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Scientific publications

2009

Long-term benefit of intracardiac delivery of autologous granulocyte–colony stimulating factor-mobilized blood CD34 + cells containing cardiac progenitors on regional heart structure and function after myocardial infarct

Stéphanie Pasquet [1], Hanna Sovalat [1], Philippe Hénon [1-2-3], Nicolas Bischoff [4],Yazid Arkam 2, Mario Ojeda-Uribe [2], Ronan le Bouar [5], Valérie Rimelen [2], Ingo Brink [6], Robert Dallemand 4 and Jean-Pierre Monassier [5]

Background

Starting from experimental data proposing hematopoietic stem cells as candidates for cardiac repair, we postulated that human peripheral blood (PB) CD34+ cells mobilized by hematopoietic growth-factor(G-CSF) would contain cell subpopulations capable of regenerating post-ischemic myocardial damages.

Methods and results

In a phase I clinical assay enrolling seven patients with acute myocardial infarct, we directly delivered to the injured myocardium autologous PB CD34 + cells previously mobilized by G-CSF, collected by leukapheresis and purified by immunoselection. In parallel, we looked for the eventual presence of cardiomyocytic and endothelial progenitor cells in leukapheresis products of these patients and controls, using flow cytometry, reverse transcription- quantitative (RTQ)–polymerase chain reaction (PCR), cell cultures and immunofl uorescence analyzes.

Mobilized CD34+ cells contain stem cells committed along endothelial and cardiac differntiation pathways which could play a key role in a proposed two-phase mechanism of myocardial regeneration after direct intracardiac delivery, probably being responsible for the long-term benefit observed .

2018

VSELs maintain their pluripotency and competence to differentiate after enhanced ex vivo expansion.

R. Lahil, M. Scrofani, R. Barbet, C. Tancredi, A.Aries, Ph. Hénon. Stem Cell Reviews and Reports, (2018), Vol.14 , 510-524

Background

Very small embryonic stem cells (VSELs) are pluripotent stem cells deposited during ontogeny in the bone marrow where they persist longlife, but they can also be found in peripheral blood and cord blood.They are small cells having similar morphologic, molecular, and phenotypic characteristics of embryonic stem cells. As well as the latters,they can give rise to cells from all three germ layers. However their small number restrict the possibility of their use for cell therapy, hence to need to develop a method allowing their ex vivo expansion.

Methods and results

In the present study, we first delineate different subpopulation of VSELs from human cord blood CD34+ cells to define their purity. We next determine genes expression levels in the whole transcriptome of VSELs expressing the pluripotent marker NANOG and control cells under the steady state condition. We found that more than a thousand of genes are downregulated in VSELs, as well as many membrane receptors, cells signaling molecules and CDKs mRNAs. In addition, we observed discordance in some pluripotent genes expression levels with embryonic stem cells (ESCs), which could explain VSELs quiescence. We then evaluate VSELs capacity to expand and differentiate in vitro in specific and appropriate media. After 12 days culture in specific medium containing a pyrimidoindole derivative (UM171), VSELs were significantly expanded for the first time without feeder cells and importantly preserve their capacities to differentiate into hematopoietic and endothelial cells. Interestingly, this stimulation of VSELs self-renewal restores the expression of some downregulated genes known as key regulators of cell proliferation and differentiation. The properties of such pluripotent expanded cells make them a potential candidate in regenerative medicine.

2019

Design and validation of an automated process for the expansion of peripheral blood derived CD34+ cells for clinical use after myocardial infarction.

C. Saucourt, S. Vogt, A. Merlin, C. Valat, A. Criquet, L. Harmand, B. Irebent, H. Rouard, Himmelspach C, Jeandidier E, Chartois-Leauté A-G, Derenne S, Koehl L, Salem J-E, Ulot J-S, Tancredi C, Aries A, Judé S, Martel E, Richard S, L. Douay, and Ph. Hénon. Stem Cells Translational Medicine (2019), Vol.8, 822-832

Background

We previously demonstrated that intracardiac delivery of autologous peripheral blood-derived CD34+ stem cells (SCs), mobilized by granulocyte-colony stimulating factor (G-CSF) and collected by leukapheresis after myocardial infarction, structurally and functionally repaired the damaged myocardial area. When used for cardiac indication, CD34+ cells are now considered as Advanced Therapy Medicinal Products (ATMPs).

Methods and results

We have industrialized the production of CD34+ stem cells by developing an automated device for ex vivo CD34+-SC expansion, starting from a whole blood (WB) sample. Blood samples were collectedfrom healthy donors after G-CSF mobilization. Manufacturing procedures included: (a) isolation of total nuclear cells, (b) CD34+ immunoselection, (c) expansion and cell culture recovery in the device, and (d) expanded CD34+ cell immunoselection and formulation. The assessment of CD34+ cell counts, viability, and immunophenotype and sterility tests were performed as quality tests. We established graft acceptance criteria and performed validation processes in three cell therapy centers. 59.4 × 106 36.8 × 106 viable CD34+ cells were reproducibly generated as the final product from 220 ml WB containing 17.1 × 106 8.1 × 106 viable CD34+ cells. CD34+ identity, genetic stability, and telomere length were consistent with those of basal CD34+ cells. Gram staining and mycoplasma and endotoxin analyses were negative in all cases. We confirmed the therapeutic efficacy of both CD34 -cell categories in experimentalacute myocardial infarct (AMI) in immunodeficient rats during preclinical studies. This reproducible, automated, and standardized expansion process produces high numbers of CD34+ cells corresponding to the approved ATMP

2020

Key success factors for regenerative medicine in acquired heart diseases.

Ph. Hénon Stem cell Reviews and Reports (2020), Vol.16, 441-458

Stem cell therapy offers a breakthrough opportunity for the improvement of ischemic heart diseases. Numerous clinical trials and meta-analyses appear to confirm its positive but variable effects on heart function.

Whereas these trials widely differed in design, cell type, source, and doses reinjected, cell injection route and timing, and type of cardiac disease, crucial key factors that may favour the success of cell therapy emerge from the review of their data. Various types of cell have been delivered. Injection of myoblasts does not improve heart function and is often responsible for severe ventricular arrythmia occurrence. Using bone marrow mononuclear cells is a misconception, as they are not stem cells but mainly a mix of various cells of hematopoietic lineages and stromal cells, only containing very low numbers of cells that have stem cell-like features; this likely explain the neutral results or at best themodest improvement in heart function reported after their injection. The true existence of cardiac stem cells now appears to be highly discredited, at least in adults.Mesenchymal stem cells do not repair the damaged myocardial tissue but attenuate post-infarction remodelling and contribute to revascularization of the hibernated zone surrounding the scar. CD34+ stem cells - likely issued from pluripotent very small embryonic-like (VSEL) stem cells - emerge as themost convincing cell type,inducing structural and functional repair of the ischemic myocardial area, providing they can be delivered in large amounts via intra-myocardial rather than intra-coronary injection, and preferentially after myocardial infarct rather than chronic heart failure.

2020

CD34+ Very Small Embryonic-Like stem cells or Induced Pluripotent stem cells for cardiac repair?

Ph. Hénon, R. Lahlil, A. Aries, Journal of Cardiac and Vascular Diseases, (2020), Vol 6.

Current research in regenerative medicine is focused on finding pluripotent or multipotent cells with lower associated risks and fewer ethical problems when used as a treatment for patients. There are unresolved ethical and technical issues that hamper the clinical use of embryonic stem cells (ESCs). Even though induced pluripotent stem cells (iPSCs) avoid ethical issues, they present the same technical issues as ESCs, mainly related to their genomic dysregulation and epigenetic instability. In parallel, evidence has accumulated that adult bone marrow harbors different primitive cells which possess the ability to repair organs outside the hematopoietic system. It has been observed that in myocardial infarctions (MI), the injection of CD34+ cells close to the injured myocardial area can achieve a significant restoration of cardiac function, suggesting their direct and/or indirect involvement in the regeneration of heart tissue. This thought was sustained when pluripotent cells known as VSELs, which are able to differentiate and regenerate different organic tissues, were identi¬fied and isolated among CD34+ stem cells; this dismissed the presumption of the possible cell plasticity or de-differentiation. However, additional clinical studies are still to be carried out to further examine this. In the present review, we focused on the biological aspects of different stem cells to shed light on which most are promising, in the hope of improving the treatment of MI by regenerative medicine, once their manipulation becomes mastered.

2021

Differential expression of the Tertraspanin CD9 in normal and leukemic stem cells.

R. Lahlil, M. Scrofani, A. Aries, Ph. Hénon, B.Drenou, Biology, 2021, Vol.10: 312-326

Background

D9 plays a crucial role in cellular growth, mobility and signal transduction , as well as in hematological malignancies. In myeloid neoplasms, CD9 is involved in the altered interactions between leukemic and stromal cells. However, apart from its role in CD34+ progenitors and myeloid and megacaryocytic differentiation, its function in normal and leukemic pluripotent cells has not yet been determined. Very small embryonic-like stem cells (VSELs) are promising pluripotent stem cells found in adult tissues that can be developed for safe and efficient regenerative medicine. VSELs express different surface receptors of the highest importance in cell functioning, including CD9, and can be effectively mobilized after organ injury or in leukemic patients.

Methods and results

In the present study, we observed that CD9 is among the most expressed receptors in VSELs under steady state conditions ; however, once the VSELs are expanded, CD9+ VSELs decrease and are more apoptotic. CD9- VSELs had no proliferative improvement in vitro compared to those that were CD9+. Interestingly, the addition of SDF-1 induced CD9 expression at the surface of VSELs, as observed by flow cytometry, and improved their migration.In addition, we observed in the phenotypically identical VSELs present in the peripheral blood of patients with myeloproliferative neoplasms compared with healthy subjects, a significantly higher number of CD9+ cells. However, in their hematopoietic stem cells (HSC) counterparts, the expression remained comparable. These results indicate that, likewise,in progenitors and mature cells, CD9 may play an important function in normal and malignant VSELs. This could explain the refractoriness, observed by some groups, of expanded stem cells to repairing efficiently damaged tissues when used as a source in cell therapies. Understanding the function of the CD9 receptor in normal and malignant CD34+ and VSELs, along with the relationship with the CXCR4/SDF-1 pathway will enable advances in the field of adult pluripotent cell usage in regenerative medicine and in their role in leukemia.

2021

Purification of CD34+ stem cells from myelodysplastic syndrome with ring-sideroblasts using immunomagnetic strategy.

R. Barbet, A. Eidenschenk, I. arzallah, Ph. Hénon, B. Drenou. Biomedical Journal of Science & Technical Research, 2021, Vol.34 (5): 27120-27125

We developed an efficient and robust procedure to isolate highly purified CD34+ cells from MDS-RS using magnetic column.Our flow cytometry analysis demonstrates the presence of erythroid and monocytes derived cells in samples purified from MDS-RS. Precleaning column did magnetically retain these cells leading to a better purification percentage of the hematopoietic stem cells. Results have been confirmed by MGG and Perls staining. Malignant clonal cells purification is a clue when working on disease such as Myelodysplastic Syndromes (MDS). We believe that these data may help for developing alternative procedure for studying MDS-RS stem cells with a higher purity.

2021

CD34+ Stem Cells and Regenerative Medicine.

Ph. Hénon and R. Lahlil. In: Stem Cells: Latest advances. 2021. K.H. Haider, Editor; Springer, Switzerland; Chapter 2, pp: 21-34

Although hematopoietic stem cell (HSC) transplantations has been performed daily for more than 50 years, the concept of « regenerative medicine » was only proposed at the beginning of the 21st century, with the goal to structurally and functionally repair damaged tissues and organs using stem cells. Despite the hype and the hope generated by the pluripotency of embryonic or induced pluripotent stem cells, ethical problems and/or risk of teratoma formation and genomic instability hinder their use in the clinic in the foreseeable future. Various types of adult stem cells, mostly isolated from bone marrow, peripheral blood, umbilical cord blood, or adipose tissue, have thus been commonly used to regenerate organs within the past years, with unequal efficacy. CD34+ cells emerge now as the more convincing cell type. From their discovery in 1984 until the end of the 1990's, they were considered as being solely HSCs. However, it was progressively demonstrated that endothelial, cardiac muscle, liver, and bone progenitor cells also bear the CD34 marker, giving rise to the transgressive concept of « cell plasticity », which postulated that HSCs can transdifferentiate into other cell lineages from different germ layers. However, this concept should be definitely rejected because evidence has accumulated that adult bone marrow harbors rare pluripotent stem cells, named very small embryonic –like stem cells (VSELs) which are also CD34+. They have been shown to be precursors of hematopoietic, mesenchymal, endothelial, cardiomyocytic, lung epithelial stem cells, and germ cells. To date, no clinical studies have been performed using VSELs. However, the clinical demonstration in recent years of the regeneration potential of CD34+ stem cells which are probably derived from VSELs, could open the way to their greater therapeutic use in the near future.

2022

Industrialized GMP Production of CD34+ Cells (ProtheraCytes®) at Clinical Scale for Treatment of Ischemic Cardiac Diseases Is Feasible and Safe

Philippe Hénon, Marc Kowalczyk, Anne Aries, Christine Vignon, Guillaume Trébuchet & Rachid Lahlil.
Stem Cell Reviews and Reports, 2022 ; Vol. 18 : 1614–1626

Background

Regenerative medicine now needs to pass a crucial turning point, from academic research to the market. Several sources/types of cells have been experimented with, more or less successfully. CD34+ cells have demonstrated multipotent or even pluripotent capacities, making them good candidates for regenerative medicine, particularly for treating heart diseases.

Methods and results

Strongly encouraged by the results, we achieved in a pilot study using CD34+ stem cells in patients with poor-prognosis acute myocardial infarcts (AMIs), we soon began the development of an industrialized platform making use of a closed automated device (StemXpand®) and a disposable kit (StemPack®) for the large-scale expansion of CD34+ cells with reproducible good manufacturing practice (GMP). This scalable platform can produce expanded CD34+ cells (ProtheraCytes®) of sufficient quality that, interestingly, express early markers of the cardiac and endothelial pathways and early cardiac-mesoderm markers. They also contain CD34+ pluripotent cells characterized as very small embryonic-like stem cells (VSELs), capable of differentiating under appropriate stimuli into different tissue lineages, including endothelial and cardiomyocytic ones. making them very good candidates for sustained heart repair after AMI. Their industrial GMP-compliant production in large numbers is feasible and safe and does not alter their biological/functional characteristics, thus fulfilling regulatory requirements and facilitating their extensive clinical use.