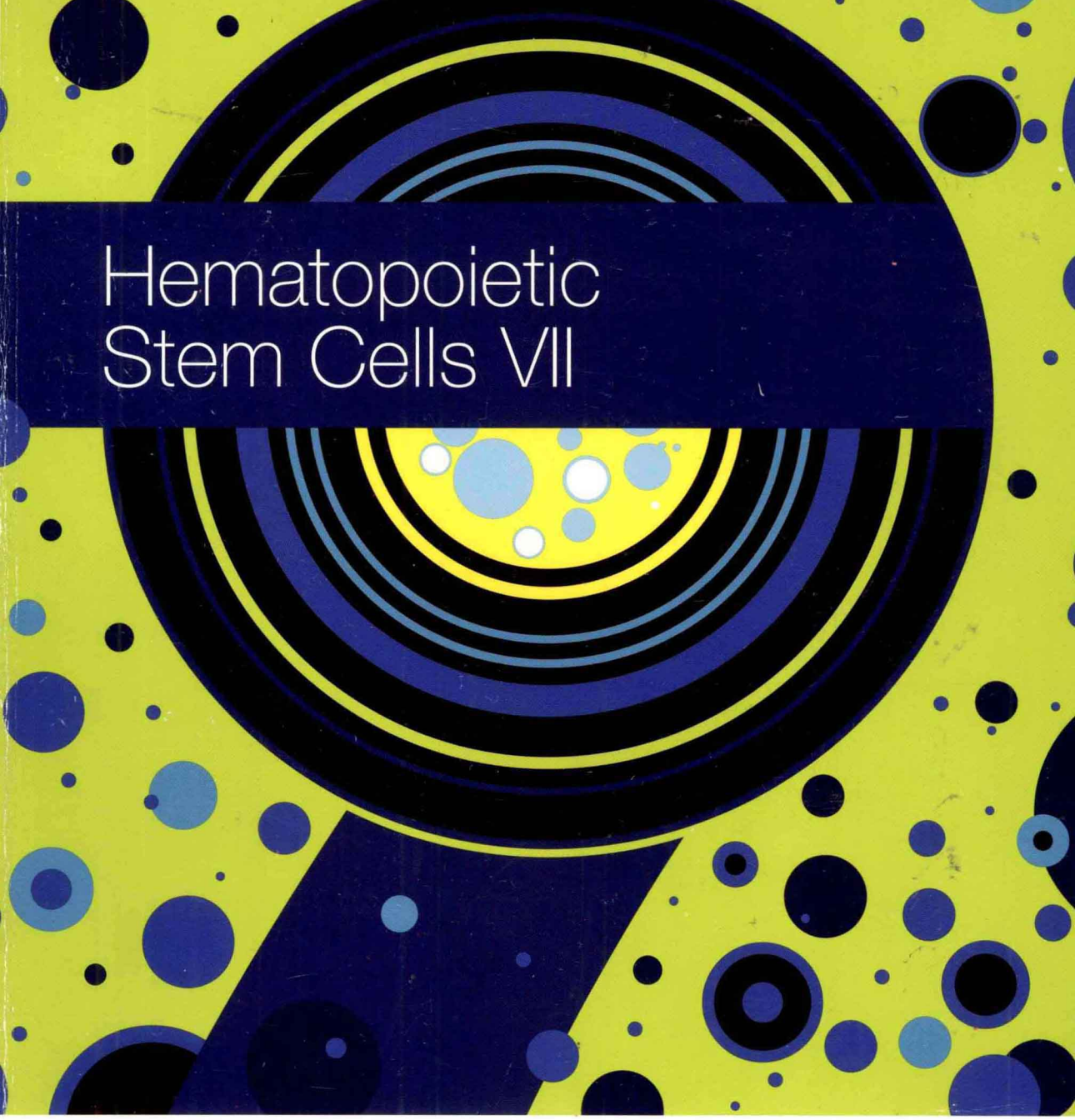

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Hematopoietic Stem Cells VII

Edited by
Lothar Kanz, Katja C. Weisel, John E. Dick, and Willem E. Fibbe

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Hematopoietic Stem Cells VII

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Hematopoietic Stem Cells VII

Editors

LOTHAR KANZ, KATJA C. WEISEL, JOHN E. DICK, AND WILLEM E. FIBBE

This volume presents manuscripts stemming from the conference entitled "Hematopoietic Stem Cells VII," held in Meersburg, Lake Constance, Germany on September 18–20, 2008.

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International Symposium and Workshop on Hematopoietic Stem Cells VII, University of Tübingen, Germany, September 18–20, 2008

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Introduction

The seventh biennial meeting on hematopoietic stem cells (HSCs), hosted by the University of Tübingen in cooperation with Amgen, was held in Meersburg, Germany, from September 18 to 20, 2008. Thirty leading scientists in the field of hematopoiesis discussed the most recent developments in HSC science and their clinical implications. This year's major topics covered various aspects of HSCs, including stem cell hierarchy, niche, and regulation. Other topics focused on mesenchymal stem cells, malignant hematopoiesis, aging, regulators, pluripotency, and both embryonic and translational research (meeting program: www.onkologie-tuebingen.de). In this meeting summary the authors outline what was considered to be of particular interest and importance to both basic biologists as well as clinically oriented scientists working in the field of experimental hematology and stem cell biology.

Stem Cell Hierachy

Hideo Ema (University of Tokyo, Tokyo, Japan) reported that the hematopoietic system is a hierarchically organized series of cell populations that range from HSCs to a variety of mature blood cells. The entire system is clonally established and maintained by HSCs. CD34-negative or low, c-Kit-positive, Sca-1-positive, and lineage marker-negative (CD34 KSL) cells are highly enriched in adult mouse bone marrow HSCs. Single-cell assays have shown that individual $CD34^-$ KSL cells have a variety of repopulating and self-renewal potentials of *in vitro* colony-forming activities and of *in vivo* repopulation kinetics following their transplantation into lethally irradiated mice,[1] suggesting that HSCs are heterogeneous in terms of their potential. About 25% of $CD34^-$ KSL cells, on average, are considered to be HSCs by competitive repopulation assays. In order to study the potential hierarchical organization of heterogeneous HSCs and to achieve greater purification of HSCs, $CD34^-$ KSL cells were subdivided and their functions were characterized. $CD34^-$ KSL cells were stained with other antibodies and were analyzed by flow cytometry. $CD34^-$ KSL cells did not stain with most of >100 antibodies examined. However, 20 or more antibodies stained all these cells stained, and 10 or more antibodies stained some of them. $CD34^-$ KSL cells stained with the last group of antibodies were separated into positive and negative fractions, and 10 cells from each fraction were transplanted into each of a group of lethally

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irradiated mice along with competitor cells. Only four antibodies proved useful for further enrichment in HSC activity. Among them, attention was focused on anti-CD150 antibody. When $CD150^{high}$, $CD150^{med}$, or $CD150^{neg}$ $CD34^{-}$ KSL cells were individually transplanted, the frequencies of repopulating cells appeared similar among these three types of cells, but the mean percent chimerism achieved using $CD150^{high}$ or $CD150^{med}$ $CD34^{-}$ KSL cells was significantly higher than that achieved using $CD150^{neg}$ $CD34^{-}$ KSL cells. Interestingly, the myeloid lineage was predominantly reconstituted by $CD150^{high}$ cells, whereas the lymphoid lineage was predominantly reconstituted by $CD150^{neg}$ cells. Secondary transplantation showed marked self-renewal potential in $CD150^{high}$ cells, but little such potential in $CD150^{neg}$ cells. Moreover, single-cell colony assays showed that $CD150^{high}$ cells were enriched in neutrophil/macrophage/erythroblast/megakaryocyte colony–forming activity, whereas $CD150^{neg}$ $CD34^{-}$ KSLcells were enriched in neutrophil/macrophage colony–forming activity. These data demonstrate that $CD150^{high}$ cells are more immature than $CD150^{neg}$ cells and that a higher degree of HSC purification can be obtained by excluding $CD150^{neg}$ cells from $CD34^{-}$ KSL cells. The data also suggest that high self-renewal potential, predominant myeloid lineage reconstitution, and full myeloid differentiation activity are tightly associated events among HSCs.

Hierarchical structures are not only restricted to hematopoietic self-renewal and differentiation but also control insertional transformation of hematopoietic cells as shown by Christopher Baum (Medizinische Hochschule Hannover, Hannover, Germany, and Cincinnati Children's Hospital, Cincinnati, Ohio, USA). Gene transfer into HSCs may be used to treat a great variety of genetic and acquired disorders; however, low efficiency and dose-limiting side effects related to insertional mutagenesis have hampered progress in this area. Christopher Baum and his group have developed murine models to address the impact of target cell type, cell culture conditions, and vector technology on the induction of insertional mutants.[2] To this end, results obtained using a cell culture–based assay with insights from studies of vector-modified HSC performed in serial transplantation with long-term follow-up (up to 20 months) were compared. In the worst case, a single insertion of a vector with a strong viral enhancer–promoter next to a proto-oncogene such as *Evi1* or *Prdm16* was found to be sufficient to induce leukemia.[3] Further experiments identified a hierarchy of factors governing the emergence of insertional mutants. By transducing sorted populations of hematopoietic cells, it was shown that an intrinsic repopulation potential is dominant over insertional mutations. Thus, insertional mutants with potential for uncontrolled clonal dominance were observed only in the progeny of multipotent cells with intrinsic HSC potential, whereas multipotent progenitor cells, more mature myeloid cells, and T cells were largely refractory to the induction of a transformation process by insertional mutagenesis. Insertional upregulation of *Evi1* in the HSC progeny restricted T lymphoid differentiation, suggesting that a myeloid differentiation bias reported for "normal" HSC may reflect epigenetic dysregulation of *Evi1* or genes with a related function. As the second most important factor in the induction of insertional mutants, the presence of a strong enhancer–promoter in the vector backbone was identified.

The third factor in this hierarchy is the insertion pattern of retroviral vectors, with the gammaretroviral insertion pattern showing a greater risk to induce insertional mutants than the lentiviral pattern. However, lentiviral vectors harboring strong internal enhancer–promoter sequences with high activity in HSC were still able to trigger insertional transformation. These studies provide the basis for rational approaches to improve the therapeutic index of integrating vectors. At the same time, insertional mutagenesis by replication-deficient vectors emerges as a tool to identify genes that regulate clonal homeostasis, which is of major

interest for hematology, oncology, and regenerative medicine.

Koichi Akashi (Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts, USA) has been working for years on the hierachical aspects in the hematopoietic developmental tree[4] and now discusses one of the major questions in hematopoiesis: Where and when HSCs begin to activate the lymphoid developmental program. Common lymphoid progenitors (CLPs) are the earliest precursors and express IL-7 receptors (IL-7R), whereas the earliest lymphoid progenitors (ELPs) are the earliest populations that express recombination activating gene (RAG) but not IL-7R. Several groups reported that the ELP is, therefore, upstream of CLP, and the major source of T cells. By utilizing "RAG history" mice, which harbor $RAG^{Cre/+}$ and the enhanced yellow fluorescent protein (EYFP)-ROSA reporter, the group has shown that the CLP is divided into YFP^+ and YFP^- populations, and only YFP^- CLP that has not activated the RAG gene has CLP activity that includes T cell potential. Furthermore, the competitive reconstitution of YFP^- CLP and ELP showed that the CLP is more potent for T cell production. The YFP^- CLP changes its phenotype into that of the earliest thymic precusors in the thymus. These data clearly show that the CLP and the ELP are independent populations, and the former is the main source of thymic T cells.

Stem Cell Niche

A hallmark of HSCs is their relative quiescence, although functional activity related to divisional history has not been demonstrated. Kateri Moore (Mount Sinai Medical School, New York, New York, USA) and her group addressed this and other questions related to the kinetics of stem/progenitor population usage during normal homeostasis.[5,6] They developed a mouse model that allows viable isolation of quiescent label-retaining cells. This is a double transgenic model in which the tetracycline (Tet)-controllable transactivator (tTA) expressed from human CD34 regulatory elements (hCD34tTA) activates a histone H2B-GFP fusion protein controlled by a Tet-responsive promoter (TetO-H2B-green fluorescent protein [GFP]). In the absence of the Tet-derivative doxycycline (Dox) these mice continually incorporate GFP into nucleosomes; the hCD34 regulatory element is active in $mCD34^{-/lo}$ HSCs, mirroring the functional activity of HSC in both species, and when mice are given Dox, the H2B transgene is turned off, allowing progressive dilution of GFP label in dividing cells. After Dox treatment, flow cytometric analyses of bone marrow revealed that GFP label was retained in the most primitive HSC populations and cell cycle analysis revealed they were in G_0. Functional analyses of Dox-treated LSK^-GFP^+ and LSK^-GFP^- cells demonstrated that virtually all of the primitive *in vitro* progenitors segregated to the LSK^-GFP^+ fraction, and 80% of mice were repopulated with as few as five LSK^-GFP^+ cells. Secondary repopulation was only achieved with bone marrow (BM) from primary LSK^-GFP^+ mice. Data from these studies indicate that divisional history can be used as an indicator of stem cell potency and allows for an assessment of the kinetic utilization of different stem and progenitor compartments during normal homeostasis. In addition, the model provides a tool for *in situ* visualization of HSCs in their niches both during normal homeostasis and after perturbation. During postnatal life, the BM supports both self-renewal and differentiation of HSCs in specialized niches.

Currently, the niche for long-term (LT)-HSCs is thought to consist conceptually of two parts: the endosteal surface (the osteoblastic niche) and a sinusoidal endothelium (the vascular niche). Fumio Arai (Keio University, Tokyo, Japan) focused his work on the characterization of the cell fraction in the osteoblastic niche cells.[7] It was found that cells isolated from endosteum were subdivided into the three populations: mesodermal progenitor cells (MPCs), immature, and mature osteoblasts. Gene

expression assays revealed that MPCs tend to highly express cytokines that affect both HSC proliferation and quiescence. In contrast, the mature osteoblastic cells express multiple cell adhesion molecules, including N-cadhein, OB-cadherin, and ALCAM. From these data, it was hypothesized that multiple mesenchymal, osteoblastic populations form a "niche complex" to support HSCs in the osteoblastic niche. Furthermore, the function of cytokines produced by the osteoblastic niche was also investigated in the regulation of HSC quiescence. The role of thrombopoietin (TPO) in the regulation of quiescent HSCs was examined. Results demonstrate that LT-HSCs expressing TPO receptor, Mpl, are quiescent populations in adult BM that interact with the TPO-producing bone lining cells. Inhibition and stimulation of TPO/Mpl pathway resulted in reciprocal regulation in the quiescence of LT-HSC: the anti-Mpl neutralizing antibody, AMM2, suppressed the quiescence of LT-HSCs and enabled HSC engraftment without irradiation, whereas exogenous TPO transiently increased the quiescent HSC population. These observations indicate that TPO/Mpl signaling is a novel niche component that plays a critical role in the regulation of LT-HSCs in the osteoblastic niche.

Stem Cell Regulation

The group of Stefan Karlsson (Lund University Hospital, Lund, Sweden) works with members of the transforming growth factor-β (TGF- β) superfamily of growth factors, which have been shown to regulate the *in vitro* proliferation and maintenance of hematopoietic stem cells. Working at a common level of convergence for all TGF-β superfamily signals, Smad4 is key in orchestrating these effects.[8] The group has clarified the role of Smad4 in hematopoiesis by utilizing an inducible model (Mx-Cre) of Smad4 deletion. Surprisingly, several transplantation approaches demonstrated a critical role for Smad4 in the maintenance of HSC self-renewal and reconstituting capacity. This was in direct contrast to earlier studies disrupting TGF-β signaling and suggests noncanonical functions for Smad4 as a positive regulator of HSC self-renewal. Furthermore, the observed downregulation of *notch1* and *c-myc* in $Smad4^{-/-}$ primitive cells places Smad4 within a network of genes involved in the regulation HSC fate options (Karlsson et al, J Exp Med, 2007). Recently, it was demonstrated that TGF-β inhibits Nup98-HoxA9 transformation of bone marrow cells, through direct interaction between Smad4 and the fusion protein, in a fashion which is surprisingly independent of active Smad signaling (EMBO J, 2006). Therefore, Karlsson and his coworkers decided to investigate further the interaction between Nup98-HoxA9 and Smad4. Using retroviral vectors to generate enforced expression of Nup98-HoxA9 in Smad4-null HSC, the transforming function was increased approximately four fold. Transplantation of these transduced cells into recipient mice confirms that a block of the TGF-β pathway leads to faster development of myeloproliferation *in vivo*. At the molecular level, a five fold accumulation of Smad4 protein in wild-type HSCs transduced by the Nup98-HoxA9 was found in comparison to untransduced cells. Therefore, the hypothesis was proposed that the oncogenic potential of Nup98-HoxA9 involves a sequestration and stabilization of Smad4 in the cytoplasm. Secondly, an approach to reactivate the TGF-β pathway was developed. The cotransduction of a small portion of Smad4, encoding a 20–amino acid peptide (competing for HoxA9 binding), reduces the expansion of Nup98-HoxA9–transduced cells more than 10 fold, as measured by colony replating. Moreover it was confirmed that administration of this "competitor molecule" allows endogenous sequestrated-Smad4 release, reactivating the TGF-β pathway. While administration of TGF-β as a therapeutic in humans remains inconceivable, due to secondary effects *in vivo*, the findings from these studies might be used to design novel therapies for leukemia that is induced by abnormal Hox protein expression.

Canonical Wnt signaling has been implicated in various aspects of hematopoiesis. During T cell development Wnt signals provide crucial proliferative signals to the most immature thymocytes. Its role in HSC is controversial due to different outcomes between various inducible Wnt signaling loss-of-function models and also compared to gain-of-function systems. Frank Staal (University of Rotterdam, Rotterdam, The Netherlands) and his group studied a mouse deficient for a Wnt gene that seemed to play a nonredundant role in hematopoiesis.[9,10] Mice lacking Wnt3a die prenatally around E12.5, allowing fetal hematopoiesis to be studied using *in vitro* assays and transplantation into irradiated recipient mice. It was demonstrated that Wnt3a deficiency leads to a reduction in the numbers of HSCs and progenitor cells in the fetal liver and to severely reduced reconstitution capacity, as measured in secondary transplantation assays. This deficiency is irreversible and cannot be restored by transplantation into Wnt3a-competent mice. The impaired long-term repopulation capacity of Wnt3a $^{-/-}$ HSCs could not be explained by altered cell cycle or survival of primitive progenitors. Moreover, Wnt3a deficiency affected myeloid but not B lymphoid development at the progenitor level, and affected immature thymocyte differentiation. These results show that Wnt3a signaling not only provides proliferative stimuli, such as for immature thymocytes, but also regulates cell fate decisions of HSCs during hematopoiesis. Overall, this mouse model provides straightforward genetic evidence that Wnt signaling regulates self-renewal of HSCs.

Anna-Rita Migliaccio (Mount Sianai School of Medicine, New York, New York, USA) reported on the role of Gata1 in long-term pathological processes revealed by knockdown mutations.[11,12] *Gata1* is a gene essential for appropriate maturation of erythroid cells and megakaryocytes. Mice carrying the X-linked hypomorphic Gata1^{low} mutation are thrombocytopenic and die of anemia at birth. However, mice with the CD1 background recover from anemia after one month and develop hematopoiesis. To clarify whether hematopoiesis in Gata1^{low} mice is stem cell autonomous, Migliaccio and colleagues analyzed the phenotypes of hemizygous Gata1$^{low/0}$ males and heterozygous Gata1$^{low/+}$ females after splenectomy. Hemizygous males died of anemia within 2 weeks of splenectomy. By contrast, heterozygous females survived for 9 months postsplenectomy with normal hematocrit and higher numbers of platelets in the blood. Their marrow stem/progenitor cells and megakaryocytes were predominantly wild type. Although the frequencies of c-Kit^{pos} cells in the marrow of Gata1$^{+/0}$ and Gata1$^{low/0}$ mice were the same, the distribution of these cells within the architecture of the bone and of the spleen differed in the two animals. In Gata1$^{+/0}$ mice, cKit^{pos} cells were rarely associated with the endosteum, while most were located in the medulla and few c-Kit^{pos} cells were located in the spleen. By contrast, most of the c-Kit^{pos} cells in the marrow of Gata1$^{low/0}$ mice were proximal to the endosteum and few were found within the medulla and numerous c-Kit^{pos} cells were detected in the spleen.

To determine how the Gata1$^{low/0}$ stem/progenitor cells overcome their genetic defect and differentiate in the spleen, tracking experiments using the expression of a reporter gene under the control of HS2, a Gata1 enhancer spared by the Gata1^{low} mutation, were performed. As expected, this reporter was expressed at low levels by stem/progenitor cells purified from the marrow and the spleen of Gata1$^{+/0}$ mice and was also poorly expressed in the stem/progenitor cells in the marrow of Gata1$^{low/0}$ mice. By contrast, the stem/progenitor cells within the spleen of the Gata1$^{low/0}$ mice expressed higher levels of the HS2-driven reporter. Progenitor cells within the spleen, but not those from the marrow of Gata1$^{low/0}$ mice, were capable of forming hematopoietic colonies *in vitro*. These results indicate that hematopoiesis in Gata1^{low} mice is preferentially promoted by the permissive microenvironment of the spleen that permits differentiation of those

stem/progenitor cells capable of an alternative organization of the *Gata1* locus, which involves activation of the HS2 enhancer. Therefore, the hematopoietic defect of the Gata1^{low} mutation is rescued by a permissive splenic microenvironment that supports maturation of stem/progenitor cells with the distinct ability to activate the alternative HS2 enhancer of the *Gata1* gene.

Focusing on circulating hematopoietic stem cells, Steffen Massberg (Harvard Medical School, Boston, Massachusetts, USA) reported on the considerable number of HSPCs that constitutively migrate out of the BM and enter the blood.[13] The residence time of individual HSPCs within the blood is on the order of minutes, implying that the turnover of HSPCs that enter and leave the bloodstream must be high. However, the ultimate fate and functional relevance of these circulating HSPCs is largely unknown. He showed data that circulating BM-derived HSPCs with short- and long-term multilineage reconstitution capacity leave the blood and traffic constitutively to multiple nonlymphoid extramedullary tissues. Here, they reside for about 36 h before entering draining lymphatics to return to the blood and eventually the BM. The egress of HSPCs from extramedullary tissues into lymph depends on sphingosine-1-phosphate (S1P) receptors, particularly $S1P_1$. Under physiological conditions migratory HSPCs contribute to the continuous restoration of specialized hematopoietic cells that reside in peripheral tissues. Upon exposure to Toll-like receptor (TLR) agonists, migratory HSPCs proliferate locally within extramedullary tissues and generate innate immune effector cells. Thus, HSPCs can survey peripheral organs to replenish tissue-resident hematopoietic cells and act as a source of mature leukocytes during host defense against pathogens.

Mesenchymal Stem Cells

The biology of mesenchymal stem cells (MSCs) is one of the main interests of Willem E. Fibbe (Leiden University, Leiden, The Netherlands). MSCs are multipotent progenitor cells that have emerged as a promising tool for clinical application. The interest in MSC therapy has been raised by observations that MSCs are able to modulate inflammatory responses *in vitro* and *in vivo*.[14] The *in vitro* properties of MSCs include inhibition of proliferation of T cells, B cells, and natural killer (NK) cells. In addition, MSCs are able to induce regulatory T cells and promote the differentiation of monocytes and $CD34^+$ cells into immature dendritic cells that might mediate tolerogenic responses *in vivo*. Although it is still unclear to what extent the *in vitro* properties of MSCs are relevant for their *in vivo* biology, MSC treatment has begun to be clinically applied. Such studies include modulation of graft-versus-host disease, promotion of hematopoietic engraftment in the setting of cord blood transplantation, and transplantation of haplo-identical stem cells.[15] New indications of MSC therapy include promotion of tissue repair in patients with inflammatory disorders, including Crohn's disease and other autoimmune disorders. The mechanisms underlying these effects of MSCs have not been clearly identified yet. Future studies will address the *in vivo* tracking of MSCs as a first step to dissect the mechanisms underlying their *in vivo* function. It will also be important to examine the hierarchy of MSC development and to identify subsets of MSCs that mediate a particular function.

Bruno Peault (Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA) and his group have documented anatomic, molecular, and developmental relationships between vascular cells and multiple mesodermal cell lineages in human fetal and adult tissues.[15,16] First, cells co-expressing myogenic and endothelial cell markers (CD56, CD34, CD144) have been identified by immunohistochemistry and flow cytometry within human skeletal muscle. These myoendothelial cells regenerate myofibers in the injured skeletal muscle of severe combined immunodeficient (SCID) mice in a much more effective manner than $CD56^+$ myogenic progenitors.

Cultured $CD56^+CD34^+CD144^+$ cells proliferate long term, retain a normal karyotype, are not tumorigenic, and survive better under oxidative stress than $CD56^+$ myogenic cells. Clonally derived $CD56^+CD34^+CD144^+$ cells were also found to be able to differentiate into myogenic, osteogenic, and chondrogenic cells in culture, and to regenerate skeletal muscle *in vivo*. These novel human myoendothelial progenitor cells display similar characteristics to the murine muscle-derived stem cells (MDSCs) and are amenable to biotechnological handling, including purification by flow cytometry and long-term expansion *in vitro*, and therefore represent a potential therapeutic cell for skeletal muscle repair.

Pericytes are perivascular cells that encircle smaller blood vessels in multiple human organs including skeletal muscle, heart, pancreas, adipose tissue, and placenta. These cells are prospectively identified through CD146, NG2, and PDGF-Rβ expression and the absence of hematopoietic, endothelial, and myogenic cell markers. Cumulative evidence demonstrates that perivascular cells purified from either skeletal muscle or nonmuscle tissues were myogenic in culture and *in vivo*. Irrespective of their tissue origin, long-term cultured perivascular cells retained myogenic ability. They also exhibited various differentiation potentials (osteogenic, chondrogenic, and adipogenic), expressed known MSC markers, and migrated in a culture model for chemotaxis. Strikingly, expression of MSC markers was also detected at the surface of native, noncultured perivascular cells. These results suggest that human capillary and microvessel walls from all studied tissues harbor a reserve of progenitor cells integral to the origin of the elusive MSCs, which have only been identified retrospectively in primary cultures of total tissues.

Hans-Jörg Bühring (University Hospital of Tübingen, Tübingen, Germany) presented data regarding immunophenotypic characterization of MSCs.[18] The "isolation" of bone marrow–derived MSCs (BM-MSCs) by plastic adherence is poorly standardized and provides little information about the starting populations. Conventionally, unfractionated BM-MSCs are cocultured not only with hematopoietic cells but also with other nonrelated cells. As a result, the growth and differentiation of MSCs are influenced by the effect of secreted soluble factors as well as by cell–cell contact with these cells. Because the frequency of MSCs in BM is very low, the influence of these unrelated cells is considerable. Another problem arises when nonadherent cells are removed from the culture, which occurs a few days after coculture. This selection procedure may also lead to the undesired removal of MSC subsets that adhere at later time points. In an attempt to define the starting population more precisely and to exclude the influence of unrelated cells on MSC expansion, a number of markers, including CD105, CD73, CD271, STRO-1, GD2, and SSEA-4, have been identified, that are suitable for primary MSC enrichment. However, most of these markers are not selective for MSCs and are additionally expressed on hematopoietic cell subsets.

In order to identify more selective markers, Bühring and collegues found two MSC-specific epitopes, W8B2 and 39D5.[19–21] These antibodies exclusively react with $CD271^{bright}CD45^-$ cells, which are known to contain the entire clonogenic (CFU-F) potential. Multicolor cell sorting and colony assays revealed a 90-fold CFU-F enrichment in the $CD271^{bright}W8B2^+39D5^-$ and a 180-fold enrichment in the $CD271^{bright}W8B2^+39D5^+$ fraction. Cells from both fractions gave rise to adherent cells with typical fibroblastoid morphology. However, $CD271^{bright}W8B2^+39D5^+$ cells proliferated faster and gave rise to larger colonies on average than $CD271^{bright}W8B2^+39D5^-$ cells. Detailed phenotypic analysis showed that cells from both fractions were positive for the established MSC markers CD13 (e.g., CD73, CD90, CD105, and CD140b). In contrast, expression of other MSC markers (e.g., CD10, CD26, CD106, and CD146) was restricted to the $39D5^-$ subset and CD166

expression was confined to $39D5^+$ cells. Also, giemsa staining showed that $39D5^-$ cells contain large and bright cytoplasmic regions with prominent vacuoles—reminiscent to the published MSC morphology—whereas $39D5^+$ cells are characterized by a smaller cytoplasm with basophilic-like granules. Expanded MSC from both subsets could be induced to the osteogenic, myogenic, and neuronal differentiation lineages. In contrast, only $39D5^+$ cells gave rise to chondrocytes and pancreatic-like islets, and likewise, only $39D5^-$ but not $39D5^+$ cells gave rise to adipocytes.

The proliferation and differentiation potential of MSC subsets was also analyzed on the single-cell level. Sorting of single cells into 96-well plates revealed that the cloning efficiency of $W8B2^+39D5^+$ cells (11/96) was approximately two fold higher compared with $W8B2^+39D5^-$ cells (5/96). The culture of single cells resulted in the appearance of colonies with variable size, distinct phenotypes, and differing proliferation and differentiation potentials, suggesting an unexpected degree of heterogeneity. Thus, one $39D5^-$ clone gave rise to adipocytes but not osteoblasts and neuronal cells, whereas the cells from a $39D5^+$ clone could be differentiated into osteoblasts and neuronal cells but not into adipocytes. In summary, the scientists identified two novel MSC subsets with distinct phenotypic and functional properties. The unique $39D5^+$ subset may represent an attractive starting population to generate chondrocytes for replacement therapies in spinal disk injuries.

Malignant Hematopoiesis

Leukemias are composed of a hierarchy of cells, a fraction of which have stem cell–like properties capable of self-renewal. Scott Armstrong (Harvard Medical School, Boston, Massachusetts, USA) reported on mixed lineage leukemia (MLL) fusion proteins, which are produced by translocations involving the MLL gene on chromosome 11q23 and confer stem cell–like properties on committed hematopoietic progenitors.[22,23] This provides an opportunity to assess changes in gene expression and epigenetic programs during the transition from a cell with minimal inherent self-renewal capability to cells capable of leukemic self-renewal. His group transduced murine $IL\text{-}7R^-Lin^-Sca\text{-}1^-c\text{-}Kit^+CD34^+$ $Fc\gamma RII/III^{hi}$ granulocyte macrophage progenitors (GMPs) or the $Lin^-Sca\text{-}1^+c\text{-}kit^+$ HSC-enriched population of cells (LSK) with retroviruses encoding the MLL-AF9 fusion protein, which led to the development of acute myelogenous leukemia (AML). From the leukemias, a population of $IL\text{-}7R^-Lin^-Sca\text{-}1^-c\text{-}Kit^+CD34^+Fc\gamma RII/III^+$ leukemic stem cells (LSCs) was isolated, which transplant the disease when as few as four cells are injected into secondary recipients. Gene expression profiling was then used to demonstrate that, independent of the cell of origin, LSCs possess a gene-expression profile most consistent with differentiating myeloid cells. However, a leukemia self-renewal signature was identified that shows significant overlap with a group of genes highly expressed in HSCs whose expression decreases during the transition to normal committed progenitors. This signature possessed a number of genes important for normal HSC and human MLL-rearranged AMLs including *HoxA*5, *HoxA7*, *HoxA9*, *HoxA10*, and *Meis1*. Next, the investigators asked whether expression of *HoxA9* and *Meis1* could transform HSC and GMP, and found that HSC transformation was quite efficient whereas GMP transformation was quite inefficient, suggesting other pathways are necessary for MLL-AF9 induced transformation of progenitor cells. Finally, the epigenetic programs controlled by MLL-fusion proteins in a recently developed model of MLL-AF4 leukemia were explored and widespread abnormalities in histone H3 lysine 79 methylation, which is correlated with aberrant gene expression, were found. These data suggest that MLL-fusion proteins transform hematopoietic progenitor and stem cells via activation of *Hox* genes and other pathways,

perhaps as a result of abnormal histone methylation.

John Dick (University of Toronto, Toronto, Ontario, Canada) and colleagues focused on how homeostasis is maintained in the hematopoietic system while still retaining the flexibility to rapidly produce large numbers of mature cells in respond to stress.[24] They identified promyelocytic leukaemia zinc finger (PLZF) as a key molecular switch that controls these biological processes. Through a series of elegant genetic studies in human stem and progenitor populations to examine gain of function and loss of function, they found that PLZF blocked cell proliferation and differentiation of progenitors using a battery of *in vitro* and *in vivo* assays. In contrast, loss of function rapidly promoted differentiation. Cytokine signaling reduced the function of PLZF inducing rapid neutrophil differentiation. These studies indicate that antidifferentiation factors are critical to the process of fine tuning developmental programs.

Markus H. Frank (Harvard University, Boston, Massachusetts, USA) reported on cancer stem cells (CSCs) that drive tumor initiation and growth through self-renewal and differentiation and have been identified in cancers of the hematopoietic lineage and certain solid tumors. Recent findings in human malignant melanoma point to a specific relationship of such tumorigenic minority populations to therapeutic resistance and neoplastic progression.[25,26] Furthermore, initial proof-of-concept has been established indicating that specific targeting of chemoresistant ABCB5$^+$ melanoma stem cells for cell killing is sufficient to halt experimental tumor growth. These findings indicate that CSC-targeted strategies involving CSC ablation, modulation of CSC-specific molecular pathways, or inhibition of CSC-dependent functions in tumor progression could be suited to improve conventional therapies, which are believed to spare refractory tumor initiators responsible for recurrence and metastasis. Additional findings indicate that ABCB5$^+$ melanoma stem cells, as opposed to melanoma bulk populations, possess a distinct immunophenotype associated with preferential T helper 2 lymphocyte activation and more efficient inhibition of T cell proliferation. These findings raise the possibility that ABCB5$^+$ melanoma stem cells may not only represent a cancer subpopulation characterized by resistance to chemotherapeutic agents, but may furthermore coincide with malignant subsets potentially responsible for the resistance to immunotherapeutic approaches in human malignant melanoma.

Gary Van Zant (Markey Cancer Center, Lexington, Kentucky, USA) recently identified latexin as a negative regulator of the size of the HSC population in mice.[27,28] Latexin regulates stem cells via effects on self-renewal and apoptosis. Thus, latexin acts as a brake on the growth in size of this important population, which raises the possibility that latexin may be downregulated in malignant cells where unrestrained growth is a common feature. Indeed, latexin was found to be absent or greatly diminished in a variety of cancer cell lines and in patients with hematologic malignancies. Ectopic expression of latexin in lymphoma cell lines via expression vectors dramatically suppressed cell growth *in vitro* and tumor formation *in vivo*. These results suggest that latexin may provide a new modality in the treatment of human malignancies.

Malignancy and Aging

Peter M. Lansdorp (British Columbia Cancer Agency, Vancouver, British Columbia, Canada) presented data confirming loss of telomere repeats in purified hematopoietic cells with each cell division.[29,30] Using single telomere length analysis (STELA), the telomere length at the Xp/Yp telomere was measured in less than a hundred purified HSCs. Interestingly, a pronounced loss of several kilobases was observed in CD34$^+$CD38$^-$ cord blood cells following transplantation into immune-deficient mice. Most likely this loss reflects