Report from the 25th Sysmex Hematology Seminar

The Sysmex Hematology Seminar was held on June 15, 2002.

This year was the memorial seminar because this seminar has continued for 25 years. We invited a speaker from USA in honor of it.

This year the main theme of the seminar was “Stem Cell Biology and Transplantation”, which is one of the latest hot topics. All the speakers gave very impressive lectures with useful and current information obtained not only in Japan but also some of the advanced countries in the world. Participants had many questions during each lectures.

We believe that all participants benefited from the lectures and discussion during this seminar.

The following is an abstract taken from the text book provided in the seminar.

Date : June 15, 2002

Place : **Kobe (Main)**
Tokyo, Sendai, Nagoya, Fukuoka (Satelite)

Lecturers : **Kohichiro TSUJI, M. D.**
Division of Cellular Therapy, The Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
: **Toshio SUDA, M. D.**
Department of Cell Differentiation, Keio University School of Medicine, Tokyo, Japan.
: **Koichi HATTORI, M. D.**
Division of Hematology-Oncology, Weill Medical College of Cornell University, New York, USA.
: **Tsuneo A. TAKAHASHI, D. Sc.**
Division of Cell Processiong, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Chairman : **Yasuo IKEDA, M. D.**
Department of Internal Medicine, Keio University School of Medicine, Tokyo, Japan.
: **Shunichi KATO, M. D.**
Department of Cell Transplantation and Regenerative Medidcine, Tokai University Hospital, Kanagawa, Japan.

In Vitro Expansion of Human Hematopoietic Stem Cells

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Proliferation and differentiation of hematopoietic stem cells (HSC) are regulated at least in part by interactions of various hematopoietic cytokines. Previous studies showed that stem cell factor (SCF) , Flk2/Flt3 ligand (FL) , thrombopoietin (TPO) , and a complex of interleukin (IL) -6 and soluble IL-6 receptor (IL-6/sIL-6R) have important roles in the development of human HSC. Recently, we have established a novel method for *in vitro* expansion for cord blood HSC using these cytokines. When cord blood $CD34^+$ cells were cultured with SCF, FL, TPO and IL-6/sIL-6R for one week, human HSC capable of repopulating in immunodeficient NOD/SCID mice expanded by four-fold. This culture system may pave the way for the *in vitro* expansion of human transplantable HSC suitable for clinical applications.

Linkage between Hematopoiesis and Angiogenesis

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Angiogenesis is an important event for embryonic organogenesis as well as for tissue repair in the adult. Here we show that hematopoietic stem cells (HSCs) are essential for angiogenesis during embryogenesis. To investigate the role of HSCs in endothelial cell (EC) development, we analyzed AML1 deficient embryos, which lack definitive hematopoiesis. These embryos showed defective angiogenesis in the head, pericardium and fetal liver. Para-aortic splanchnopleura (P-Sp) explant cultures on stromal cells (P-Sp culture) did not generate definitive hematopoietic cells and showed defective angiogenesis in the AML1 null embryo. Disrupted angiogenesis in P-Sp cultures from AML1 null embryos was rescued by addition of HSCs. HSCs specifically produce angiopoietin-1 (Ang1) . Thus, HSCs, which express Ang1, directly promoted migration of ECs. These findings suggested that HSCs prepare the hematopoietic microenvironment by themselves.

Recruitment of Hematopoietic Stem Cells from the Bone Marrow Niche

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In adults, hematopoietic stem cells (HSCs) are localized within the bone marrow (BM) microenvironment where they are maintained in an undifferentiated status. Following physiological and chemical stressors, they can be instructed to selfrenew or be recruited to an environment where they can differentiate or get mobilized to the peripheral circulation. Such stressors include the administration of cytotoxic agents or hematopoietic growth factors using in the treatment for solid and liquid tumors. Granulocyte colony stimulating factor (G-CSF) or glanulocyte/macrophage colony stimulating factor (GM-CSF) is a hematopoietic growth factor being widely used to prevent life threatening infections following chemotherapy or total body irradiation. It has been reported that other cytokines/growth factors, such as soluble Kit-ligand (sKitL; stem cell factor) , interleukin-1 (IL-1) , IL-3, IL-11 and Flt-3 ligand, and angiogenic factors just like vascular endothelial growth factor (VEGF) and angiopoietin, might be used as HSC mobilizing agents in the near future. Recently, others and we demonstrated that chemokines such as macrophage inflammatory protein-1α (MIP-1α) , IL-8 or stromal cell-derived factor-1 (SDF-1) , mobilize HSCs into circulation *in vivo*. However, the mechanism by which HSCs get recruited from their BM niches (microenvironment) and get launched into circulation is still unknown. Cytokines/chemokines and adhesion molecules must be key players in processes of HSC recruitment from their BM niche and in the HSC mobilization into circulation. It has been reported that G-CSF treatment results in downregulation of the adhesion molecule very late antigen-4 (VLA-4) and vascular cell adhesion molecule-1 (VCAM-1) on human CD34 positive BM cells. Additionally, anti VLA-4 or anti VCAM-1 monoclonal antibody treatment significantly increased the number of HSCs and progenitor cells in circulation. The secretion of enzymes including neutrophil elastase and matrix metalloproteinases (MMPs) following cytokine/chemokine stimulation are another important mediator during HSCs mobilization. MMPs degrade connective tissue and basement membrane, thereby facilitating HSCs to cross the extracellular matrix. MMPs are also known to cleave cytokines and/or receptors, such as tumor necrosis factor-α (TNF-α) or Fas ligand, in processes that can either activate or inactivate cytokines. Others and we also showed that MMP-9 was upregulated in BM cells after exposure to hematopoietic stressors like chemokines/cytokines (G-CSF, VEGF, SDF-1) and myeloablation. Our study demonstrated that hematopoietic stressors (total body irradiaiton, chemotherapy) increased local production of SDF-1 in the BM, thereby facilitating homing of HSCs to the BM. We found increased plasma levels of SDF-1 after myeloablation, concomitant with local upregulation of MMP-9 in the BM which resulted in the shedding of sKitL. In MMP-9 – / – mice, recruitment of HSCs from their quiescent into a permissive environment was profoundly impaired resulting in hematopoietic recovery failure and in increased mortality after BM suppression. Exogenous sKitL fully restored hematopoiesis and survival of BM ablated MMP-9 – / – mice. Thus, release of sKitL by MMP-9 in response to stressors enables HSCs to reconstitute the stem cell pool and to recruite HSCs to a permissive niche favoring differentiation and mobilization. Because modulating the bioavailability of local cytokines changes the HSC fated, regulators of enzyme activity leading to proteolytic cleavage are critical elements in the determination of the HSC fate and therefore a critical step during the HSC recruitment and mobilization.

Cord Blood Transplantation and Banking

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Unrelated allogeneic bone marrow transplantation can cure hematopoietic disorders including leukemia, lymphoma, immunodeficiency syndrome and some metabolic disorders. But its success depends on how rapidly suitable donors who have full matched HLA can be identified, how fast the transplant can be accomplished, and how well acute GVHD can be controlled.

Unrelated umbilical cord blood transplantation has been overcoming these problems, although other problems remain to be solved. Since Tokyo Cord Blood Bank started cryopreserving cord blood, more than 4,000 units have been collected and 1,800 units have been registered with the Japan Cord Blood Network.

In the world, 9,000 units are now registered in this Network and more than 600 cord blood transplants have been performed since 1997 to April 2002.

Tokyo Cord Blood Bank has provided 118 units for patients in Japan, United States and New Zealand and Vietnam. In the beginning of cord blood transplantation, the patients were limited to children, but now adult patients are successfully transplanted, i.e., overall survival of adult patients demonstrated no significant difference from child patients. For successful cord blood transplantation, supply of quality controlled cord blood units is necessary. We process cord blood by the HES method, reducing the volume to 25 mL and cryopreserve them at -196ºC using BioArchive system.

We obtained ISO 9002 for cord blood processing and that helps to insure the safety of the units internationally. Tokyo Cord Blood Bank is one of the organising members of the International Cord Blood Bank Network, NETCORD, with other major banks in the world, and also serves as a virtual office of the organisation of cord blood banks in Asia called AsiaCord.