Nuovi orientamenti nel “cord blood banking”

Laura Salvaneschi
Banca del Sangue del Cordone Ombelicale
Università degli Studi di Pavia
IRCCS Policlinico San Matteo Pavia
Il network internazionale delle Banche di CSE da SCO

139 Banche/reti di Banche SCO

106 Banche agiscono attraverso i Registri
11 Banche indipendenti
Unità SCO in ciascun continente

Totale 537.276
New directions in Cord Blood Banking

• Cord blood (CB) transplantation is successfully used to treat malignant and non-malignant disorders

• The national and international inventories need to be expanded in order to provide CB units with high cellular dose and an increased chance of matching for ethnic minorities
Current research is focused on

– increasing the limited number of CB stem cells, as a low cell dose may cause:
  • granulocyte and platelet recovery delay (increased infection risk)
  • graft failure
  • disease relapse

– finding new therapeutic uses of CB stem cells
The Network of CB Banks

Quality control shall be planned and regularly performed to ensure the quality of products for clinical use.

Written policies and procedures (SOPs) covering all relevant aspects of CB Banking (from collection to transplantation) in compliance with international and national standards guidelines and laws shall be in place to achieve FACT-Netcord accreditation (after on-site inspection).
CELL DOSE = number of total nucleated cells

OK

pediatric patient

1. Double CB transplantation (Minneapolis)
2. Single CB transplantation with reduced intensity conditioning (Minneapolis)
3. ex vivo cellular expansion (Huston)
4. Intra-bone CB transplantation (Genova)

adult patient
Immunological privileges

Reduced risk for immunomediatted complications (naïveté of CB immune system)

- transplantation is possible between non relatives
- transplantation is possible between mismatched donor and recipient (up to 2 mismatches)
Advantages of Cord Blood

- LOW incidence and severity of graft versus host disease (GvHD)
- LOW risk of viral contamination (CMV, EBV)
- AVAILABILITY for unrelated patients (reduced search times)
Compared to the register of bone marrow donors, the CB inventory has a low number of units, but a greater genetic variability (partially due to the higher number of donors from non-Caucasian ethnic groups)
• **EASY TO COLLECT**

• **SAFE FOR THE DONOR**
How to overcome the disadvantages of CB transplantation

Disadvantages:
• limited number of cells obtained from a single CB collection
• slower time for neutrophil and platelet engraftment

Improvement in CB transplantation:
• collecting more haematopoietic stem and progenitor cells from placental and CB
• ex vivo expansion of haematopoietic stem and progenitor cells
• enhancing homing and engrafting of CB stem cells

Broxmeyer HE, 2011
Collecting more cells from placental and CB

• Collect as much blood as possible from the umbilical cord and placental blood vessels
• Perform the perfusion of the placenta after collecting the greatest volume of blood from the umbilical cord
• The placenta is also rich of progenitor cells, and represents a new source of stem cells

**Critical:**
– The perfusion of the placenta may cause the collection of maternal cells. This contamination must be carefully monitored as an excess maternal cell has the potential to elicit higher levels of graft-versus-host disease
Perfusion of the placenta

- Using solutions able to mobilize stem/progenitor cells (AMD3100 is used to enhance mobilization of stem cells from adult peripheral blood)

**Critical:**
- Need to check and set the method
- Compare the cell yield obtained by perfusion with the yield obtained by traditional collections to demonstrate that the mobilizing agent really increases the cell number of collection
- Because of great variability in numbers of cells collected between CB units from different donors, we must use the same placenta and cord as internal control to show that after perfusion the placenta has exhausted further release of cells
Ex vivo expansion of haematopoietic stem cells

- The ex vivo expansion of long-term engrafting human haematopoietic stem cells has not been yet adequately demonstrated.
- Research efforts are focused on enhancing the environment in vitro to improve survival, proliferation and self-renewal of the stem cells, including use of potent combinations of cytokines and growth factors.
- Identifying receptors and intracellular signals that are selectively involved in enhancing hematopoietic stem cell expansion will allow us to bypass the need for stromal cell interactions of the niche and specific cytokines.
Pharmacological-induced expansion

- In mice, the over-expression of Rheb2 enhances the haematopoietic progenitor cell growth in vitro and a transient short-term repopulation in vivo.

- In mammalian cells, Rheb1 and Rheb2 are able to activate the signal of the target of rapamycin (mTOR)

- The means to modulate the signalling pathway that incorporates rapamycin-sensitive mTOR pathway may, in the presence of cytokines, enhance functional capacity of and/or expand, human haematopoietic stem cells in vitro.
Enhancing homing and engraftment capabilities of haematopoietic stem cells

Ligand–receptor interaction is strongly implicated in homing and engraftment of haematopoietic stem and progenitor cells:

stromal cell-derived factor-1 (SDF-1 /CXCL12) acts as a chemotactic (directed cell migration) agent, but also enhances survival of early haematopoietic cells
Other uses of cord blood

There are numbers of papers reporting presence and/or generation of non-haematopoietic cell types from cord blood for **regenerative medicine**.

Included in such reports are references to mesenchymal stem/stromal cells, endothelial progenitor cells and induced pluripotent stem cells. Such laboratory and pre-clinical animal studies are of interest, and can provide us with insights into biological processes.

However, there is little or no rigorous evidence yet that such studies can or will translate into relevant clinical treatments. These clinical studies will need to be well controlled before any conclusions regarding clinical efficacy are put forth for non-haematopoietic uses of cord blood.
Hot topics in cord blood banking

- Provide high quality certified products for clinical use
- Provide units with high cellular dose for clinical use
- Give a prompt genomic analysis of each CB unit typing HLA-A, -B, -C, and -DRB1 at high resolution
- Investigate the HLA-G immunosuppressive potential on transplantation outcomes
- Study the NIMA and NImMA immunotolerant effects on transplantation outcomes
Immunosuppressive effect of HLA non classical class I molecules in Cord Blood

HLA-G
Pregnancy is an immunological balancing act in which the mother's immune system has to remain tolerant of paternal antigens and yet maintain normal immune competence for defense against microorganisms.
Changes at the feto-maternal interface

• Uterine decidual and placental cells produce a huge array of cytokines which, in part, contribute to the deviation of the immune response from Th1 to Th2

• The trophoblast expresses Fas ligand conferring immune privilege: maternal immune cells expressing Fas will undergo apoptosis at the placenta/decidua interface

• During pregnancy there is a low peripheral NK activity, and early pregnancy the majority of uterine lymphocytes are CD56(bright) granulated NK cells, which do not express CD16 or CD3
Fetal protection against maternal rejection

- Trophoblast cells fail to express HLA class I or class II molecules to prevent normal T cell responses.
- Cells lacking HLA molecules undergo cytolysis by Natural Killer cells, thus the trophoblast cells strongly express the non classic class I HLA-G, -E, -F molecules, which may downregulate Natural Killer cell function.
HLA-G functions against maternal rejection

- inhibits the cytolytic function of uterine and peripheral blood NK cells

- stimulate the production of cytokines and angiogenic growth factors that allow implantation and placental vascularization
HLA-G differs from classical class I molecules

- lower polymorphism
- restricted tissue distribution (trophoblast, cornea, timus)
- particular expression pattern as seven protein isoforms generated from alternative splicing of a single mRNA transcript (transmembrane isoforms: G1, G2, G3, G4; soluble forms: G5, G6, G7)
- Further soluble molecule, namely G1 shed, that is proteolitically clived from the G1 transmambrane isoform
HLA-G inhibitory functions

HLA-G binds to:

- **KIR2DL4** inhibiting cytotoxic function of Natural Killer cells, stimulating the production of pro-angiogenic factors after internalization

- **ILT2** inhibiting the proliferation of natural killer cells, CD8+ and CD4+ lymphocytes, inhibiting the alloreactivity of CD4+ lymphocytes

- **ILT4** inhibiting the alloreactivity of CD4+ lymphocytes and growth of dendritic cells (DC)

- **CD8** stimulating the apoptosis of natural killer cells and CD8+ lymphocytes

- **CD160** stimulating the apoptosis of endotelial cells
HLA-G enhances tolerance in transplantation setting

- Soluble HLA-G levels in sera and bioptic tissues from heart transplanted patients correlate with lower incidence of rejection.

- High soluble HLA-G levels correlate with lower incidence of rejection in kidney and kidney/liver transplanted patients.

- Pre-transplantation high soluble HLA-G levels correlate with lower incidence of acute GvHD after hematopoietic stem cell transplantation.
HLA-G and xeno-transplantation

- Low genomic polymorphism
- Soluble proteins isoforms
- Immunosuppressive function as ligand for inhibitory receptors of T cells, Natural Killer cells, and APC

HLA-G tollerogenic potential in xeno-transplantation
Current research

- Measurement (immunoassay) of soluble HLA-G molecules in CB plasma before and after cryopreservation, to verify if these molecules are stable also after a long time storage.

- Analysis of the HLA-G 14 bp insertion/deletion polymorphism (rs16375, 3’UTR), affecting mRNA stability, to verify a possible correlation between soluble HLA-G expression and genotype in CB donors.
Best Poster Session Award
EFI Congress 2010
Impact of fetal-maternal tolerance in hematopoietic stem cell transplantation

Takanori Teshima\textsuperscript{1}, Ken-ichi Matsuoka\textsuperscript{2} and Tatsuo Ichinohe\textsuperscript{3}

\textsuperscript{1} Center for Cellular and Molecular Medicine, Kyushu University Hospital, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
\textsuperscript{2} Biopathological Science, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
\textsuperscript{3} Department of Hematology/Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

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Non inherited maternal antigens
NIMA
Haploidentical siblings may provide an alternative transplant option when HLA-matched related donors are not available, the disadvantages being essentially the increased risk for GvHD and graft rejection.
ASCT from an inherited paternal HLA antigen (IPA)/non inherited maternal antigen (NIMA) – mismatched donor has been previously demonstrated to be feasible, decreasing GvHD and preserving graft-versus-leukaemia (GvL) effect.

*Exposure to IPA during pregnancy/delivery

van Rood et al., 2002
Maternal cells passing the placenta during pregnancy express HLA molecules which are acknowledged by the foetus, who develops a tolerance to NIMAs maintained in adulthood.

The maternal haplotypes are easily available for cord blood (CB) units while this information is not provided for adult donors.
As NIMAs should be tolerated by the CB immune system, the knowledge of the third donor’s NIMA haplotype could further augment the chance for finding a compatible CB donor and reduce immune complications of ASCT.
Current research on NIMA
Pavia Cord Blood Bank

EUROCORD multicenter study:
“IMMUNOGENETIC DETERMINANTS OF OUTCOMES OF UNRELATED CORD BLOOD TRANSPLANTATION: KIR AND NIMA EFFECT”

EUROCORD records and updates data regarding patients transplanted with CB stem cells
EUROCORD sets procedures and guidelines to improve outcomes after CB transplantation

manuscript submitted
Non inherited maternal MINOR antigens NIMmA
Minor histocompatibility antigens (mHAg)

- minor histocompatibility antigens (mHAg) are polymorphic peptides derived from the proteolitic cleavage of intracellular proteins encoded by genes mapping on autosomes and Y chromosome.

- in transplantation setting, all intracellular peptides may be considered virtually mHAg if these peptides are able to bind HLA class I and II molecules and be recognized by alloreactive T cells.

- as mHAg are expressed by genes mapping on chromosomes following the mendelian segregation, HLA-identical brothers may have a different mHAg repertoire. This genetic diversity, due to the natural genetic variability, influences the production of polymorphic mHAg.
Besides major histocompatibility complex antigens (HLA), minor histocompatibility antigens (mHAGs) may be considered to play a role in transplantation setting.

In HLA identical sibling transplantation, mHAGs mismatches can lead to T-cell activation with specific alloreactivity against recipient’s or donor’s antigens, thus supporting the GvHD or rejection response, respectively.

Naturally acquired tolerance and sensitization to minor histocompatibility antigens in healthy family members

Blood 2009;114(11):2263-72

Cord blood comprises antigen-experienced T cells specific for maternal minor histocompatibility antigen HA-1

Current research on NImMA
Pavia Cord Blood Bank

- Evaluate the NImMA effect in CB sibling transplantation considering the post transplantation complications, such as acute and chronic GvHD, relapse and total-related mortality in patients affected by non malignant diseases

- Analyze the influence of NImmA on anti-tumoral GvT effect in malignant patients transplanted the CB of their siblings
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Banca del Sangue del Cordone Ombelicale
Università degli Studi di Pavia
IRCCS Policlinico San Matteo Pavia