INTRODUCTION*

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In the last decade, remarkable advances have been made in bone marrow transplantation (BMT), which is now becoming a powerful strategy in the treatment of various intractable diseases, such as leukemia, aplastic anemia, congenital immunodeficiency, and autoimmune diseases.

This is a collection of the abstracts presented at the Symposium. The Symposium focused on the development of new strategies (including gene therapy) for the treatment of intractable diseases, such as autoimmune diseases and cancer, using bone marrow cells and ES cells.

The Organizing Committee believes that this collection of abstracts will provide new insights into hematopoietic stem cells, mesenchymal stem cells, ES cells, virus infection, gene regulation and gene therapy, and BMT.

SOME NEW APPROACHES TO ANTI-HIV THERAPY. Robert C. Gallo, Professor and Director, Institute of Human Virology, University of Maryland Baltimore, 725 W. Lombard Street, Suite S307, Baltimore, MD, USA

HIV pathogenesis involves both direct pathogenic effects on some HIV infected cells and indirect effects on uninfected (bystander) cells. It has been self-evident for more than 20 years that reducing the amount of virus would be beneficial to infected persons, and this was documented by appropriate pharmacological attacks on HIV enzymes since the mid-1990s. Reduction in HIV levels hinders both the direct and indirect pathogenic effects.

Because of HIV high mutation rates and thus HIV drug escape mutants, long term toxicity with some current protocols, and the desirability of more feasible therapies for patients in developing nations, we are in continuous need for new approaches to therapy. One such approach is blockage of HIV entry. This can be achieved by targeting any of the major steps involved in HIV entry. A particularly attractive step to target is CCR5 since: (a) the bulk of HIV variants utilize this receptor, (b) genetic mutations leading to loss of CCR5 are not harmful to modern people, (c) a cellular protein is less variable than a viral protein. This story began with our observations that CCR5-Chemokine ligands (RANTES, MIP-1, and MIP-1) were potent inhibitors of HIV, and the results of others soon after, which provided the mechanism for this effect (by E Berger, P. Murphy, J. Moore, D. Littman and others) when they showed CCR5 to be a second and critical HIV receptor. Targeting CCR5 with small drugs (agonists or antagonists of these ligands) has already been shown to be effective in patients. In this report I will show that induction of elevated - Chemokine ligands for CCR5 is associated with resistance to HIV infection and resistance to progression once infected. Enhancement of chemokines results in diminished CCR5, and studies from our group (Heredia, Redfield, et al.) have shown that agents which increase chemokines and diminish CCR5 synergize with drugs which target CCR5 in reducing HIV. Results with new agents directly targeting CCR5 will also be summarized.

Attempts to diminish the indirect effects of HIV on uninfected cells (other than by HIV reduction) strictly depend upon our knowledge of pathogenesis. Pathogenesis studies with D. Zagruy, A. Burny and others led us to conclude that IFN-α is a major factor in the perturbation and premature killing of bystander cells. A simple and practical approach to diminish this effect is the use of therapeutic vaccines against IFN-α and the HIV Tat protein, which is directly involved in promoting the overproduction of IFN-α.

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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

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.

While lung cancer remains the leading contributor of cancer mortality in the U.S., benefits from first- and second-line treatments for patients with non-small cell lung cancer (NSCLC) are limited. Improved outcomes for NSCLC may lie in the development of agents with novel mechanisms of action that are directed at specific molecular targets. Activation of receptor-tyrosine kinases is a pivotal molecular mechanism for driving tumor growth, invasion, metastasis, and angiogenesis. Gefitinib, a tyrosine kinase inhibitor that targets the activated epidermal growth factor receptor (EGFR)-tyrosine kinase, shows promise in the treatment of NSCLC and other solid tumors. Although phase III trials of gefitinib (*Iressa*®; AstraZeneca, Wilmington, DE) combined with doublet platinum-based chemotherapy showed no clinical benefit, phase II studies with *Iressa* monotherapy ($n = 142$) demonstrated a benefit in objective tumor response rate in advanced NSCLC patients who had failed first- and second-line treatments. At the 250-mg dose, the objective response rate was 13.6% (95% confidence interval: 6.4%–24.3%). *Iressa* appears to be reasonably well tolerated by this patient population; most adverse events were grade 1 or 2. Iressa received approval for treatment of advanced NSCLC following failure of first- and second-line treatments.

Recent research demonstrates that specific activating mutations in the EGFR gene correlate with clinical responsiveness to gefitinib in a subgroup of patients with NSCLC. Screening for these mutations could guide clinical decisions in the future. Further trials may establish additional indications and reveal other benefits of this new chemotherapeutic agent.

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 targeted therapies in the future. The promise for a future of molecular therapies for molecular targets appears, therefore, promising indeed.

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

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 appears to be closer to B lineage than myeloid lineage. PDCs/IPCs are continuously produced from hematopoietic stem cells within the bone marrow, and then released into the peripheral blood stream. Unlike myeloid cells, which enter the secondary lymphoid nodes from afferent lymphatics, PDCs/IPCs enter the lymph nodes through HEV (similar to 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/IPCs 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.

PROGRESS IN DEFINING THE ROLE OF RSV IN ALLERGY AND ASTHMA: FROM CLINICAL OBSERVATIONS TO ANIMAL MODELS. Laurel J. Gershwin, Department of Pathology, Microbiology, & Immunology, School of Veterinary Medicine, University of California, Davis, CA 95616

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; theses 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, TNFα, IL-8, and IL-1β 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.

AAV (ADENO-ASSOCIATED VIRUS) VECTORS AND THEIR APPLICATION TO GENE THERAPY. Keiya Ozawa, 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: 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 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.

THE MOLECULAR BASIS OF PRIMARY BILIARY CIRRHOSIS: FROM INDUCTION THROUGH DESTRUCTION. M. Eric Gershwin[1], Zhe-Xiong Lian[1], and Susumu Ikehara[2], [1]Division of Rheumatology, Allergy and Clinical Immunology, University of California at Davis, Davis, California, 95616, USA; [2]First Department of Pathology, Kansai Medical University, Moriguchi, Osaka, 570-8506, Japan

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.

ADVANCES IN THE USE OF STEM CELLS FOR TYPE I DIABETES: RESTORATION OF PANCREATIC FUNCTION AND ANTIOXIDANT GENES. Nader G. Abraham, Director of Gene Therapy, Professor of Pharmacology and Medicine, New York Medical College, Valhalla, NY 10595

Type I diabetes mellitus is caused by an autoimmune destruction of the insulin-producing beta cells. Type I (insulin-dependent) diabetes affects as many as 1 in 300 persons in the United States. Despite advances in disease management and insulin preparations, lifespan is shortened by one-third in those developing Type I diabetes under the age of 30, and many patients develop significant vascular complications. 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). We used an animal model, the nonobese diabetic (NOD) mouse, in this study of Type I diabetes. NOD mice are characterized by the development of autoimmune disease as a result of a striking infiltration of T-cells into the pancreatic islets and destruction of β-cells. We assessed the effect of stem cell transplant on immune and pancreatic function, and on the expression of the antioxidant gene, HO-1. When NOD mice (2 months old) were irradiated and reconstituted with bone marrow cells from young BALB/c mice (<2 months old), the NOD mice did not exhibit insulitis or 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 aortic 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 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 μmol/mg protein compared to 1.16 ± 0.37 μmol/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 the mechanism by which bone marrow transplant prevents vascular complications in diabetes includes restoration of pancreatic function, prevention of T-cell-mediated destruction of islets, and increasing the levels of the antioxidant gene, HO-1. These results demonstrate that stem cell transplant restores HO-1 and pancreatic function, and provide a novel strategy to support the concept of using stem cells to cure Type I diabetes.

A NOVEL STRATEGY FOR ENHANCED REGENERATION OF BONE MARROW. Il-Hoan Oh, Director, Catholic High-Performance Cell Therapy Center, The Catholic University of Korea, Seoul, Korea

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

Here, we present two strategies for enhanced repopulation of transplanted HSCs, one increasing input amount of graft 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 recipient 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 additional 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, implying immunological competition between the grafts. Importantly, cotransplantation of culture-expanded mesenchymal stromal cells obtained from a 3rd party alleviated one-donor predominance without the need for lineage depletion of the grafts. Furthermore, MSC mediated alleviation of donor predominance was well correlated to 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 with higher level engraftments. Interestingly, STAT3-activated HSCs, while exhibiting higher regenerative potential, did not override the normal physiological feedback mechanism, 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.

TRANSPLANTATION OF CIRCULATING STEM CELLS IN THE HEART IN SEVERE CARDIAC FAILURE. Philippe R.G. Hénon, Institut de Recherche en Hématologie et Transplantation and Université de Haute-Alsace, Mulhouse, France

There is a growing and tremendous interest in 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. However, recently acquired knowledge regarding adult stem cells might prompt their use for regeneration of wounded or degenerative tissues. Among them, hematopoietic stem cells (HSCs), which are 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 repermeabilized, 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 ($CD34^+/VEGFR\text{-}2^+$) or immature ($CD34^+/133^+/VEGFR\text{-}2^+$) endothelial progenitor cells – thus potentially capable of neoangiogenesis –, or as muscle progenitors ($Desmin^+$) and even more as cardiomyocytes ($Troponin\text{-}T^+$). In a phase-I trial benefiting from 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 repermeabilization of the coronary artery involved in the infarction; age $\leq$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 $\geq$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.

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, Houston, Texas

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.

ALLOGENEIC STEM CELL TRANSPLANTS FOR AUTOIMMUNE DISEASE. Richard K Burt, 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.

BONE MARROW TRANSPLANTATION FROM HLA MISMATCHED MOTHER. Li Chunfu, He Yuelin, Wu Xuedong, Fong Xiaoqin, Qian Xinhua, and Zhang Yuming, Dept. of Pediatrics, Nanfang Hospital, The First Military Medical University, Guangzhou China 510515

Objective: To study the possibility of bone marrow transplantation (BMT) for β-thalassemia major and Acute lymphocyte leukemia (ALL) using an alternative donor from HLA mismatched mother.

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 who were phenotypically matched mothers. The median age at transplant was 6.5 years, ranging from 1-13 years. All but one of the recipients were boys. Three patients with β-thalassemia major were class III in the Pesano grouping and others were class I-II. Patients with β-thalassemia major received TBI (3Gy), Busulphan (Bu, 10-14mg/kg), Cyclophosphamide (Cy, 120-160mg/kg), Antithymocyte globulin (ATG-F, 30-90mg/kg) as conditioning, but one who was youngest was given Fludarabine 150mg/m^2 instead of TBI. Of patients with ALL, one received TBI (10Gy), VP16 (60mg/kg), Ara-C (4g/m^2) and ATG-F(45mg/kg), while the other received Bu (16mg/kg), Ara-C(12g/m^2), Cy(120mg/kg) and ATG-F(45mg/kg). Graft-versus-host disease (GVHD) prophylaxis was cyclosporin and Mycophenolate mofetil. Additionally, three patients received low dose 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: Eight of ten patients showed engraftment. 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 engraftment. The others died from GVHD and severe infection. Four patients with β-thalassemia major were transfusion-independent, who was younger than 7 years. 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 the significant factor associated with survival for patients with β-thalassemia major.

IDENTIFICATION OF A NOVEL CLASS OF CD34-NEGATIVE HEMATOPOIETIC STEM CELLS USING THE INTRA-BONE MARROW INJECTION. Yoshiaki Sonoda, Department of Molecular-Targeting Cancer Prevention, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto City, Kyoto 602-8566, 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. 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.

A NEW STRATEGY FOR TREATMENT OF VARIOUS INTRACTABLE DISEASES: "PERFUSION METHOD" + "INTRA-BONE MARROW - BMT". Susumu Ikehara, First Department of Pathology, Transplantation Center, Regeneration research Center for Intractable Diseases, Center for Cancer Therapy, Kansai Medical University, Osaka Japan

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.