3rd Workshop ITERA

24th – 27th October 2007

UNIVERSITY OF NEWCASTLE UPON TYNE

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International Tissue Engineering Research Association

Castle Vaeshartelt, Maastricht

Stem-Cells of the Cord, Cord-blood, Placenta:
Scientific approaches, pre-clinical and clinical applications
3rd ITERA Life-Sciences Forum Workshop
24th – 27th October 2007

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Program 3rd ITERA Workshop **25 October 2007**

Stem-Cells of the Cord, Cord-blood and Placenta: Scientific approaches, Pre-clinical and Clinical applications

Chairmen: **Andreas Zisch, Barbara Lukomska, Trine Fink, Harald Klueter**

08.30 – 08.50	**Registration**
09.00 – 09.15	**Welcome**, Albert Ramon, Chairman ITERA Life-Sciences-Forum Dept. of Gastroenterohepatology, University Hospital Antwerp / UZA, Belgium Institute of Genetics and Molecular Biology , Cologne, Germany
09.15 – 09.45	**Introduction and Overview** Colin McGuckin, Director Newcastle Centre for North-East Stem Cell Institute Newcastle University, United Kingdom
09.45 – 10.15	**Mesenchymal cells as vehicle for therapeutic genes: analysis of cell survival and transgene expression after transplantation in rat spinal cord.** Peter Ponsaerts, Experimental Hematology, University Antwerp, Belgium
10.15 – 10.45	**Cells from cord blood and placenta for in-utero and perinatal tissue regeneration.** Daniel Surbek, Head of Dept. Gynecology, University Bern, Switzerland
10.45 – 11.15	**Coffee / Tea Break**
11.15 – 11.45	**Cells isolated from the mesenchymal region of the amniotic membrane: stem cell potential and immunological features** Ornella Parolini., Fondazione Poliambulanza , Brescia, Italy
11.45 – 12.15	**Comparative characterization of cultured human term amnion epithelial and mesenchymal stromal cells for prospective application in cell therapy.** Steffen Zeisberger, Obstetrics, Univ. Hospital Zürich, Switzerland
12.15 – 12.45	**Fetoscopic closure of puncture fetal membranes with acellular human amnion plugs.** Andreas Zisch, Obstetrics, Univ. Hospital. Zürich, Switzerland
12.45 – 13.00	**Comparison of different mesenchymal stem cells from different origins.** Guy Wouters, Research and development, Cryo-Save Labs, Mechelen, Belgium.
13.00 – 13.15	**Human Mesenchymal Stromal (stem) cells derived from umbilical cord as a potential source for cell-based therapies: in vitro and in situ characterization.** Marjan Moreels, Histology, Univ. Hasselt, Belgium
13.15 – 14.00	**Lunch**

Program 3rd ITERA Workshop 25 October 2007

Stem-Cells of the Cord, Cord-blood and Placenta: Scientific approaches, Pre-clinical and Clinical applications

Chairmen: **Colin McGuckin, Daniel Surbek, Peter Ponsaerts, Viggo Vantendeloo**

14.00 – 14.30	**Human alternatives to bovine serum for the isolation and expansion of mesenchymal stem cells.** Karen Bieback, Cell biology - Institute of Transfusion Medicine and Immunology, DRK-Manheim, Germany
14.30 – 15.00	**Human Cord blood mononuclear cell as a source of functional neurons and glial cells in vitro.** Krystyna Domanska - Janik, Polish Academy of Sciences, Warsaw, Poland.
15.00 – 15.30	**Molecular Mechanisms of Blood cell mediated vascular growth.** Gina Schatteman, Integrative Physiology, Univ. Iowa, U.S.A.
15.30 – 16.00	**Coffee / Tea Break**
16.00 – 16.30	**Cord and Cord Blood stem cells for non hematopoietic applications (kidney and lung).** Lorenza Lazarri, Cord Blood Bank, Milano, Italy
16.30 – 17.00	**Human CB – CD133^{+} cells injected into nod-scid mice made deaf after ototoxic treatment provide conditions for the resumption of the inner ear structure and function.** Roberto Revoltella, CNR-ITB, Pisa and Research Foundation: ”Stem Cells & Life”,. Pisa, Italy
17.00 – 17.30	**Hepatobiliary Tissue Engineering from Cord Blood Stem Cells: First Steps towards Regenerative Medicine for Liver Disease.** Nico Forrraz, Newcastle Stem Cell Institute, United kingdom
17.30 – 18.00	**Discussions – Meet the experts**
19.30	**Dinner**

Program 3rd ITERA Workshop 26 October 2007

Stem-Cells of the Cord, Cord-blood and Placenta: Scientific approaches, Pre-clinical and clinical applications

Chairmen: **Simon-Philipp Hoerstrup, Christoph Stamm, Marc Ramael, Matthias Steinwachs**

08.30 – 09.00	**Visions from the European Commission.** Gwennael Joliff-Botrel, Principal Scientific Administrator Assistant to the Health Research Director European Commission, Brussels
09.00 – 09.30	**GMP-Conform Generation and Cultivation of Unrestricted Somatic Stems Cells (USSC) from cord blood using the SEPAX©-Separation Method and a closed culture system applying cell stacks.** Gesine Koegler, ITZ Univ. Düsseldorf, Germany
09.30 – 10.00	**Cord blood – and other – cells for myocardial regeneration** Christof Stamm, Deutsches Herzzentrum Berlin, Germany
10.00 – 10.30	**Coffee / Tea Break**
10.30 – 11.00	**Prenatal cells for cardiovascular tissue engineering.** Simon-Philipp Hoerstrup, Cardiovascular surgery, Univ. Hospital Zürich, Switzerland
11.00 – 11.30	**Bone marrow and adipose tissue derived Mesenchymal stem cells for clinical use.** Trine Fink, Laboratory for Stem Cell Research, Aalborg, Denmark
11.30 – 12.00	**Animal models for orthobiologic applications of stem cells, including state of the art imaging procedures.** Martijn van Griesven, Ludwig Boltzman Institute, Vienna, Austria
12.00 – 14.00	**Lunch**

Program 3rd ITERA Workshop 26 October 2007

Stem-Cells of the Cord, Cord-blood and Placenta: Scientific approaches, Pre-clinical and clinical applications

Chairmen: **Kathleen DE Greef, Dirk Ysebaert, Jan Vandroogenbroeck, Desmond Schatz, Gesine Koegler**

14.00 – 14.30	**About HLA and KIRs** Melanny Hidajat, AZ St. Jan, Hematology, Brugge, Belgium
14.30 – 15.00	**GVHD** Jan Vandroogenbroeck, AZ St. Jan, Clin. Hematology, Brugge, Belgium
15.00 – 15.30	**Coffee / Tea Break**
15.30 – 16.00	**Umbilical cord blood transplantation - a single centre experience.** Pedro Pimentel, Fernando Compilho , BM Transplantation Institute, Oncology Institute, Lisboa, Portugal
16.00 – 16.30	**Autologous umbilical cord blood infusion as immunotherapy for type 1 diabetes.** Michael Haller, Pediatric Endocrinology, Univ. Florida, U.S.A.
16.30 – 17.00	**Cord – Blood – Banking: Functioning and logistic.** Joan Garcia Lopez, Francesc Godia, Banc de Sang I Teitits, Barcelona, Spain
17.00 – 17.30	**GMP requirements for market authorization principles of consideration for clinical trials.** Peter De Waele, Head regulatory affairs, Cryo-Save Institute, Mechelen, Belgium
17.30 – 18.00	**Discussions – Meet the experts.**
18.00 – 18.30	**Poster Session**
19.30	**Candle-light Dinner**

Welcome,

Albert Ramon, Chairman ITERA Life-Sciences Forum
Dept. of Gastroenterohepatology, University Hospital Antwerp, Belgium

Introduction and overview

Colin McGuckin, Director Newcastle Centre for North-East England Stem Cell Institute, Newcastle University, United Kingdom

Nico Forraz, Marcin Jurga, Christina Basford, Saba Habibollah, Marko Strbad, Hamad Ali, Kendall Harger, Priti Dhareshwar
+44 7971 266 764
E-mail: c.mcguckin@newcastle.ac.ukProfessor Colin P. McGuckin

Newcastle Centre for Cord Blood
Regenerative Medicine Research

Facilities at Newcastle

The Royal Victoria Infirmary & Medical School

The International Centre for Life

Incorporating Hospital, University, Industry Stem Cells

Why are we here?
Global Strategy for Transplanting patients.+

Basis: Embryonic stem cells, adult stem cells and cancer stem cells
Aim – Transplanting patients.

Stem Cell Sources:
Embryonic - Cord Blood – Adult

Not all are equally available:
Fat – Skin – Bone marrow – organ tissues
Cord Blood – Embryonic cells

Cord related Cell Sources:
Placenta – Cord Blood – Cord (Wharton's Jelly)

<u>Stem/Progenitor Cells from Cord Blood:</u>
Easily accessible – Ethically sound -
100 million babies born each year!
Immature & immunonaive cells
Experimental and Clinical background: Bone Marrow Transplant

Autologous: Treating You with Your stem cells
Allogeneic : Treating You with Donor stem cells.

Today 85 conditions mostly in blood and immune diseases
Tomorrow needs regenerative medicine research and Cell expansion technologies

Uses of Cord Blood in our centre:
• Clinical Reconstruction of bone marrow in cancer patients

• Clinical Reconstruction of immune system in patients with immune deficiency
• Research into Regenerative Medicine

What we do is cell harvesting out of normal human tissues for future therapies

Clinical Research:

- **Communication between Cord Blood Stem Cells by Scanning Electron Microscopy**
- **Stem Cells project extensive pseudopodia**
- **World First No.1 Production of Stem Cells with Embryonic Characteristics from Human Umbilical Cord Blood.**
- **CBE's Cord Blood derived Embryonic like cells, up to 168 fold expansion achieved by now.**
- **World First No.2 Liver from Cord Blood.**
- **World First No.3 Pancreatic Cord Blood.**
- **Tissue engineering from Cord Blood.**
- **World's first multi-lineage progenitor cell line from Cord Blood. (with Minnesota USA)**
- **Produces @20 tissue types**

Promote Cord Blood Stem Cells!

Contact us: c.mcguckin@newcastle.ac.uk **+44 7971 266 764**

Mesenchymal cells as vehicles for therapeutic genes: analysis of cell survival and transgene expression after transplantation in rat spinal cord.

Peter Ponsaerts[1,6,*], Mark W Ronsyn[2], Jasmijn Daans[1], Gie Spaepen[3], Shyama Chatterjee[4], Katrien Vermeulen[1], Patrick D'Haese[3], Viggo FI Van Tendeloo[1,6], Eric Van Marck[4], Dirk Ysebaert[5,6], Philippe G Jorens[2,6], Zwi N Berneman[1,6].

(1) Laboratory of Experimental Hematology, (2) Division of Clinical Pharmacology, (3) Laboratory of Physiopathology, (4) Laboratory of Pathology, and (5) Laboratory of Experimental Surgery, Antwerp University, Universiteitsplein 1, 2610 Wilrijk, Belgium.
(6) Centre for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium.

*** presenting author**

Background: Bone marrow-derived stromal cells (MSC) are attractive targets for *ex vivo* cell and gene therapy. In this context, we investigated the feasibility of a plasmid-based strategy for genetic modification of human (h)MSC with enhanced green fluorescent protein (EGFP) and neurotrophin (NT)3. Three genetically modified hMSC lines (EGFP, NT3, NT3-EGFP) were established and used to study cell survival and transgene expression following transplantation in rat spinal cord.
Results: First, we demonstrate long-term survival of transplanted hMSC-EGFP cells in rat spinal cord under, but not without, appropriate immune suppression. Next, we examined the stability of EGFP or NT3 transgene expression following transplantation of hMSC-EGFP, hMSC-NT3 and hMSC-NT3-EGFP in rat spinal cord. While *in vivo* EGFP mRNA and protein expression by transplanted hMSC-EGFP cells was readily detectable at different time points post-transplantation, *in vivo* NT3 mRNA expression by hMSC-NT3 cells and *in vivo* EGFP protein expression by hMSC-NT3-EGFP cells was, respectively, undetectable or declined rapidly between day 1 and 7 post-transplantation. Further investigation revealed that the observed *in vivo* decline of EGFP protein expression by hMSC-NT3-EGFP cells: (i) was associated with a decrease in transgenic NT3-EGFP mRNA expression as suggested following laser capture micro-dissection analysis of hMSC-NT3-EGFP cell transplants at day 1 and day 7 post-transplantation, (ii) did not occur when hMSC-NT3-EGFP cells were transplanted subcutaneously, and (iii) was reversed upon re-establishment of hMSC-NT3-EGFP cell cultures at 2 weeks post-transplantation. Finally, because we observed a slowly progressing tumour growth following transplantation of all our hMSC cell transplants, we here demonstrate that omitting immune suppressive therapy is sufficient to prevent further tumour growth and to eradicate malignant xenogeneic cell transplants.

Conclusions: In this study, we demonstrate that genetically modified hMSC lines can survive in healthy rat spinal cord over at least 3 weeks by using adequate immune suppression and can serve as vehicles for transgene expression. However, before genetically modified hMSC can potentially be used in a clinical setting to treat spinal cord injuries, more research on standardisation of hMSC culture and genetic modification needs to be done in order to prevent tumour formation and transgene silencing *in vivo*.

Novel research directions:

Currently, our research is focussing on two novel topics: (1) characterisation of human fat and cord-derived mesenchymal stem cell transplants in rat spinal cord, and (2) the use of sleeping beauty transposons for genetic modification of mesenchymal stem cells. Preliminary data will be presented and discussed !

Placenta stem cells as autologous grafts for peripartum neuroregeneration? The neural differentiation potential of human placental mesenchymal stem cells

Professor Daniel Surbek, M.D.

Department of Obetstrics and Gynecology, University Hospital, University of Berne, Switzerland

Objective

We have recently identified mesenchymal stem cells (MSC) in placental tissue and showed their mesodermal and neuroectodermal differentiation potential. This study was focused on the induction of neural stem or progenitor cells in placental MSC via spherical culture and subsequent neuronal, oligodendrocytic and astrocytic differentiation

Study Design

Fetal MSCs from first trimester placental chorionic villi and term gestation chorion were isolated and grown in the presence of EGF and FGF-2 as floating spheric clusters. After plating on collagen, neural differentiation was initiated with retinoic acid and growth factors. Differentiation into neurons, oligodendrocytes and astrocytes and their progenitors was monitored immunohistochemically and by RT-PCR of neural genes. 2D-PAGE followed by high performance liquid chromatography (HPLC) with subsequent tandem mass spectrometry (MS/MS) was used for protein identification..

Results

After 5-7 days placental MSC formed rapidly proliferating neurosphere-like structures which stained strongly positive for nestin.60-80% of the cells outgrowing from stimulated neuropheres were positive for Tuj-1 and TUC-4, both markers specific for immediately postmitotic neurons (untreated controls: 4%), also confirmed in the Proteomics analyses.10% of the neurons expressed markers for more mature postmitotic neurons (NeuN;MAP1B; NF-M; NSE). Mature (MAP2+/TAU1+/NF200+) neurons were rarely found (1%). A part of the neurons had dopaminergic, another serotonergic or glutamatergic (but not GAGAergic) character. A fraction of 5-10% of the neurally differentiated cells had oligodendrocytic character: We mostly found immature oligodendrocytes staining for O1/O4 and GalC and O4 single positive late oligodendrocyte progenitors. A small amout of were identified as mature, MBP expressing oligodendrocytes or NG2- oligodendrocyte progenitors.GFAP-positive astrocytes were never found.

Conclusion:

Placental MSCs can differentiate into early neural progenitors and might be an ideal source for autologous stem cell graft for peripartum neuroregeneration

Cells isolated from the mesenchymal region of the amniotic membrane: stem cell potential and immunological features

Marta Magatti, Silvia De Munari, Elsa Vertua, Lucia Gibelli, Maddalena Soncini, ***Ornella Parolini****.*

Fondazione Poliambulanza, Brescia, Italy

Cells derived from the amniotic membrane of human placenta have been receiving particular attention because of their stem cell potentiality and immunomodulatory properties, which make them an attractive candidate source for cell therapy approaches.

We have previously reported that cells isolated from human fetal membranes of term placenta are able to successfully engraft in the bone marrow, lung and brain of newborn swine and rats, suggesting that they are non- immunogenic. *In-vitro* studies demonstrate that these cells not only fail to induce allo- or xenogeneic lymphocyte activation, but also actively suppress T-cell proliferation in an allogeneic mixed lymphocyte reaction (MLR), in both direct contact and transwell settings. Amniotic mesenchymal cells retain their immunosuppressive properties after culturing, and optimal inhibition is achieved at a 1:1 cell ratio of AMC to responders. The observation that MLR was still inhibited by AMC when cultured in a transwell system is indicative of soluble inhibitory factor(s). In addition, we have characterized cells isolated from the mesenchymal region of amnion and identified two different subpopulations, both of fetal origin. One subpopulation exhibits a mesenchymal stem cell-like phenotype (CD105+, CD29+, CD44+, CD54+, CD90+, CD73+, CD34-, CD45-), demonstrating *in-vitro* differentiation potential toward osteogenic, chondrogenic and adipogenic lineages. The other subfraction exhibits a monocytic phenopyte. We have investigated the immunological properties of these two populations and found that they differ in their immunododulatory characteristics.

In conclusion, evidence of progenitor phenotype, together with their immunological properties, makes cells derived from amniotic and chorionic fetal membranes an extremely attractive alternative source of progenitors for cell therapy approaches, even though caution should be taken when using fractionated versus unfractioned cell populations obtained from the mesenchymal region for transplantation.

Comparative characterisation of cultured human term amnion epithelial and mesenchymal stromal cells for prospective application in cell therapy

Zeisberger SM, Bilic G, Mallik AS, Zimmermann R, and Zisch AH

Departement of Obstetrics, University Hospital Zurich

Emerging evidence suggests human amnion tissue as a valuable source of two distinct types of pluripotent and immunoprivilegged cells, amnion epithelial cells (hAECs) and mesenchymal stomal cells (hAMSCs), for applications in cell therapy. No comparative systematic attempt has been made to determine the quantity and quality of amnion cells after isolation and culture. Amnion cells were isolated from 27 term placentas. Yields were 6 x 10^6 hAECs and 1.5 x 10^6 hAMSCs per gram amnion. All 27 cases led to vital cultures of hAMSCs, while one third of hAEC cultures (9 of 27) failed to grow out. Primary cultures contained significantly more proliferating than apoptotic cells. hAEC morphology changed towards mesenchymal phenotype during subculture. Flow cytometric characterization showed expression of mesenchymal progenitor markers CD73, CD90, CD105 and CD166, and embryonic stem cell markers STRO-1, SSEA-3 and -4 on both amnion cell types; levels of SSEA-4 markedly decreased during subculture. Expression of CD90 and SSEA-4 was confirmed by immunocytochemistry on both cell types. Reverse transcriptase-PCR analysis showed transcripts of Oct-3/4 and stem cell factor in primary cultures of all donors, but no of telomerase reverse transcriptase. Notably, protein Oct-3/4 was detectable only in hAECs of some but not all donors, and undetectable in hAMSCs.

In conclusion, both amnion cell types exhibit and maintain similar marker profile of mesenchymal progenitors in culture. hAECs are a less reliable cell source compared to hAMSCs and alter morphology during subculture.

Fetoscopic closure of punctured fetal membranes with acellular human amnion plugs in a rabbit model

Andreas H. Zisch. Department of Obstetrics, University Hospital Zurich, and Zurich Center for Integrative Human Physiology, Switzerland. e-mail: andreas.zisch@usz.ch

Iatrogenic preterm premature rupture of the fetal membranes (iPPROM) occurs in approximately 1-2% of patients after amniocentesis, and approximately 3% to 5% after diagnostic fetoscopy, and approximately 5% to 8% of patients after operative fetoscopy. To date, the interventional options to reseal fetoscopic entry wounds have remained very limited. Wounds in the fetal membranes are extremely difficult to seal, for technical as well as biological reasons. An appealing strategy for closure of iatrogenic puncture wounds in the fetal membranes is to deploy biopolymeric plugs that immediately seal, perhaps even help anatomic healing of leaky membranes. For this study, we established surgical plugs made of collagen scaffold that we prepared from decellularized term human amnion membrane (DAM). We explored utility of DAM plugs for fetoscopic closure of iatrogenic defects in fetal membranes in a rabbit model. Our study was performed in 8 rabbit does. Punctures were created at midgestational day 23 by 14-gauge needle fetoscopy on surgically exposed rabbit amniotic sacs. The entry sites were fetoscopically plugged either with DAM (n=10) or a commercial TissuFleece E® collagen foil (n=10), followed by their primary fixation with fibrin glue and myometrial suturing. Seven punctured sacs without any plugging and 31 sacs without any manipulation served as two reference groups. Amniotic integrity and fetal parameters were assessed at gestational day 30, one week after surgery. DAM sheets could be delivered precisely and controlled by fetoscopy as compact plug into amniotic defects. The surgical handling characteristics of DAM were significantly better than TissuFleece E®. Both treatment with DAM or TissuFleece E® compared regarding efficiency for restoring amniotic integrity. 75% and 71.4% of amniotic sacs treated with DAM or TissuFleece E®, respectively, showed amnion integrity, compared to 25% in the left-open study group. Histology at the one week experimental endpoint showed no evidence for inflammation, but also no beginning of anatomic healing of grafted DAM.

In conclusion, fetoscopic delivery of plugs made of DAM presents a surgically practical method to restore amniotic integrity of punctured fetal membranes. DAM plugs possesses several benefits for the clinic: They are easy to manufacture, cell-compatible and non-immunogenic, and suitable for off-the shelve use. Further, scaffolds made of DAM may become useful as surgical wound dressing or vehicle for cell transplantation in a broad range of tissue repair applications.

This study was supported by Swiss National Science Foundation grant no. 31000-108270, EU commission FP6-grant 'EuroSTEC', and the Zurich Center for Integrative Human Physiology.

EX VIVO GENERATION AND EXPANSION OF MESENCHYMAL STEM CELLS FROM DIFFERENT TISSUES FOR CLINICAL APPLICATION.

Guy Wouters
R&D Department Cryo-Save Group

Mesenchymal stem cells (MSC) are of great therapeutic potential due to their capacity of self-renewal and multilineage differentiation. They support hematopoiesis and enhance the engraftment of haematopoietic stem cells after co-transplantation. Currently, bone marrow (BM) represents the major source of MSC for cell therapy, though aspiration of BM involves invasive procedures, and the frequency and differentiation potential of BM-MSC decrease significantly with age. Therefore, other sources are studied from amniotic fluid ,umbilical cord (Wharton Jelly) and adipose tissue.

The growth kinetics have been studied of these different sources using the doubling population levels. The growth of MSc from fresh WJ, adipose tissue and amnion fluid is compared. We observe a growth of 10 doubling levels within a period of 7 weeks for WJ and adipose MSc. When the tissue was cryopreserved a delay in the onset of growth of about ten days is observed due to cryobiological damage. As a first observation MSc from amnion fluid have a very high growth potential in the first two passages.

Comparing different media there is no difference between common DMEM and DMEM/F12 media though using MesenPro ^{R} there is a significant higher growth rate.

It can be concluded that MSc can be isolated in different tissues of the human body and the growth kinetics of the MSc cells are different, but other characteristics (markers, morphology) are considered similar.

Cryopreservation and banking of this type of cells are the logic consequence and research on this is promising.

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Human mesenchymal stromal (stem) cells derived from umbilical cord as a potential source for cell-based therapies: in vitro and in situ characterization

Moreels Marjan, Theunissen Evi, Ramael Marc, Lambrichts Ivo, Hellings Niels

The adult central nervous system (CNS) possesses a limited capacity for regeneration. However, new possibilities for repairing nervous tissue have arisen since the discovery of stem cells. Mesenchymal stem cells (MSCs) are one of the most promising stem cell types for therapeutic uses due to their accessibility and the relatively simple requirements for in vitro expansion and genetic manipulation. Upon tissue injury, MSCs have been shown to migrate to the damaged brain. These results suggest that MSCs could provide an ideal cell source for repairing CNS diseases such as multiple sclerosis. Besides the well-characterized MSCs, which are derived from bone marrow and cord blood, a growing body of evidence suggests that human umbilical cord matrix (Wharton's jelly) contains a substantial amount of cells having properties similar to MSCs. In the present study the mesenchymal phenotype and differentiation abilities of cultured umbilical cord matrix cells were analyzed. Ultrastructural characterization was assessed by transmission and scanning electron microscopy. Our observations demonstrate that stromal cells derived from umbilical cord show immunophenotypical and ultrastructural similarity with MSCs. In addition, their differentiation potential to multiple mesenchymal lineages including adipocytes, osteoblasts, and chondrocytes clearly indicates their stem cell properties. Immunohistochemical analysis of human umbilical cord using mesenchymal stem cell markers further defined the umbilical cord stem cell niche. These results support the applicability of the umbilical cord as an excellent alternative to bone marrow as a source for MSCs for cell-based therapeutic strategies.

Human Alternatives to Bovine Serum for the Isolation and Expansion of Human Mesenchymal Stem Cells

Karen Bieback[1], Andrea Hecker[1], Asli Kocaömer1, Heinrich Lannert[2], Harald Klüter[1]

[1]Institute of Transfusion Medicine and Immunology, German Red Cross Blood Service of Baden-Württemberg - Hessen, Medical Faculty Mannheim, University of Heidelberg, Germany
[2]Medical University Hospital Heidelberg Dep. V (Hematology, Oncology and Rheumatology), University of Heidelberg, Germany

Mesenchymal stromal cells (MSC) are promising candidates for novel cell therapeutic applications. Clinical exploitation of these cells necessitates a pharmaceutical manufacturing according to international Good Manufacturing Practice (GMP) guidelines. At the moment, however, most protocols for clinical-scale production of MSC use culture media supplemented with Fetal Calf Serum (FCS) which poses a potential risk for infections as well as immunological reactions. Our goal was to develop a standardized isolation and expansion protocol for MSC replacing FCS. Human factors derived from serum, plasma or platelets have been discussed as potential alternatives to FCS. We compared the effects of different human supplements to FCS on the isolation and expansion of MSC isolated from both adipose tissue (AT) or bone marrow (BM). Human alternative supplements were either: Pooled human serum derived from blood group AB donors (HS-AB), thrombin-activated platelet releasate in plasma (tPRP) or platelet lysate (PL). All human supplements supported the isolation and expansion of MSC derived from AT and BM. However, strikingly the effects were different for the two tissue MSC: Whereas in AT both AB-HS and tPRP were superior to FCS in supporting long-term expansion, in BM only PL allowed for a superior expansion. Nevertheless, MSC of all isolation and culture conditions displayed a MSC typical fibroblastoid morphology, immune phenotype and differentiation potential. In AT, MSC expanded in the human alternatives contained a small proportion of contaminating hematopoietic cells at early passages. Thus, we applied two different enrichment protocols to deplete contaminating cells from BM: depletion of cells using an antibody cocktail against mature hematopoietic lineages (RosetteSep™) or sorting of CD271 expressing cells. Purification of BM-MSC by either depletion or enrichment strategies showed no improved expansion capacity of BM-MSC, in contrast the doubling time increased and cultures showed early proliferative senescence. Importantly, spontaneous cell transformation was not observed in any of the culture conditions: Proliferative senescence was associated by ceased proliferation and lack of telomerase activity at any passage. Concluding, we demonstrate that human MSC derived from human BM display different susceptibility to human alternatives to FCS when compared to AT-MSC. Whereas HS-AB and tPRP proved to be superior in expanding AT-MSC, PL seems to be optimal for BM-MSC.

Human Cord Blood mononuclear Cell as a source of functional neurons and glial cells in vitro.

Krystyna Domanska

Our research has been focused on feasibility of deriving therapeutically applicable human neural stem cells (hNSC) in way that avoid any ethical controversies. Previously we have shown (Bużańska et al 2002, 2006) that human umbilical cord blood cells (HUCB) can be selected and committed to neural lineage in vitro. The non-immortalized, stable cell line with neural stem cell properties and ability to differentiate toward electrophysiologically functional neurones as well as to astrocytes and oligodendrocytes have been developed from non-hematopoietic (CD34 minus) mononuclear fraction. HUCB-NSCs proliferates continuously, are diploid and clonogenic but not tumorogenic upon transplantation into NOD-SCID mice. Thus, these observations strongly suggests that the canonical for tissue-specific SC balance between symmetrical and asymmetrical cell division leading to senescence of culture, can be omitted in cord blood derivatives under certain promoting conditions. However, the mechanism underlying this phenomenon needs to be elucidated what is crucial for standardization of the procedure which still proven elusive.

We have decipher the first steps of neural stem/progenitor genes induction in aggregating culture of cord blood mononuclear cells, their rapid phenotypic changes and under influence of mitogen activation (bFGF, EGF) their variable, time-limited expansion as a cohort of low-differentiated neural progenitors (NP). These NPs, when seeded on rat brain primary cultures or on HUCB-NSC monolayer culture can further grow and differentiate suggesting their utility for neural cell replacement therapy.

However, initial transplantation of HUCB-NSCs into intact rat brain striatum or cerebral cortex revealed that only a small fraction of the cell population can survive the first few days, independently of CsA-induced immunosuppression of the host animal. Moreover, the cell implant evoked acute local tissue reaction with strong infiltration by host inflamatory (ED1-reactive) cells. Importantly, HUCB-NSCs express Human Leukocyte Antigen - HLA ABC (MHC- class I) molecules, whereas highly immunogenic HLA-DR (MHC- class II) or CD45-antigen-presenting cells are almost completely absent from this cell population (unpublished data). The animals compromised by a focal brain lesion were rather more tolerant of HUCB-NSC transplantation than intact recipients. The migrating donor cells which accumulated in the peri-infarct area were found over 7 days after transplantation, while it was possible to show the appearance of differentiated phenotypes with expression of neuronal (NF 200) but only moderate astrocytic (GFAP) cell markers (Kozłowska et al. 2007). However, the paucity of HUCB-NSCs detected within the post-ischemic parenchyma at the end of one month following transplantation combined with the acute rejection of grafted cells by intact (yet immunosuppressed) rat brain tissue, strongly suggests development of the immunological graft/host conflict.

Acknowledgments: This work was supported by Polish Ministry of Scientific Research and Higher Education, grant #: 2P05A17729

An ubiquitous reserve of perivascular multi-lineage stem cells within human tissues

Bruno Péault

Multi-lineage stem cells were believed to be present exclusively in developing embryos, and from these early stages be lost progressively at the expense of committed tissue progenitors. This idea was challenged by the discovery that multipotent stem cells can be derived from diverse adult organs such as the brain, bone marrow, skeletal muscle or even fat tissue. However, adult multipotent stem cells were exclusively derived from long-term cultured cell suspensions. The identity, anatomic localization and even existence within native adult tissues of such multi-lineage stem cells, therefore, remained obscure. We have attempted to prospectively identify, purify and characterize such broadly committed progenitors within fetal and adult human human tissues that include placenta, pancreas, skeletal muscle, lung, bone marrow and adipose tissue. We now present evidence for the presence in all human tissues of multi-lineage progenitor cells associated with the walls of blood vessels.

First, we have documented anatomic, molecular and developmental relationships between endothelial and myogenic cells within human skeletal muscle. Cells co-expressing myogenic and endothelial cell markers (CD56, CD34, CD144) have been identified by immunohistology and flow cytometry. These *myo-endothelial* cells form myotubes *in vitro* and regenerate myofibers in the SCID mouse muscle about ten times more efficiently than CD56+ myogenic progenitors. Cultured CD56+CD34+CD144+ cells proliferate long-term, retain a normal caryotype, are not tumorigenic and better survive under oxidative stress than CD56+ myogenic cells. Clonally derived CD56+CD34+CD144+ cells were also found to differentiate into bone and cartilage cells in culture.

Next, we have identified, by immunohistochemistry and flow cytometry, perivascular cells know as pericytes in a variety of pre- and postnatal human tissues including skeletal muscle, pancreas, bone marrow, brain, adipose tissue, skin and placenta, according to CD146, NG2, CD133 and PDGF receptor expression and absence of CD56, CD45, CD34 and other endothelial cell markers. Perivascular cells purified to homogeneity by flow cytometry from skeletal muscle, as well as non-muscle tissues, were robustly myogenic in culture and when transplanted into the cardiotoxin-injured skeletal muscles of immunodeficient mice. Purified human perivascular cells were established in long-term culture where they retained their markers and myogenic developmental ability but, also, exhibited osteogenic, chondrogenic and adipogenic potentials. Strikingly, long-term cultured human perivascular cells expressed all the known markers of mesenchymal stem cells (MSC) such as CD44, CD73, CD90 and CD105. These data suggest the existence of perivascular cells at the origin of the elusive mesenchymal stem cells, which have been so far identified only retrospectively in primary cultures of whole organs.

Finally, we show that following transplantation into the infarcted hearts of SCID mice both human myoendothelial cells and pericytes can significantly improve cardiac anatomy, as measured by scar area and ventricle diameter reduction, and contractility, assessed by percent fractional shortening and percent fractional area change.

Altogether, these results confirm the key role that blood vessel walls appear to play as progenitor cell providers in development and post-natal life. These novel human blood-vessel associated progenitor cells are amenable to biotechnological handling, including purification by flow cytometry and clonal multiplication *in vitro*, and may therefore represent convenient therapeutic cells for human tissue repair.

Cord and Cord Blood stem cells for non hematopoietic applications (kidney and lung)

Lorenza Lazzari, PhD

Cell Factory, Centre for Transfusion Medicine, Cell Therapy and Cryobiology, Department of Regenerative Medicine - Fondazione Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milano, Italy

Stem cell research is divided into two major camps: one focused on cells from adults (bone marrow, adipose tissue, peripheral blood), the other on the controversial technique that destroys embryos. But there is another category of stem cells that are readily available, ethically trouble-free and possibly as powerful and flexible in function as their embryonic counterparts: "cord and cord blood stem cells". In this context, we successfully isolated, characterized and expanded mesenchymal and perivascular stem cells from umbilical cord and from cord blood. These cells express high percentages of pericyte markers: CD146+/34-/45-/56-, show stemness markers (Oct-4, Runx-1, SSEA-4) and explain the ability to differentiate towards chondrogenic, osteogenic, adipogenic and muscular lineages if in presence of appropriate stimuli. These stem cells could be potentially used in several clinical applications but we decided to focus our attention on bronchopulmonary dysplasia (BPD) and on acute renal failure. Despite the new pharmacological treatments, these diseases still afflict a large number of patients: in the acute renal failure (up to 7% of all hospitalized patients) the mortality rate remains greater than 50%; and in the BPD, infants with severe BPD remain at high risk for pulmonary morbidity and mortality during the first 2 years of life.

In this context, we evaluated the ability of these cells to migrate when co-cultured with alveolar or tubular epithelial cell lines damaged by bleomycin or cisplatin, respectively. After 2weeks of co-culture, as a result of the chemiotactic migration, we observed that PKH26+ umbilical cord and cord blood stem cells migrated and were found in contact with the damaged epithelial cell layer. Moreover, we collected at different times (24, 48, 72, 96, 120 hours after damage) the supernatants from the co-cultures. The level of angiogenetic and inflammatory factors was quantified in all the supernatants by a multiplexed sandwich ELISA that allows a chemiluminescent quantitative measurement of 9 proteins per well. We observed that angiogenic factors are highly expressed when umbilical cord and cord blood stem cells are present in the co-culture confirming the role of mesenchymal/perivascular cells as “drugstores” of soluble factors. At the same time, inflammatory factors are statistically reduced showing the immunomodulatory effects of these cells. For the acute renal failure these results have been confirmed in an animal model of renal failure.

Our results confirm that it is possible to isolate and to maintain in long-term culture mesenchymal/perivascular cells from umbilical cord and cord blood. Moreover, these stem cell populations are able to migrate towards alveolar/tubular epithelial cell layer previously damaged with bleomycin or cisplatin. Potentially these new stem cells can provide a valuable resource for tissue repair and for engineered organs as well. Our data encourage further investigations in order to evaluate the feasibility of therapeutic applications.

HUMAN CORD BLOOD-DERIVED CD-133^{+} CELLS INJECTED TO NOD-SCID MICE MADE DEAF AFTER OTOTOXIC TREATMENT PROVIDE CONDITIONS FOR THE RESUMPTION OF THE INNER EAR STRUCTURE AND FUNCTION

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Alessandro Martini, Andrea Ciorba, Lucia Bertolaso, Sara Magosso, Dept. Audiology, University Hospital, Ferrara, Italy
Richard Poulsom, Rosemary Jeffery, Cancer Research UK, London, UK

We investigated whether human CD133^{+} stem cells (HSC) engraft the cochlea and contribute to the restoration, *in vivo*, of the organ of Corti in nod-scid mice after ototoxic treatment with kanamycin and noise. By PCR, we tested the presence of HLA.DQα1, used for the traceability of the engrafted cells, demonstrating that the HSC injected i.v. migrate to various tissues of the host, including the organ of Corti. In oto-injured mice, HSC contributed to the stimulation *ex novo* of the morphological recovery of the organ of Corti, while the control oto-injured mice that were not transplanted, remained seriously damaged. Partial functional recovery was demonstrated after transplantation. Dual color-FISH analysis provided further evidence of the positive engraftment. in the organ of Corti as well as in different mouse tissues, and also revealed a small proportion of hybrids probably derived from fusion of donor with endogenous cells.

These observations offer the first evidence that, HSC migrating to the cochlea and contributing to the organ of Corti's structure restoration, may provide conditions for the resumption of the inner ear.

Cord blood stem cells for regenerative medicine and tissue engineering.

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With over 130 million births per year worldwide, cord blood is perhaps the largest untouched stem cell source available for human bio-medical research and clinical development. The non-invasive nature of its collection confers cord blood a distinct advantage over any other sources of adult and embryonic stem cells, since its cells can be easily characterized, cryopreserved in, and retrieved from bio-banks worldwide. Cord blood has been used for over thirty years in the treatment of mostly blood and immune disorders. Recent research, including from our group, suggests that cord blood stem cells will play an increasing role in regenerative medicine strategies.

We have further developed routine protocols for isolation of defined cell populations from extremely immature stem cell compartments, through to more mature progenitor cells, which have allowed defined tissue engineering and regenerative procedures to be advanced. We were the first research group in the world to develop a protocol for the production of karyotypically normal Cord Blood-derived Embryonic-like stem cells (CBE's), which have the ability to form embryoid body-like structures and to express the majority of Embryonic Stem Cell markers. It is also possible to subculture these cells for ex vivo expansion for over 12 weeks, whilst maintaining their immaturity. This is the first production of a clone of embryonic-like cells from a non-embryonic tissue. Our work has shown that cells from all three germ layers can be produced from cord blood and bone marrow stem cells. We further confirmed the pluripotential of cord blood stem cells that can be differentiated into tissues of endodermal, mesodermal and ectodermal origins including neural, endothelial, hepatic, and pancreatic tissues. Conventional cell culture methods employ 2-dimensional culture systems to propagate cells in vitro. Although this approach is useful to understand key biological features like the cell cycle, growth factor stimulation, proliferation rates and signal transduction, the large scale clinical grade ex-vivo tissue expansion generation calls for 3-dimensional Tissue Bio-engineering. Our preliminary work with NASA-engineered RCCS bioreactors has demonstrated that they allow continuous rotation, expansion and endogenous extracellular matrix production in a shear stress-free environment favouring development into functional 3-D neural, liver and pancreatic-like tissues. Expansion, differentiation and tissue engineering of cord blood stem cells offer exciting prospects in the development of tomorrow's cures.

Stem Cell Research at EU level

Dr. Gwennaël Joliff-Botrel

Health Research Directorate

European Commission

- EU strategy policy
- SC research funded at EU level
- Health Programme
- The future ⇒ FP7

EU Strength/ Policy

- *Collaborative research* ***between scientists from different countries***
- *Various Instruments:*
  coordination, small research projects ***(5-10 partners € 1.5-2.5m / 4 years),***

  large projects ***(15-25 partners € 8-12m / 4-5 years).***

EU Strategy/ Policy
⇒ Concentrate on topics where cooperation is of clear added value to increase European competitiveness in:

- *Basic research* ***(e.g. characterization of human embryonic stem cell lines , understanding differentiation, epigenetics, comparison of stem cells from different origin)***
- *Applied research*

–Translational research

– for the High tech Biotech industry (15% of the EU budget is targeted to Small and Medium sized Enterprises / EC contribution up to 75% of the costs)

EU Strategy/ Policy

- ***Coordination:*** *e.g. Creation of a European hESC registry (€1m for 3 years):*
  - to gather the information on all the European human embryonic stem cell lines in order to make the most effective use of existing ones (then extension to non-European lines)
  - to disseminate the information to all the scientific community
  - to favour the comparability of results (standardisation)
  - to increase transparency

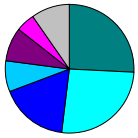
Every EU country that allows the creation of new lines is involved plus Turkey, Israël, Switzerland, US, Australia

EU Strategy/ Policy on stem cells

- EU research on stem cells from all origin need to be supported
- Clear rules for hESC research supported by EU funds *(only research on hESC in culture). 100% transparency as regards the projects supported.*
- Support has to be provided to alternatives to hESC
- EU should continue to promote the dialogue between scientists and the society on this issue

SC research funded at EU level

104 FP6 projects involving stem cells ~ €500 millions



UNDERSTANDING
Fundamental knowledge relevant to human health
DEVELOPING
Tools for new therapies and medicines
BUILDING
Tissue engineering
104 FP6 projects involving stem cells ~ €500 millions

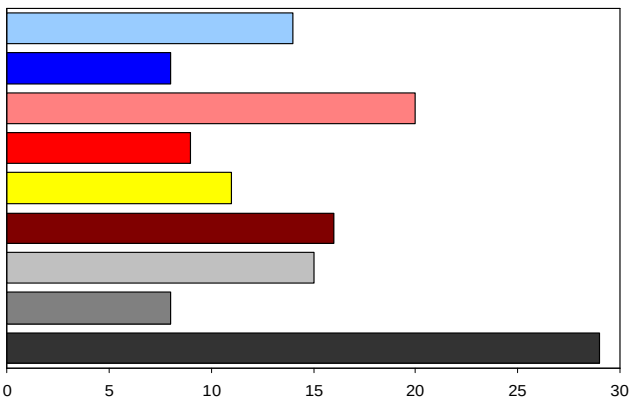
MODELLING
Mathematical & biology models, alternatives to animals testing
REPAIRING
Pre-clinical & clinical studies for diseases and impairments
TREATING
Improvement of standard hematopoietic SC transplantation
INTEGRATING
Ethics, legal, societal aspects, training

FP6 projects: type of cells/tissues/organs

Nervous system & annexes
Skin/ other epithelium tissues
Bone/ other connective tissues
Heart & Vasculature

Digestive & urogenital tracts
Blood & Immune system
Cancer

Heritable & Rare diseases
Basic or horizontal issues



Health Programme

Activities in 3 main areas:

- Biotechnology, generic tools and technologies for human health
- Translating research for human health
- Optimising the delivery of healthcare to citizens

Main features of transition from FP6 to FP7:
Continuity; broader scope; less focus on genomics;
emphasis on translational research;
health policy driven research strongly reinforced.
New: emerging epidemics, obesity, chronic diseases, biomedical technology & engineering;

1: Biotechnology, generic tools and technologies

- High-throughput research
  enhancing data generation, standardisation, acquisition & analysis
- Detection, diagnosis and monitoring ↑
  with emphasis on non-invasive or minimally invasive approaches
- Predicting suitability, safety and efficacy of therapies develop and validate parameters, tools, methods and standards (**mainly through IMI**) and alternatives to animal testing ↑
- Innovative therapeutic approaches and interventions
  gene and cell therapy, regenerative medicine, immunotherapy
  and vaccines. ↑

2: Translating research for human health

- Integrating biological data and processes:
  large-scale data gathering, systems biology ↑
- Research on the brain and related diseases,
  human development and ageing ↑
- Translational research in major infectious diseases:
  to confront major threats to public health
  antimicrobial drug resistance, HIV/AIDS, malaria and TB,
  emerging epidemics, neglected infectious diseases
- Translational research in other major diseases:
  cancer, cardiovascular disease, diabetes and obesity,
  rare diseases, and other chronic diseases ↑

3: Optimising the delivery of health care to citizens

- Translating clinical research into clinical practice
  patient safety, better use of medicines, benchmarking, pharmacovigilance, etc. ↑
- Quality, efficiency and solidarity of health care systems organisational and financial aspects, health systems, etc.
- Enhanced health promotion and disease prevention providing evidence of best public health measures – life styles, interventions, special focus on mental health, etc.

Special emphasis and measures for:

- **SMEs**
  - Participation encouraged in all areas
  - Special topics for SMEs
  - Support actions
- **International Cooperation**
  - Participation possible in all areas
  - Special International Cooperation Actions (SICA)
  - Coordination or Support actions

Health Programme

Response to the 1st call - Overview

- **1st call of Health theme** (FP7-HEALTH-2007-A)
  - 87 topics, € 637 million, deadline 19 April 2007

- **914 proposals received**
  - 21 declared ineligible

1st call outcome of evaluation

- **425 proposals (48%) above all thresholds**
- **152 proposals short-listed for funding**
  - **i.e. 17% of proposals received were short-listed**
  - this represents 36% of proposals above threshold

2nd call (FP7-HEALTH-2007-B)

- € 549 million, deadline 19 September 2007

3rd call

- Planned for the 1st semester of 2008

Workshop 13 September 2007**: "GMP facilities and non-commercial clinical trials"**

- ***Early Clinical trials on vaccines/immunotherapy - related GMP facilities***
- ***Early Clinical trials on Cell and Gene therapies - related GMP facilities***
- ***Early Clinical trials on other bioproducts "Orphan drugs/ off-patent paediatric medicines, etc. - related GMP facilities***
- ***Public-Private GMP facilities and translational research***

Main Recommendations

- ***Sufficient number of GMP facilities in Europe: effort on networking-dissemination of the existing facilities (e.g. FP6 Clinigene project-FP7 ECRIN/EATRIS)***
- ***To support Clinical Trials from pre-clinical to Phase I/II except in specific cases (e.g. rare diseases/off-patent paediatric medicines)***
- ***Dedicated project solely on clinical trials***
- ***To consider GMP development/production for EU funding***
- ***To consider one site or multi-centres clinical trials***
- ***To continue to support innovation steps: new GMP products/process***
- ***Separate the issue of «external» GMP facilities and GMP facilities within the hospitals that also need support***
- ***Not so many «serious» non-commercial clinical trials are ready (topics have to be broad)***

Workshop 20 September 2007: "Regenerative Medicine"

- To delineate the scope of regenerative medicine (definition)
- To identify trends in research and allied industry including breakthrough results
- To identify research priorities in regenerative medicine and European strengths in the field (European added value)

Outcome:

Definition: *«Repair of functionally compromised cells, tissues or organs by biological substitutes or stimulation of endogeneous processes going beyond standard therapies»*

Trends in research:

- ***Very active research area with redefining new results (e.g. induced pluripotent SC)***
- ***Shift from gene therapy to cell-based therapies***
- ***Shift from in-vitro tissue engineering to stimulating the body to repair***
- ***Bioprocessing needs to be better developed***
- ***Last stages of translation needs more support (i.e. GMP, clinical studies)***

Outcome:

Research priorities with EU added value

- ***Support of translational research (demonstration of proof of concept/safety-toxicology/laboratory & manufacturing protocols, standardisation)***
- ***Understanding mechanisms of actions***
- ***Reprogramming***
- ***Directed differentiation***
- ***New hESC sources***
- ***Coordination activities (e.g. such as International SC Initiative)***

SC Research the Future: FP7 (2007-2013)

Collaborative research:

- Health (€6.1 billion)
  - **Basic research and applied research (emphasis on translational research)**
- Nanonosciences, nanotechnologies,

Materials/New Production

Technologies (€3.5 billion)

- **⇒Tissue engineering**
- **European Technology Platform on Nanomedicines ⇒ strategic research agenda that includes Regenerative Medicine**
  http://cordis.europa.eu/nanotechnology/nanomedicine.htm

European Research Council

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Experts: http://cordis.europa.eu/research_openings/

GMP-Conform Generation and Cultivation of Unrestricted Somatic Stems Cells (USSC) from cord blood using the SEPAX©-Separation Method and a closed culture system applying cell stacks

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Generation and characterization of unrestricted somatic stem cells (USSC) from cord blood (CB) was described by our group and has been well established under laboratory conditions [1-4]. Due to their proliferative and differentiation capacity, USSCs are an interesting candidate for the future development of cellular therapy for tissue repair and tissue regeneration as well as a supportive cell layer to support hematopoietic reconstitution.

Since generation and expansion under GMP-grade conditions is mandatory for use in clinical application, the automated cell processing system Sepax (BIOSAFE) with the CS900 separation kit was used for mononuclear cell separation and the subsequent generation of the USSC colonies in the presence of 30% GMP-grade fetal calf serum, low- glucose DMEM-medium/10^{-7}M dexamethasone. Expansion of USSC was performed in a closed system applying cell stacks. Results achieved so far indicate that the generation frequency and quality of generated USSC under GMP conditions are equal or even superior (45%) to manual generation under laboratory conditions (43%). 20 cord-blood units have been processed, resulting in 9 USSC-colony formations and lines within 14-28 days. Growth kinetics is equal to the previously established USSC-lines (~48-36 h / doubling). Analysis of the immunophenotype as well as the differentiation potential towards the mesenchymal , neural and endodermal lineages also showed no difference to those lines generated manually using Ficoll-separation and normal cell culture flasks (T225). The closed system applied here is perfectly suitable to ensure safe and easy handling of the USSC, including seeding, trypsination and harvesting. In combination with the cell stack system (1, 2 and 5 and 10 layers), cell amounts of $1.5x10^9$ USSC can be achieved within 4 passages. These USSC products were temperature controlled cryopreserved in the presence of 10%DMSO, HSA and dextran. USSC can be thawed and further expanded in clinical grade quality. On the basis of their pluripotency and expansion under GMP-conditions into large quantities, these USSC from cord blood, when pretested for infectious agents and matched for the major transplantation antigens, may serve as a universal allogeneic stem cell source for tissue repair and tissue regeneration.

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3. Kogler G, Sensken S, Wernet P (2006) Comparative generation and characterization of pluripotent unrestricted somatic stem cells (USSC) with mesenchymal stem cells (MSC) from human cord blood. Exp. Hematology in press

4. Sensken S, Waclawczyk S, Knaupp A, Trapp T, Enczmann J, Wernet P, Kogler G (2007) In vitro differentiation of human cord blood-derived unrestricted somatic stem cells towards an endodermal pathway. Cytotherapy 9:362

Christof Stamm, Deutsches Herzzentrum Berlin

Cord blood – and other - cells for myocardial regeneration

Aside from organ transplantation or “artificial hearts”, traditional therapeutic approaches designed to treat heart failure merely enable the organism to survive with a heart that is working at a fraction of its original capacity. It is therefore no surprise that the prospect of cardiovascular regenerative medicine has raised many hopes. Early reports on myocardial repair by adult bone marrow stem cells in rodent models promoted an unparalleled boost of clinical and experimental cell therapy studies. The phenomenon of stem/progenitor cell-induced angiogenesis in ischemic myocardium has ever since been reproduced in a variety of small and large animal models, as well as in clinical pilot trials. “Myogenesis”, however, is an altogether different matter. Many of the initial clinical studies were fuelled by the suggestion that early pre-haematopoietic stem cells have plasticity high enough to permit cross-lineage differentiation into cells of cardiomyocyte phenotype, but the initial enthusiasm has largely faded. The myogenic potential of stroma cell-derived mesenchymal stem cells is much better documented, but transfer to the clinical setting faces a variety of obstacles.

Cord blood has been known to contain haematopoietic stem cells for several decades. In the context of regenerative medicine it is often emphasized that the use cord blood cells obviates the need for invasive bone marrow collection, although this is not a very strong argument for the clinician. Allogenic cord blood cell transplantation is possible in patients with compromised immune system, but it is unlikely that human cord blood cells are sufficiently naive in terms of their antigenic surface markers to allow for allogenic use in immunocompetent individuals. A stronger argument in favour of cord blood cells is their alleged immaturity, which is believed to translate into a greater potential for trans-differentiation into any desired target cell type. Moreover, both proliferation rate and functional capacity are generally believed to be higher the younger the cell and the healthier its donor is. Whether this is true for all cord blood cell subpopulations cannot be said with certainty. Detailed comparative analyses regarding the non-haematopoietic regeneration capacity have just begun, and preliminary data vary greatly between different subsets cord blood cells.

As with many other advances in biomedical research, it has become clear that the knowledge gained in animal models is not readily applicable to the clinical situation. Novel cell therapy approaches therefore have to be adjusted according to human pathobiology, and, last but not least, to a very complex regulatory framework. Integrating cell therapy strategies in a conventional cardiologic or cardiovascular therapy algorithm is in itself a challenging task. It is further complicated by the fact that physicians and surgeons often legally become part of the manufacturing process of a medicinal product.

Based on their extensive experience with patients suffering from end-stage heart failure, physicians, surgeons, and scientists at the German Heart Institute Berlin (DHZB) have recognized the potential of cell-based regenerative medicine for heart disease. Together with its local and international partners in academics and industry, DHZB is developing novel strategies for cardiac regenerative medicine and pursuing their safety and efficacy evaluation in the clinical setting. Those include clinical pilot trials in patients with end-stage ischemic heart disease as well as patients with non-ischemic heart failure who require implantation of a ventricular assist device. Specific surface marker-defined bone marrow progenitor cells, mononuclear cell preparations, and *ex vivo* manipulated cell products are being currently being evaluated. In addition to reviewing our preclinical and clinical data, the presentation will highlight the logistic and legal challenges that occur whenever novel or modified cell therapy protocol is to be evaluated in the clinical setting.

(Simon-Philipp Hoerstrup)

Prenatal Progenitors For Autologous Cardiovascular Tissue Engineering

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[5]Department of Biomedical Engineering, Technical University Eindhoven, The Netherlands

A major limitation for the repair of congenital malformations is a lack of viable autologous replacement material with the capacity of growth and regeneration. Current surgical treatment of cardiac defects commonly requires non-autologous, non-living replacement materials that are associated with disadvantages including obstructive tissue ingrowths and calcification. This typically causes re-operations over the patient's lifetime. Alternatively, cardiovascular tissue engineering aims at the fabrication of autologous living replacements with the potential to grow, to repair and regenerate. Ideally, such a living replacement would already be available at or shortly after birth in order to prevent secondary damage of the immature heart. Thus, the tissue engineering process has to be initiated prior to birth as soon as the cardiovascular defect has been detected. Here, a novel concept providing prenatally tissue engineered human autologous heart valves based on routinely obtained fetal prenatal progenitor cells is presented.

Prenatal progenitor cells were isolated from extra-embryonically situated tissues including cord blood, umbilical cord tissue, placenta or amniotic fluid. After differentiation and characterization, cells were expanded in vitro into the cell types that are required for cardiovascular tissue engineering: myofibroblast-like cells and endothelial-like cells. Thereby, cell phenotypes were analyzed by immunohistochemistry and flowcytometry. For fabrication of cardiovascular tissues, biodegradable scaffolds (PGA/P4HB) were seeded with either mesenchymal-like cells derived from Wharton's Jelly, placenta or amniotic fluid. For tissue maturation constructs were implanted in a pulse duplicator system and subsequently coated with endothelial progenitor cells derived from cord blood or amniotic fluid. Resulting engineered heart valve tissues demonstrated endothelialized layered tissue formation in histology and immunohistochemistry, respectively. Analysis of extracellular matrix elements and cell number revealed production of gycosaminoglycans and collagen (GAG 80%, HYP 5%) and cell numbers up to native tissue values.

In conclusions, the use of prenatal progenitor cells as single, prenatal cell source is a promising strategy enabling the fabrication of heart valves ready to use at birth for the early repair of congenital malformations.

Bone Marrow and Adipose Tissue-Derived Mesenchymal Stem Cells for Clinical Use

Trine Fink

The aim of these workpackages is to run develop isolation and expansion protocols for human bone marrow derived and adipose tissue-derived stem cells. We will compare the two types of stem cells for their ability to proliferate and differentiate; especially the stem cells ability to differentiate into endothelial cells. In particular, methods how to isolate and expand adipose stem cells under cGMP conditions will be developed. The isolated stem cells will be grown in different hypoxic environments to test how this affects the proliferation and differentiation. The project will provide new information about the use of adipose tissue-derived stem cells in stem cell treatment, the differences between bone marrow derived stem cells and adipose tissue-derived stem cells in proliferation rate, differentiation potential and new techniques of preconditioning stem cells before their use i.e. in the treatment of acute myocardial infarction or in tissue engineering. Aditionally, the bone marrow and adipose-derived stem cells will be compared in a rat-model of myocardial infarction, to determine which cell type has the greater regenerative potential.

Research plan

WP 1: Generation of therapeutically suitable stem cells

The first part of the project will be focused on the development of isolation and culture protocols, in order to obtain stem cells from adipose tissue, which are suitable for autologous treatment. At the Laboratory for Stem Cell Research, we have refined the previously described procedure [21] with respect to enzymatic dissociation and *in vitro* culture. The stem cells in the adipose tissue will be released by digestion with collagenase. The resulting cell population will be further enriched by selecting for plastic adherence, and the cell culture media will be supplemented with autologous serum. The procedure will be carried out using materials conforming to cGMP rules and the isolated cells will be characterized by flow cytometry. The cells will be divided into groups that each will be continuously passaged at various oxygen tensions ranging from 21% (ambient) to 1% (hypoxic). To select for the oxygen tension that best preserves the characteristics of the stem cells by each passaging, the cell proliferation will be monitored by a colony-forming and BrdU-labeling proliferation assays, and the phenotypic profiling will be done for surface markers and gene transcriptional activity using Q-RT-PCR and flow cytometry, respectively.

WP 2: Assessment of the angiogenic properties of ASCs

The stem cells isolated and characterized in WP1 will be assessed for angiogenic potential. First, the capacity to firm endothelial structures will be determined in an *in vitro* tube assay. The cells will be seeded sparsely onto matrigel and supplemented with basic fibroblast growth factor and vascular endothelial growth factor. The differentiation into endothelium will be followed microscopically for up to five days, after which the cells will be dispersed with dispase, seeded onto coverslips and stained for von Willebrand factor immunocytochemically.

Secondly, as vascular endothelial growth factor is responsive to hypoxia, the above mentioned endothelial tube formation assay will be carried out at reduced oxygen tension with and without the supplementation of growth factors, to determine how the stem cells respond to growth conditions more mimicking those found in the damaged myocardium. In addition, the secretion of VEGF into the media at different oxygen tensions will be determined. Finally, conditioned media of hypoxia-treated cells will be used to induce endothelial tube formation. The knowledge obtained from this workpackage will help ascertain whether the beneficial effects of stem cells in the treatment of tissue ischemia is a result of *de novo* angiogenesis or a secretion of angiogenic growth factors leading to recruitment of resident endothelial precursors.

WP 3: Testing of cells in an animal model of myocardial infarction

Acute myocardial infarction will be induced in rats by permanent ligation of the left coronary artery and a number of rats will be sham operated. All rats will be treated with cyclosporine A (which enables transplantation of human adipose tissue-derived stem cells to the rats without rejection). After 1 week the rats will be re-operated and hypoxic preconditioned adipose tissue-derived stem cells injected intramyocardially. The functional effect will be evaluated by echocardiography. Blood samples measuring BNP will be drawn before adipose tissue-derived stem cells treatment and 4 weeks after the treatment.

In addition, as rat cells are markedly less sensitive to diphtheria toxin than human cells, the human adipose tissue-derived stem cells can be removed from the rat myocardium in by injection of diphtheria toxin, thus removing any direct functional effect in the rat myocardium visualized by echocardiography 1 week after last toxin injection. This will enable us to determine if any positive effects on the cardiac function is directly mediated by the stem cells, or are a result of paracrine effects.

In conclusion, our ambition is to compare and enhance the regenerative potential of adipose tissue-derived stem cells and bone- marrow derived stem cells for the treatment of damaged myocardium following an infarct.

Ethical considerations

The principal investigator has obtained permission from the regional ethical committee to use adipose tissue from liposuction procedures. The use of adipose tissue-derived stem cells received acclaim by a member of the ethical council Peter Øhrstrøm. During the animal experiments, all animals will receive care in compliance with the European Convention on Animal Care (Journal of the European Community L 358/1, November, 24th 1986). The animal experiments are necessary prior to translation of the results into a clinical setting, however, the number of animals will be kept to a minimum.

Animal models for orthobiologie applications of stem cells, including state of the art imaging procedures.

Martijn van Griesven, Ludwig Boltzman Institute Vienna, Austria

Animal models for tissue engineering purposes – emphasis on amnion

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It is important to test tissue engineering approaches before they are transferred to the clinical arena. This testing is first performed in vitro. However, in vivo studies are necessary and demanded by federal institutions. The studies have to be performed according to animal welfare guidelines causing as least suffering as possible for the experimental animals involved.

Depending on the subject area of interest, different models have to be applied. Concerning whole amnion application, skin models are appropriate. Full thickness wounds are made in the dorsal skin of pigs resembling deep wounds or excised wounds after burning. Amnion can be applied for this application to enhance skin regeneration. However, not only complete amnion can be used. It is also possible to isolate stem cells from amnion. Two different populations can be isolated: epithelial and mesenchymal cells. These stem cells can be used in an array of different applications.

A possible application is bone regeneration. For this purpose, several models are available. First, a simple drill hole model in the femur of rats is applicable for screening purposes. Having assessed the best concentration etc. the cells can be applied in an non-union femur defect model in the rat. In this model, a segment of the femur is removed and replaced by a silicon spacer for 4 weeks. After this period, the femur doesn't heal anymore. The stem cells are administered and first results are promising. In the last phase, a sheep segmental defect model in the tibia can be used. An intramedullary nail is inserted and locked in the tibia. A 2.5 cm segment is excised causing a non-union defect. The defect can be filled with substances and healing can be assessed by µCT and histology.

The faith of the stem cells can be traced by a Xenogen camera. For this technique the cells are labelled with the luciferase gene. Upon injection of luciferin, photons are emitted. These photons can be visualized and measured by the Xenogen device.

It is important to choose the optimal animal model for the hypothesis tested. It is also important to use sophisticated methods to obtain good quality data. Furthermore, one should have experience in applying the animal models to obtain consistent and reliable data.

About HLA and KIRs

Melanny Hidajat, AZ St. Jan, Hematology, Brugge, Belgium

KIRs (Killer cell Immunoglobulin-like Receptors) are expressed on NK (Nature Killer) cells and subset of effector/memory $CD8^+$ T cells. The KIRs family consists of activating and inhibitory receptors that control the function of NK cells. KIRs recognise groups of HLA (Human Leukocyte Antigen) class I molecules as their ligands. NK activity is partially controlled through the interaction between KIRs and their HLA ligands. The NK cells are regulated by a net-balance of activating or inhibitory signals as to whether a target cell is to be killed or not. The function of these NK cells is negatively regulated by inhibitory KIRs for HLA class I molecule on the target cells. Cells lacking specific inhibitory KIR ligands in the presence of additional activating signals are exposed to NK cell action. Even in the presence of an activating ligand, when inhibitory ligands are also expressed on target cells, the inhibitory signals may deliver overriding signals that culminate in a net suppression of NK cell function.

NK cells play an important role in host-response to infectious pathogens and tumor cells. NK cells first recognize and kill tumour or virally infected cells without prestimulation.
The expression of KIR on NK cells implies that NK cell-deficient individuals are susceptible to herpes virus infections. NK cells are a vital component of the immune response that constantly protects an individual from life-threatening infections. A number of autoimmune disorders have been also associated with specific KIR genes.

Several studies report that KIRs may affect the outcome of Haematopoietic Stem Cell Transplantations. Allogeneic NK cells can mediate anti-leukemic effects against Acute Myeloid Leukemia after allogeneic haploidentical Stem Cells Transplantation when KIR/KIR ligand incompatibility exist in the Graft versus Host direction. In this case an HLA class I KIR ligand is absent in the recipient. Failure to recognize the appropriate KIR ligand on a mismatched cell can trigger NK elimination of the target cell. In this setting, donor NK cells can expand and kill the target cells, by which the risk for disease relapse, graft failure, and Graft versus Host disease will be reduced substantially.

Abstract: Clinical aspects of GVHD.

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Haematopoietic stem cell transplantation has become standard treatment in several haematologic malignancies. Despite major improvements during the last decades, clinically significant graft-versus-host disease (GVHD) still occurs in about 10 to 50 percent of patients who undergo allogeneic stem cell transplantation (aSCT).

A short overview of the current insights in the development of GVHD will be given. The core of this talk will focus on the clinical manifestations and diagnosis of this debilitating and often life-threatening complication after aSCT.

The risk factors, the different settings of myelo- or non-myeloablative conditioning, recommendations for prevention and treatment of this disorder will be discussed.

Especially steroid refractory acute GVHD is associated with high rates of morbidity and mortality; current treatment and emerging possibilities will be discussed. New drugs have been tested in the last few years; mesenchymal stem cells (MSCs) have an immunomodulatory function and are also evaluated as a possible therapy.

Umbilical cord blood transplantation – a single centre experience.

Pimentel Pedro, **Fernando Campilho**

Cord blood transplantation: a single center experience

Fernando Campilho on behalf of BMT Unit staff (Director: Pedro Pimentel; F. Campilho, A. Campos, C.P. Vaz, Rosa Branca)) and Immuno-Hemotherapy Department (Director: Alzira Carvalhais; Susana Roncon)
Serviço de Transplantação de Medula Óssea, Instituto Português de Oncologia do Porto, Portugal
Porto, September 2007

Since the first cord blood (CB) transplantation performed in 1988 in a 6 years old child with Fanconi anemia by the group of Eliane Gluckman this modality of treatment has been increasingly used. The use of CB as a source of hematopoietic progenitors in allogeneic stem cell (SC) transplantations has several potential advantages: 1) they are almost immediately available; 2) for the same degree of HLA disparity there is a decreased incidence and severity of graft versus host disease (GVHD) which permits the use of donors with more HLA disparities; 3) there is a lower viral contamination of SC. However several disadvantages have emerged in comparison with bone marrow or peripheral blood transplants: 1) graft failure is more frequent; 2) hematological and immunological recovery is slower; 3) further infusions of graft or donor lymphocytes infusions, if needed, are not possible; 4) graft loss is possible if any problem occurs with the bag or the storage process.

Currently at our center CB transplants represent about 40% of allogeneic SC transplants performed in younger patients. Between October 1996 and August 2007 we performed 30 transplants in 16 males, 14 females, with a median age 6 (range: 1 – 42) years old. In 27 of these patients the donor was unrelated, all with HLA mismatches; in 1 of this the patient was transplanted with CB of 2 donors. In the 3 remaining patients the donor was an HLA matched sibling; one of this the graft was the product of the CB of 2 identical twins, that due the small number of nucleated cells was complemented with bone marrow harvested of 1 of the twins (the transplant from this patient is not included in this analysis). Patients diagnosis: acute myeloid 19 (lymphoid: 13, myeloid: 5; biphenotypic: 1); myelodysplastic syndrome: 3; immunodeficiency: 2; other diagnosis: 5. Graft characteristics are shown in the table. The median viability was 90% (range: 50 – 100%).

	Nucleated cells*	***CD34+ cells*****	***Nucleated cells****	***CD34+ cells*****
	preserved		*infused*	
median	4.8	2.2	3.3	1.1
range	1.1 – 15	0.4 – 8.5	1.2 – 9.8	0.1 – 5.2

**) x 10^7/kg; **) x 10^5/kg*

Conditioning regimen was myeloablative in 26 (busulphan, cyclophosphamide plus antilymphocytic serum in unrelated transplants) and non-myeloablative in 3 patients. A calcineurin inhibitor (tacrolimus or cyclosporine) was used in all patients, associated to mycophenolate mofetil in unrelated transplants.

Graft failure occurred in 6 patients (20%), with 2 deaths, 1 spontaneous autologous hematopoietic reconstitution and 2 rescued after infusion of autologous stem cell progenitor backup. One patient was rescued after transplant from an alternative donor. In our patients we didn't find a significant difference related to the nucleated or CD34+ cells collected or infused in patients with graft failure.

GVHD grade 2 occurred in 12 patients (41%), 1 with evolution to chronic extensive GVHD. One patient had grade 3-4 GVHD, with evolution to extensive chronic GVHD and death at day 156 of invasive aspergillosis.

With median follow-up of patients alive of 1.7 years (range: 2 months – 8.4 years) the overall survival at 5 years is 40 ($\pm$12)%. There was a trend of better survival if infused or collected nucleated or CD34+ cells was greater than the median.

The transplant related mortality to the entire population was 22 ($\pm$8)%, with a lower mortality if the infused nucleated cells was higher than the median (8% versus 38%, p=0.04). The median time to neutrophils $0.5x10^9$/L and platelets $20x10^9$/l was 30 and 37 days respectively.

The use of CB is an alternative source of SC for allogenic transplant that is almost immediately available and permits the use of donors with some degree of HLA mismatch, allowing to enlarge the pool of potential donors to more patients. Graft failure is one serious problem after CB transplants and several ways to diminish this event are being studied (use of multiple CB units, in vitro expansion, co infusion of mesenchimal or regulator T cells, intra osseous infusions). Other important caveat of these transplants is, if needed, the impossibilities of using further graft or donor lymphocytes infusions. A better definition of graft quality with widely accepted studies and composition of the graft is also needed. The issue of the use of public or private CB banking deserves further analyses in order to better define the role of each.

Finally we report a HLA sibling transplant in a 2 years old patient with SCID (CD8 deficiency) where the graft source was a CB unit of his brother, previously collected, intended for autologous use and criopreserved in a private bank. Seven months after transplant the child is well with good immunological recovery.

Autologous cord blood infusion as immunotherapy for type 1 diabetes. Michael Haller

Changes in Regulatory T Cells Following Autologous Umbilical Cord Blood Transfusion in Children with Type 1 Diabetes

H Viener, T Brusko, C Wasserfall, K McGrail, S Staba, C Cogle,
D Schatz, M Atkinson, **M Haller**

Type 1 diabetes (T1D) is an autoimmune disease characterized by failure of the immune system to maintain tolerance. Regulatory T cells (Treg) play a pivotal role in immune regulation as they can suppress the activity of effector T cells (Teff) reactive to both self and non-self antigens. Umbilical cord blood (UCB) has recently been shown to provide an excellent source of highly functional Treg. Previously, we observed that autologous UCB infusion led to prolonged honeymoon and preservation of C-peptide in a young child with T1D. We hypothesized that transfusion of autologous UCB in patients with T1D would impart tolerance demonstrated by improvements in metabolic control and increased frequency of peripheral blood Treg. We transfused autologous UCB in 7 additional children with T1D who had average: age at infusion - 5.29yr ± 1.8 (range 2.4-7.3); diabetes duration - 0.84yr ± 0.8 (0.19-2.6); HbA1c - 6.3% ± 0.7 (5.2-7.5); number of infused nucleated cells/kg - $1.9x10^{7}$ cells ($2.8x10^{6}$-$3.6x10^{7}$); and cell viability - 96% ± 0.02 (92-99%). Peripheral blood samples were obtained on all subjects before infusion, 3 mo, and 6 mo after infusion. FACS was used to identify cells based on markers for CD4, CD25, CD3, and FOXP3. The percentage of CD4+CD25+FOXP3+ (Treg) decreased from 7.21% ± 3.0 (3.53-11.66) at infusion to 6.57% ± 2.59 (2.19-9.73) at 3 mo (P=0.028) but then increased significantly by 6 mo to 9.0% ± 3.1 (5.64-14.3)(P=0.032). In addition, the percentage of CD25-FOXP3+ cells increased from 8.6% ± 4.55 (2.9-16.1) at baseline to 17.4% ± 8.2 (6.8-24.15) at 6 mo (P=0.014). The change in the percentage of FOXP3+ cells in peripheral blood at 6 mo demonstrated a significant correlation with the quantity of UCB cells infused/kg (r=0.44, P=0.031).The percentage CD25+, CD25+CD4+, and CD25+CD4+FOXP3- all decreased significantly from infusion to 6 mo (P=0.041, P=0.04, and P=0.02 respectively). The initial decline in peripheral Treg during the 3 mo following UCB infusion and the rapid increase in Treg seen between 3 and 6 mo post UCB infusion could be due to the temporary sequestration and eventual peripheral distribution of increased Treg provided by or induced by autologous UCB infusion. The observed changes in Treg demonstrate the potential of autologous UCB as immunomodulatory therapy for T1D. Further studies are needed to fully characterize the role of autologous UCB in the induction of tolerance and as a potential therapeutic modality for T1D.

Autologous cord blood infusion as immunotherapy for type 1 diabetes. Michael Haller

Insulin Requirements, HbA1c, and Stimulated C-peptide following Autologous Umbilical Cord Blood Transfusion in Children with T1D.

MJ Haller, HL Viener, T Brusko, C Wasserfall, K McGrail, C Cogle, S Staba, M Atkinson, **DA Schatz**

T1D is characterized by failed self-tolerance, autoimmune beta cell destruction, and in very young patients, a rapid decline in endogenous insulin production. Since umbilical cord blood (UCB) is an excellent source of stem cells and regulatory T cells, we hypothesized that transfusion of autologous UCB would lead to enhanced metabolic control over time as compared to children receiving standard intensive insulin. Previously, we observed that autologous UCB infusion led to prolonged honeymoon and preservation of C-peptide in a young child with T1D. We have now transfused 7 additional young T1D patients with average: age at infusion - 5.29yr ± 1.8 (range 2.4 - 7.3 yr); T1D duration - 0.84 ± 0.8yr (range 0.19 - 2.6); HbA1c - 6.3% ± 0.7 (range 5.2 - 7.5%); number of infused nucleated cells per kilogram - $1.9x10^{7}$ cells ($2.8x10^{6}$ - $3.6x10^{7}$); and cell viability - 96% (92-99%). A group of randomly selected intensively treated T1D subjects of similar age and duration of disease (n=13, age 4.5 ± 2.2 yr; duration 0.77 ± 0.6 yr) served as controls. Unpaired t-tests were used to compare HbA1c and total daily insulin use between the two groups from diagnosis to 6 months after infusion. Children who received UCB had lower average HbA1c [7.0% ± 1.77 (n=28 measurements) vs 8.04% ± 0.8 (n=108)] than children who received insulin therapy alone (p=0.0031). Children who received UCB also required lower average total daily insulin requirements [0.45 ± 0.23 (n=29) vs 0.69 ± 0.24 (n=129)] than children who received insulin therapy alone (p<0.0001). Mixed meal stimulated C-peptide levels were determined prior to infusion, 3 months after infusion, and 6 months after infusion in those receiving UCB infusion. Three months after infusion, the average maximal stimulated C-peptide values increased in children who received autologous UCB (1.8 ng/ml prior to infusion, 1.9 ng/ml 3 months after infusion) with a gradual decline to 1.39 ng/ml 6 months after infusion. Importantly, no treatment associated adverse events were observed. Collectively, these preliminary data demonstrating a lower HbA1c, lower average insulin requirements, and possible preservation of C-peptide suggest a beneficial effect of autologous UCB infusion in patients with T1D. While the size of this study requires cautious interpretation of these initial data, our observations justify the expansion of studies aimed at exploring the potential of UCB as therapy for children with T1D.

Cord Blood Banks Networks

J. Garcia

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After two decades of clinical experience, there is general acceptance that cord blood (CB) transplants, related and unrelated, are equivalent or might compare favourably, especially in children, with bone marrow (BM) transplants

As a consequence, there is an overall increase in annual CB transplants, clinical results continue to improve and, in some highly experienced transplant centers, CB is equally indicated or even given priority over other sources of unrelated hemopoietic progenitor cells.

A significant contribution to this improvement may be objectively attributed to the cord blood banks activity. The mission of CB banks CBB is to obtain, store and supply safe and high quality CB units, to promote the use of CB as an allogeneic source for transplants, and enhance their therapeutic possibilities by promoting research on CB biology.

Efficient CBB must have a well organized data base and a communication system which enable them to provide suitable units in the shortest possible time. Due to the growing numbers of CBB, the search for efficiency has driven them, more or less simultaneously, to constitute or adapt, consolidated data bases and access systems, and to develop a number of registries or networks to support progressively improved the access to inventories.

Cord blood bank networks are constituted at different levels:

National Networks

Australia

AusCord is one of the first integrated CBB program. The CBB of the Australian program are situated in the Sydney Children's Hospital, the Royal Children's Hospital, Melbourne and in the Mater Misericordiae Hospital in Brisbane .AusCord is currently cooperating and sharing inventories with the USA National Marrow Donor Program (NMDP), Bone Marrow Donors World Wide (BMDW) and Netcord (see below). This network has adopted the Netcord/FACT standards.

Belgium

Belgium has a very active per capita based CBB program. Housed in four locations: Brussels (2 locations), Gant and Liege. Leuvencord is an independent program with an inventory. The Belgium program operates with their National Marrow Donor Registry which cooperates with BMDW. The Belgium CBB are members and integrated in Netcord. The Liege program has achieved the NETCORD/FACT accreditation.

France

France has developed a very efficient program: *France Cord*, started in 1999 in Paris (currently not operative), with Besançon, Bordeaux and Annemasse serving as storage centers. From the beginning this program has collected and stored CB units with a high content of cells and progenitors, this being one of the reasons for its high transplant / stored units rate and for the high proportion of units delivered for adult transplants. The entire French unrelated donors activity has been integrated in the recently created "Agence de la Biomedicine". *France Cord* program has adopted the FACT/Netcord standards, and to date, Besançon has maintained this quality certification.

Italy

The Milan CBB has pioneered the concept of cord blood inventory networking under a single quality assurance system (ISO 9000). GRACE (Group for the collection and expansion of hemopoietic cells) was created in 1996 under the Milan CBB leadership, including a number of CBB in Milan, Bologna, Florence, Turin, Padova, Rome (two).

The Milan CBB has pioneered the concept of cord blood inventory networking under a single quality assurance system (ISO 9000). Furthermore Milan, in collaboration with Duesseldorf and Barcelona CBB has generated the embryo of what later developed into the Netcord Organization. The entire GRACE program has adopted the FACT/Netcord Standards, and, to date, Milan (the first) has achieved certification. This program runs in collaboration with the Italian Marrow Donors program and BMDW and, as pointed out above, is a very active member of Netcord. More recently, efforts have been developed at the national level to integrate the 16 Italian active public CBBs into a single network, and to develop a single hemopoietic stem cell donor search access point for clinicians.

Spain

Spain is a very active in CB banking. There have been up to eight active CBB: Barcelona (two), Madrid (two). Malaga, Santiago, Tenerife and Valencia. During the last several years, the Barcelona and Madrid programs have consolidated. These CBB operate through The Spanish Marrow Donors registry (REDMO) supported by the José Carreras Foundation and through NETCORD. Barcelona (Netcord founding member), All Spanish programs have adopted the FACT/Netcord standards. The Barcelona program is already accredited and Santiago and Malaga CBB are to be inspected in the near future. The Spanish CBB are currently being organized under a single national program. Due to the particularities of the National health system the current trend is to organize regional donation programs around established CBB. The Barcelona CBB is pioneering this initiative, developing collaborations with neighboring autonomous communities such as Balearic Islands and Aragon, Navarra and Extremadura, which have developed their own CB programs.

United States

The USA National Marrow Donor Program (NMDP), the largest bone marrow donor registry in the world, has developed a growing network of eighteen USA and non-USA CBB (San Diego CA, Denver CO, Durham CN, Orange CA, Altamonte Springs FL, Glenview IL, Detroit MI, Gainesville FL, Houston TX, Camden NJ, Seattle WA, Tel-Hashomer (Israel), St. Louis MO, Arcadia CA, LinKou (Taiwan), San Antonio TX, Orlando FL and Allendale NJ). This network operates in conjunction with the marrow donor search system and is accredited according to its own quality standards. Very recently, NMDP and Netcord have established cooperative contracts for sharing searches and inventories (see below), in fact CBB in Arcadia, Durham and Houston have already obtained the FACT/NETCORD certification.

INTERNATIONAL NETWORKS

Netcord

NETCORD Foundation, was established in 1998 with the aim of promoting high quality banking and clinical use of umbilical cord blood for allogeneic stem cell transplantation. It has issued statutes and guidelines in order to perform studies and research on the collection, processing, characterization, preservation, and *ex-vivo* expansion of placental blood with the primary aim of improving the quality of such components for hematopoietic transplants at an international level. NETCORD has established specific quality standards in collaboration with the Foundation for the Accreditation of Cellular Therapy (FACT).

NETCORD has established an on-line search and allocation program, the Virtual Office (VO). This program practically allows for real time searches and provides a unified report of compatible and available units from an inventory of more than 150000 available cord blood units from the worldwide active CBB.

In 2006 NETCORD and NMDP developed an agreement for sharing search requests, inventories and quality standards (NETCORD/FACT) as well as developing a common reporting system.

Asia Cord

The adoption of CB transplant technology has been particularly rapid in Asia because of smaller family size, which makes it difficult to find matched bone marrow donors for patients requiring hemopoietic transplantation.

The members of AsiaCORD represent the leading cord banks in Asia, including Bangkok, Beijing, Ho Chi Minh City, Seoul, Taipei, Tianjin and Tokyo. AsiaCORD has established statutes and standards for cord blood banking that are based on the FACT/NETCORD International Standards.

Bone Marrow Donors Worldwide (BMDW)

The BMDW is a voluntary collaborative effort of stem cell donor registries and cord blood banks with the goal of providing centralized information on the HLA phenotypes and other relevant data of unrelated stem cell donors and cord blood units, and making this information easily accessible to the physicians of patients in need of a hemopoietic stem cell transplant. Its mission is essentially informative, and it is achieved by receiving information from all the national and international registries.

Other Organizations

There are a number of additional organizations or registries involved in the search of cord blood units for unrelated transplants. Among them, Europdonor Foundation (the pioneer on registering volunteer adult donors)' The Anthony Nolan Trust' and the Caitlin Raymond Registry are the more active.

International hemopoietic transplant registries

The follow-up of patient outcomes is a precious source of information which promotes the continuous improvement of CBB activity. The vast majority of CBB and programs cooperate with transplant registries to obtain and analyze clinical information.

EUROCORD

EUROCORD is an international registry of the European Blood and Marrow Transplant group (EBMT), that includes European and non European centers, that perform either related or unrelated cord blood transplants.

In addition, EUROCORD collaborates with CBB by sending a statistical analysis of the cord blood transplants performed by their respective units to every associated CBB, annually or when requested.

The International Bone Marrow Registry (IBMTR)

The IBMTR has recently been incorporated in a consolidated organization that includes The Center for International Blood and Marrow Transplant Research (CIBMTR) including the National Marrow Donor Program, the International Bone Marrow Transplant Registry and Autologous Blood and Marrow Transplant Registry.

Its current mission is to collect patient data on allogeneic blood and bone marrow transplants worldwide, and patient data on autologous blood and marrow transplants performed in North and South America as well as to design, conduct and support clinical studies that involve large numbers of patients from multiple transplant centers.

GMP requirements for market Authorization - principles of Consideration for clinical trials.

Dr. Peter De Waele, MSc. PhD., Clinical & Regulatory Affairs, Cryo-Save AG

When reviewing the scientific literature (PubMed), the website of the US FDA covering clinical trial information (www.ClinicalTrials.gov) as well as company websites (Osiris, …), one can clearly conclude that stem cells have attracted a great deal of attention for clinical research.

Analysing the data from these individual clinical trials (phase I and II) and case studies, one observes a great variety in the clinical setting. The variety is not only illustrated by the different clinical indications (GvHD, MS, diabetes, ischemic stroke, osteogenesis imperfecta, …), but even more so differences in origin of the cells used (autologous versus allogeneic), cell source (bone marrow, (mobilised) peripheral blood, adipose tissue, umbilical cord blood), method to isolate the cells (enzymes (collagenase, trypsin, …)), manipulation of the cells (expanded, sorted, selected, depleted) and materials (monoclonal Ab's) and equipment (magnetic beads, …) used, composition of media used for cell culture (containing phenol red, DMEM, -MEM, … (bovine) serum or serum free, buffer (Hepes, …), additives used (activin, LIF, BMPs, …), other culture conditions (pH, pO2, pCO2, temperature (37°C), …), quality of plastics used fo r culturing (collagen coated, …), method of culturing (stationary phase, perfusion, fed batch, stirred tank, method of agitation in stirred tank, shear forces (stress) applied, ….), seeding densities, phase of harvesting of cells (log-phase, sub-confluent, confluent, …), number of passages, cryopreserved and methods and solutions (DMSO, …) used for cryopreservation or fresh, … .

In addition to these differences, on top of the enormous individual variability of the donor and acceptor in allogeneic conditions or differences between individuals in autologous cases for one indication, one notices great differences in the human application as cell numbers injected is concerned (cells per kg, ...), regimes used for injection (bolus, short time infusion, infusion, …) sit of injection (iv, ip, intra-tissue), frequency of treatment (once, repeated injections), time between different treatments, … !

From a regulatory perspective this is a real nightmare, which obviously indicates clear need for tighter regulation, with the intention to increase the standardization to improve the safety and the quality of the materials used, the design of the trials with the sole objective to have better guarantee for safety and quality for patients involved, reducing the risk and increasing the quality and hence potential efficacy, also further increasing the comparability and analysis and further optimization and improvement of the results. Some organizations such as the International Society for Cellular Therapy (ISCT) started already by proposing e.g. minimal criteria for defining mesenchymal stem cells. In Europe, with the Advanced Therapy medicinal Products Regulation 1394/2007 (from November 13th, 2007) finally coming in place, cellular therapy, including clinical trials, will be controlled more tightly.

They will legally need to comply with the Eudralex, the rules governing the medicinal products in the EU. During the presentation, the Eudralex chapters applicable on investigational medicinal products (used in clinical trials) are indicated, starting with the legislation (directives) and guidelines which are involved. Special attention to Good Manufacturing Practices (GMP; Volume 4) for investigational medicinal products (Annex 13) is given in the presentation, covering personnel involved, premises, equipment, starting materials. Not only patients but all stakeholders involved (clinical investigators, manufacturers, …) will no doubt benefit from a tighter control of clinical trials, hence will significantly contribute to the efficacy of stem cell therapy for several indications, now and in the nearby future.