Institut de Recherche en Hematologie et Transplantation" Mulhouse, France, since 1987
- Vice-Chairman of the Medical Board of the Mulhouse Hospitals, 1999-2003

3. MEMBERSHIPS IN SCIENTIFIC SOCIETIES

- Membership in 10 National and International Scientific Societies including ASH and ISEH
- Corresponding member of the New York Academy of Science

4. MEMBERSHIPS IN JOURNAL EDITORIAL BOARDS

- **Stem Cells and Development:** (Editor in chief: D. English) European Editor since January 2004
- **Journal of Hematotherapy and Stem Cell Research:** European Editor 1999-2003
- **Journal of Hematotherapy and Graft Engineering** (until December 1998)
- **Bone Marrow Transplantation** (Editor: J. Goldman, London, U.K. (since 1988)
- **Stem Cells** (Editor: M.J. Murphy, Jr. Dayton, USA) (1993-2002)

5. SCIENTIFIC DISTINCTIONS

Member of over 20 scientific committees in International Meetings since 1990 (France, Europe, USA, and Australia), out of which 5 ISEH meetings: Frankfurt 1995, New York 1996, Cannes 1998, Tampa 2000, and Paris 2003

Laureate of the "Excellency Achievements Awards of the Millenium" in the field of Hematopoiesis, and New York, USA, July 2000

6. SCIENTIFIC PUBLICATIONS AND PARTICIPATION IN MEETINGS

- 142 publications in National and International journals
- 3 scientific books (focused on PBSC transplant and stem cells)
- 180 communications in International Meetings, out of which 70 as invited speaker
- 35 chairmanships in International Meetings
- Organizer of 7 International Meetings on Hematopoietic Stem Cells and/or Transplantation

Advances in Stem Cell Transplantation for Lymphoma: Role of Monoclonal Antibodies

High-dose chemotherapy with autologous stem cell transplantation (ASCT) is a potentially curative therapy for younger patients with relapsed aggressive non-Hodgkin's lymphoma. However, between 40% and 70% of all patients relapse after ASCT because of contamination of the stem cell product or persistence of residual tumor cells. Evidence is emerging that the administration of the anti-CD20 monoclonal antibody, rituximab, as an *in vivo* purging agent before ASCT is effective in eliminating lymphoma cell contamination, as measured by the clearance of bcl-2-positive cells from stem cell harvests. Furthermore, *in vivo* purging with rituximab does not adversely affect the stem cell yield or function. Maintenance therapy with rituximab post-transplantation has also been explored as a means of eliminating residual tumor cells. Results suggest that rituximab may eradicate minimal residual disease post-transplant and help prevent relapse. Ongoing trials will confirm the full potential of rituximab in ASCT.

Unlike aggressive lymphomas, there is little evidence that ASCT is curative for patients with indolent lymphoid malignancies or in patients with recurrent mantle cell lymphoma. Instead, a much lower rate of relapse has been observed with allogeneic transplantation. High doses of myelosuppressive chemotherapy or radiation have been used in preparative regimens with the goal of preventing graft rejection and eradicating malignancy. Much of the benefit of transplantation, however, results from graft-versus-malignancy effects, mediated by donor immunocompetent cells. An alternative approach is to utilize less toxic, non-myeloablative preparative regimens to achieve engraftment and allow graft-versus-malignancy effects to develop. This strategy reduces the risk of treatment-related mortality and allows transplantation for elderly or medically infirm patients not eligible for myeloablative therapy. Non-myeloablative preparative regimens appear promising in diagnoses sensitive to graft-versus-malignancy effects and provide a platform for further development of cellular immunotherapy.

Most non-myeloablative preparative regimens have utilized purine analogs, alkylating agents, or low-dose total body radiation. Purine analogs have activity against a wide range of hematologic malignancies and are sufficiently immunosuppressive in standard doses to allow engraftment of HLA-compatible hematopoietic progenitor cells.

More recently, rituximab was studied in this setting. There are several observations that have led to the concomitant use of rituximab with non-myeloablative conditioning regimen and immunomodulation. Several studies conducted both *in vitro* and *in vivo*, have demonstrated an augmented activity of the monoclonal antibody when used concurrently with chemotherapy. This activity may be further enhanced by the infusion of donor stem cell or donor lymphocyte infusions through an increased antibody-dependent cytotoxicity. With this, effector cells bind to the Fc portion of the monoclonal antibody, leading to increased lysis of the tumor cells. Alternatively, it is possible that this increased efficacy is related to a "cross-priming" of cytotoxic T cells promoted by apoptosis-inducing tumor cell-reactive antibody. In a recent report, it has been demonstrated that anti-CD20 antibodies may promote uptake and cross-presentation of cell-derived peptides by antigen-presenting dendritic cells, allowing the generation of specific cytotoxic T cells.

Another reason for the inclusion of rituximab in the treatment plan is related to the potential role of B-cells as antigen-presenting cells, thus having probably an important role in the pathogenesis of graft-versus-host-disease (GVHD). Using a B cell deficient mouse model in which mice received either control rabbit immunoglobulin or rabbit anti-IgM from birth, Schultz et al reported a lower incidence of GVHD in B-cell deficient animals, and the rate of GVHD was even lower if the grafts were depleted of B cells. Inclusion of rituximab as part of the preparative regimen or prior to donor lymphocyte infusion would act to deplete both recipient and donor derived B cells and thus may decrease the severity of GVHD.

Issa Khouri
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Dr. Khouri joined the University of Texas M.D. Anderson Cancer Center, Department of Blood and Marrow Transplantation as Medical Oncology/Hematology Fellow in 1989. He is board certified in internal medicine and oncology. He currently holds the title of Professor of Medicine and Internist and serves as supervisor of the Outpatient Ambulatory Allogeneic Transplant Center. Dr. Khouri is currently a member of the American Society of Clinical Oncology, American Society of Hematology, International Society for Experimental Hematology, and American Society for the Advancement of Science. He also serves on the International Bone Marrow Transplant Registry and Autologous Bone Marrow Transplant Registry Chronic Lymphocytic Leukemia and Lymphoma Joint Working Committee. Dr Khouri is a reviewer for numerous journals, including Blood, Journal of Clinical Oncology, Bone Marrow Transplantation, Annals of Oncology, Clinical Research, and Leukemia. His research interests includes: autologous and allogeneic transplantation for chronic lymphocytic leukemia, indolent and mantle cell lymphomas and mini-allotransplantation. He is a highly sought after speaker and author, and has published over 100 journal articles, abstracts and book chapters.

Allogeneic stem cell transplants for autoimmune disease.

**Richard K Burt, M.D. Northwestern University Medical School,
Chicago, Ill, USA**

Autologous HSCT for autoimmune diseases has been ongoing in patients since 1996. The rationale for an autologous HSCT is to regenerate a new or antigen naïve immune compartment during exposure to self-antigens, similar to normal fetal ontogeny. Recently, we have performed allogeneic HSCT from matched siblings for rheumatoid arthritis as well as scleroderma. The concept of allogeneic HSCT is to change genetic predisposition to disease by changing the hematopoietic stem cells and differentiated immune cells to the disease-resistant phenotype of the donor. The goal of allogeneic HSCT is to use non-myeloablative stem cell transplantation (NST) with donor lymphocyte depletion to achieve stable hematopoietic mixed chimerism without graft versus host disease (GVHD). Early results in rheumatoid arthritis and scleroderma suggest that matched sibling NST achieves mixed chimerism without GVHD and complete remission of autoimmune disease. Data from ongoing human studies will be reviewed. In addition, recent data will be presented on embryonic stem cell transplants (ESCT) in animal models to induce hematopoietic mixed chimerism across MHC barriers without GVHD. Finally, unpublished experiments performed in collaboration with Professor Ikehara (Osaka, Japan) have demonstrated that ESCT prevents diabetes in NOD mice.

Curriculum Vitae

Richard K Burt, M.D. is a Tenured Associate Professor at Northwestern University Feinberg School of Medicine in Chicago, Illinois. Dr Burt performed the first autologous hematopoietic stem cell transplantation (HSCT) in America for multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, relapsing polycondritis, Wegners granulomatosis, Behçets, and the first autologous HSCT in the world for myasthenia gravis, pemphigus, neurovascular Sjögrens and Crohn's disease. Dr Burt has also recently performed the world's first allogeneic HSCT for rheumatoid arthritis. In animal models, Dr Burt has used both adult hematopoietic stem cells and embryonic stem cells to induce tolerance in autoimmune diseases. Dr Burt is PI on a \$10m National Institutes of Health contract to develop use of stem cells for tolerance.

Bone Marrow Transplantation from HLA Mismatched Mother

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Dept. of Pediatrics, Nanfang Hospital, The First Military Medical University,
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[Abstract] Objective: To study the possibility of bone marrow transplantation (BMT) for β -thalassemia major and acute lymphocyte leukemia (ALL) using an HLA-mismatched mother as donor. Methods: From Aug. 1999 to March 2004, eight patients with β -thalassemia major and two patients with high risk ALL received allogeneic BMT. The donors were HLA one-antigen-mismatched mothers except for two patients who received BMT from phenotypically-matched mothers. The median age at transplant was 6.5 years, ranging from 1-13 years. All but one were boys. Three patients with β -thalassemia major were class III in the Pesano grouping and the others were class I or II. Patients with β -thalassemia major received TBI (3Gy), Busulphan (Bu, 10-14mg/kg), Cyclophosphamide (Cy, 120-160mg/kg), and Antithymocyte globulin (ATG-F, 30-90mg/kg) as conditioning, but the youngest was given Fludarabine 150mg/kg instead of TBI. One patient with ALL received TBI (10Gy), VP16 (60mg/kg), Ara-C (4g/m²) and ATG-F (45mg/kg), while the other received Bu (16mg/kg), Ara-C (12g/m²), Cy (120mg/kg) and ATG-F (45mg/kg). Graft-versus-host disease (GVHD) prophylaxis was cyclosporin and Mycophenolate mofetil. Additionally, three patients received low doses of Methylprednisolone, and six patients received Daclizumab. All but two of the donors were given G-CSF for three days before transplant. The mean dose of infused mononuclear cells was 2.6 (2.14 -2.85) 10e8/kg. Results: In eight of the ten patients, engraftment was successful. The youngest rejected the graft. Three class III patients with β -thalassemia major died from treatment-related mortality. One died from severe veno-occlusive disease at day 10 after BMT, which was too early to evaluate the status of the engraftment. The others died from GVHD and severe infection. Four patients with β -thalassemia major were transfusion-independent, who was younger than 7 years. The two patients with ALL were in complete remission. Conclusions: We conclude that BMT from HLA one-antigen mismatched mothers may be used for patients with β -thalassemia major and high risk ALL. Age may be a significant factor associated with survival for patients with β -thalassemia major.

Key words: BMT, beta-thalassemia, ALL, HLA-mismatched mother.

Curriculum vitae

Name: Li, Chun-fu (given name: Chunfu, family name: Li)

Sex: Male.

Date of Birth: May 3, 1955

Family Status: Married, with one son.

Permanent Domicile: Hunan, China

Place of Birth: Hunan, China

Present Address: 501 Block 140/B, Nanfang Hospital, Guangzhou, China

Office Address: Department of Pediatrics, Nanfang Hospital, Guangzhou 510515, China

Qualifications and courses taken:

Graduated from The First Military Medical University in 1977.

Finished two-year basic medical course at The Guangzhou Medical College in 1983.

Finished three-year masters course at The First Military Medical University in 1988.

Attended one-year advanced studies for monoclonal antibodies at The Basic Research Institute of Beijing Military Medical Academy of Science in 1989.

Finished three-month course on the cryogenics and refrigeration of hematopoietic stem cells at The Beijing Military Medical Refresher Course College in 1990.

Visited and studied at Centre of Children's Cancer, Department of Paediatrics, Prince of Wales Hospital, Hong Kong, from January 1999 to March 1999 as visiting doctor.

Visited and studied at St. Jude Research Children's Hospital, USA, from November 1999 to December 1999.

Visited the Children's BMT Centre of MD Anderson, Houston, December 1999

Visited the Bone Marrow Transplantation Centre, Pesano, Italy, April 2001

Visited the Kansai Medical University, Japan, and gave a lecture, April 2002

Positions held:

Sept. 1977-Sept.1981: Pediatrician, Department of Pediatrics, Guangzhou Second Hospital.

Sept. 1983-Sept.1985: Pediatrician, Department of Pediatrics, Guangzhou Second Hospital.

Aug. 1988-Feb. 1989: Pediatrician, Department of Pediatrics, Nanfang Hospital, The First Military Medical University.

Feb. 1989-Dec. 1994: Lecturer, Paediatrician, Department of Paediatrics, Nanfang Hospital, The First Military Medical University.

Jan. 1995-Dec. 1998: Associate professor, vice-director, Department of Pediatrics, Nanfang Hospital, The First Military Medical University.

Jan. 99- now: Professor, Director, Department of Paediatrics, Nanfang Hospital, The First Military Medical University.

Committees

1. Member and Secretary, Paediatrics Subcommittee, Military Medical Committee of China.
2. Standing Member, Committee of Paediatrics, Guangdong-Province Subcommittee, Medical Committee of China
3. Scientific Collaborators of Thalassemia International deration(TIF)
4. Chairman of Thalassemia Federation of Guangdong-Province

Identification of a novel class of CD34-negative hematopoietic stem cells using the
intra-bone marrow injection

Yoshiaki Sonoda

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Abstract

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. Here, we successfully identify a novel class of human cord blood (CB)-derived CD34⁻ SCID-repopulating cells (SRCs) with extensive lymphoid and myeloid repopulating ability using the intra-bone marrow injection (IBMI) technique.

CB-derived lineage-negative (Lin⁻) cells were sorted into CD34^{high}, CD34^{low}, and CD34⁻ cells. We first tested the SRC activity of these three purified fractions of cells by conventional tail vein injection (TVI). However, only Lin-CD34^{high} cells showed distinct SRC activity. Since Lin-CD34⁻ cells expressed the lower levels of homing receptors including CXCR4 compared with CD34⁺ cells, we hypothesized that these Lin-CD34⁻ cells cannot home into the BM niche. To overcome these difficulties, we analyzed the SRC activity of Lin-CD34⁻ cells using the IBMI technique. Surprisingly, all mice that received transplants of Lin-CD34⁻ cells were repopulated. These results clearly indicate that the CB-derived Lin-CD34⁻ cell population contains SRCs detected only by IBMI. In the above-mentioned mice, we separately analyzed the human cell repopulation in the injected left tibiae and the other bones, indicating that the human CD45⁺ cells were clearly detected not only in the injected left tibia but also the other bones. In addition, significant numbers of CD34⁺ progenies were generated at both sites. These results indicate that CD34⁺ SRCs as well as CD34⁻ SRCs could migrate from the injected site to the other bones. Moreover, CD34⁻ SRCs show delayed or slower differentiating and reconstituting kinetics than CD34⁺ SRCs. These results suggest that CD34⁻ SRCs are in a more profoundly dormant state than CD34⁺ SRCs. These *in vivo* generated CD34⁺ cells showed a distinct SRC activity after secondary transplantation, clearly indicating the long-term human cell repopulating capacity of our identified CD34⁻ SRCs.

We further investigated the HSC characteristics of CD34⁻ SRCs. The absolute numbers of CD45⁺ and CD34⁺ cells generated by 1 CD34⁻ SRC are significantly higher than those generated by 1 CD34⁺ SRC. It is interesting that CD34⁻ SRCs have significantly higher migratory and proliferative abilities than CD34⁺ SRCs. Moreover, only 2 CD34⁻ SRCs transplanted to primary recipients consistently showed secondary reconstitution capacity. This finding suggested the more homogeneous nature of CD34⁻ SRCs than that of the population of CD34⁺ SRCs. These results provided further evidence that CD34⁻ SRCs are functionally different from CD34⁺ SRCs and that they are a distinct class of primitive HSCs.

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. We anticipate that the utilization of the IBMI technique will have a great impact on basic science of HSCs as well as clinical transplantation in the near future.

Curriculum Vitae

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A New Strategy for Treatment of Various Intractable Diseases: “Perfusion Method” + “Intra-Bone Marrow – BMT”

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Bone marrow transplantation (BMT) is becoming a powerful strategy for the treatment of hematologic disorders, congenital immunodeficiencies, metabolic disorders, and also autoimmune diseases.

Using various animal models for autoimmune diseases, we have previously shown that allo BMT can be used to treat autoimmune diseases. In addition, we have found that autoimmune diseases are stem cell disorders.

Using chimeric-resistant and radiosensitive autoimmune-prone mice, we have recently discovered a new BMT method (intra-bone marrow [IBM]-BMT), in which whole bone marrow cells (BMCs) are directly injected into the bone marrow cavity.

For the application of this method to humans, using long bones of cynomolgus monkeys, we have recently developed a new “Perfusion Method (PM)” for harvesting bone marrow cells (BMCs) while minimizing the contamination of BMCs with T cells from the peripheral blood. When thus-collected BMCs, which contain not only pluripotent hemopoietic stem cells (P-HSCs) but also mesenchymal stem cells (MSCs), are directly injected into the bone marrow cavity of recipients (IBM-BMT), the donor-derived hemopoietic cells quickly recover even when the radiation doses used as the conditioning regimen are reduced. Recipient mice, rats, and even monkeys show no GvHD.

We show that this new method (“PM” + “IBM-BMT”) will become a valuable strategy for the treatment of various currently intractable diseases, including age-associated diseases such as osteoporosis and emphysema.

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