Expansion of committed progenitors with maintenance of hematopoietic stem cells at low oxygen concentration

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بسم اللة الرحمن الرحيم

"ءان اللة لا يضيع أجر من احسن عملا"

صدق اللة العظيم

Abstract

In the present work we tested the hypothesis that liquid cultures (LCs) of cord blood $CD34^+$ cells at an appropriate low O2 concentration (3%) could simultaneously allow colony forming cell (CFC) expansion and long term culture initiating cell (LTCIC) preservation.

We found that 3% was the minimal O2 concentration, still allowing the same rate of CFC expansion as at 20%. We report here that 7 days LCs(liquid culture) of cord blood $CD34^+$cells at 3% O2 maintain LTCIC better than 20% O2 and allow a similar amplification of CFCs (20-55 folds) without modifying the $CD34^+$ cell proliferation.

The fold expansion of both the $CD34^+$ and the $CD38^+$ cell population was higher in 20% O2 concentration, this denotes the expansion in 20% O2 concentration involved a certain degree of maturation without preservation of the early subset ($CD34^+$, $CD38^-$).

These results suggest that low O2 concentration similar to those found in bone marrow participates in the regulation of haematopoiesis by favouring stem cell-renewing divisions. This expansion method that avoids stem cell exhaustion could be of paramount interest in hematopoietic transplantation allowing the use of small sized graft in adults.

Key words: Long term culture initiating cells- Hypoxia- Umbilical cord blood- Ex-vivo expansion.

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List of abbreviations

ATG	Anti Thymocyte Globulin
BFU	Burst forming unit.
BM	Bone marrow.
BMHS	Bone Marrow Hematopoietic cells.
BMSC	Bone marrow stem cells.
BFU-E	Burst-forming unit erythroid.
CBT	Cord blood transplantation.
CFC	Colony forming cells.
CFU-GEMM	Colony-forming unit granulocyte, erythroid, monocyte, megakaryocyte
CFU-GM	Colony-forming unit –granulocyte-macrophage.
GM-CSF	Granulocyte-macrophage colony-stimulating factor.
GVHD	Graft versus host disease.
HLA	Human leucocytic antigen.
HPC	Hamatopoietc progenitor cells.
HPP-CFC	High proliferative potential colony forming cells.
HIV	Human immunodeficiency virus.
HSC	Haematopoietic stem cells.
HSPC	Haematopoietic stem and progenitors cells.
hTR	Human telomerase.

LAM	Leukocyte adhesion molecule.
LFA-1	Leukocyte function associated antigen.
LTCIC	Long term culture initiating cells.
M-CSF	Macrophage-colony stimulating factors.
MNC	Mononuclear cells.
MSC	Mesenchymal stem cells.
MAPC	Multipotent adult progenitor cells.
NK	Natural killer.
NMDP	National Marrow Donors Program.
SCID	Severe combined immunodeficiency repopulating cells.
SCR	SCID-repopulating cells.
TGF-B	Transforming growth factor beta.
UCB	Umbilical cord blood.
URD	Unrelated donor.

Introduction & Aim of the work:

In the past few years, there has been a growing interest in cord blood transplantation as an abundant, cheap source of haematopoietic stem cells (HSC). Cord blood transplantation (CBT) has two major disadvantages:

1. The low number of HSCs and progenitor cells (Colony forming cells "CFC").
2. The long period of post transplantation cytopenia.

Simultaneous ex-vivo expansion of the HSCs and CFCs could resolve both problems ***(Piacibello & Sanavio; 1999)***.

Cord blood stem cells are comprised of two types of cells: true stem cells which are capable of long term repopulation of bone marrow "Late engraftment" (called long term culture initiating cells, LTCIC or SCID repopulating cells SCR); and the stem progenitor cells (CFC) which are the $CD34^+$ cells and are responsible for the immediate engraftment, hence shortening post transplantation cytopenia ***(Piacibello & Sanavio; 1999)***.

Most ex-vivo expansion protocols expand CFCs on the expense of the number of LTCIC of which a significant number differentiates into CFCs. This may cause late graft failure ***(Abkovitz et al;1998)***.

In 2001, ***Chow et al*** demonstrated that the oxygen concentration present in the bone marrow environment (1%) is more suitable for HSC maintenance than the 20% oxygen concentration prevalent in most liquid culture system. They demonstrated that a 4 days culture of human BM $CD34^+$ cells at 1.5%

oxygen concentration insured a transient ex-vivo expansion of human BM stem cells without substantial amplification of CFCs. This positive effect of low oxygen concentration on stem cell maintenance in-vitro was not limited to cells issued in marrow environment but also, in mobilized peripheral blood stem cells.

Aim of the work:

The aim of this work was to evaluate the effect of oxygen tension and subsequent ex-vivo expansion of cord blood on long term culture initiating cells using liquid culture and low oxygen concentration.