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Medical perspectives of adults and embryonic stem cells

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Abstract – In the last 30 years, allogeneic bone marrow transplantation has become the treatment of choice for many hematologic malignancies or inherited disorders and a number of changes have been registered in terms of long-term survival rate of transplanted patients as well as of available sources of hematopoietic stem cell (HSC). In parallel to the publication of better results in HSC transplantation, several recent discoveries have opened a scientific and ethical debate on the therapeutical potential of stem cells isolated from adult or embryonic tissues. One of the major discoveries in this field is the capacity of bone marrow-derived stem cells to treat a genetic liver disease in a mouse model, thus justifying the concept of transdifferentiation of adult stem cell and raising hopes on its possible therapeutical applications. We have tried here to summarise the advances in this field and to discuss the limits of these biological data. ***To cite this article: M. Cavazzana-Calvo et al., C. R. Biologies 325 (2002) 1053–1058.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

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Résumé – **Perspectives médicales de la cellule souche adulte et embryonnaire.** Depuis 30 ans, la greffe de moelle osseuse allogénique représente le traitement de choix des hémopathies malignes et de certaines maladies héréditaires. Parallèlement à des améliorations notables en termes de survie à long terme, les sources de cellules souches hématopoïétiques (CSH) possibles se sont multipliées. Des découvertes récentes sont à l'origine d'un débat à la fois scientifique et éthique sur le potentiel thérapeutique des cellules souches isolées à partir de tissus embryonnaires et adultes. Parmi elles, la mise en évidence de la capacité des cellules souches de la moelle osseuse à guérir une maladie génétique du foie dans un modèle murin constitue une découverte majeure, justifiant le concept de plasticité de la cellule souche adulte et laissant espérer de possibles applications thérapeutiques. Nous avons tenté de résumer les avancées dans ce domaine et de discuter les limites des résultats publiés. ***Pour citer cet article : M. Cavazzana-Calvo et al., C. R. Biologies 325 (2002) 1053–1058.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

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Version abrégée

Le recours à la greffe de moelle osseuse allogénique repose sur l'idée de remplacer le système hématopoïétique malade par celui d'un individu sain. Depuis la première tentative réussie à la fin des années 1960, un nombre croissant de greffes de moelle osseuse allogénique, depuis une douzaine en 1975 jusqu'à 800 en 2001, ont été effectuées pour des patients atteints d'hémopathies malignes ou d'autres maladies affectant le système lympho-hématopoïétique. L'ouverture d'un fichier international de donneurs a joué un rôle majeur dans cette augmentation au cours de ces dix dernières années. Actuellement, les cellules souches hématopoïétiques (CSH) sont isolées à partir de la moelle osseuse, du sang – après mobilisation à l'aide de cytokines – ou du sang de cordon. La survie a pu être considérablement améliorée grâce aux progrès effectués en termes de soins, en particulier au niveau de la gestion des complications infectieuses. L'utilisation de doses croissantes de CSH purifiées a de plus autorisé le recours à des donneurs partiellement incompatibles au niveau du système HLA.

Jusqu'à récemment, le traitement par greffe de moelle osseuse de maladies héréditaires autres que celles affectant le système hématopoïétique paraissait inconcevable. Deux essais cliniques ont modifié ce point de vue. Ainsi, Horwitz a montré que la greffe de moelle osseuse allogénique chez cinq enfants atteints d'*osteogenesis imperfecta* améliorait de manière significative les symptômes associés à cette maladie. Cet essai reposait sur l'éventuelle possibilité d'induction de différenciation ostéoblastique des cellules souches mésenchymateuses présentes dans la moelle osseuse. L'essai de Shapiro a, quant à lui, démontré le potentiel thérapeutique de la greffe de CSH dans le traitement de l'adrénoleucodystrophie liée à l'X, une maladie dégénérative du système nerveux central, caractérisée par une perte progressive de myéline. Ces deux essais ouvrent le champ d'application de la greffe de moelle osseuse allogénique à des maladies n'affectant pas directement le système hématopoïétique.

Grâce aux avancées obtenues à la fois dans le domaine du transfert de gène et dans la compréhension de la physiopathologie de certains déficits immunitaires, le potentiel thérapeutique des CSH s'est également ouvert à la thérapie génique. Les déficits immunitaires combinés sévères (DICS) présentent de multiples avantages pour la mise en œuvre de ce type de stratégie : avantage sélectif des cellules transduites, absence de réaction immunitaire vis-à-vis du transgène et du vecteur, accessibilité des CSH. Neuf enfants DICS présentant un déficit lymphocytaire sévère ont été traités par thérapie génique. Cette stratégie a permis le développement d'un compartiment lymphocytaire normal et fonctionnel chez sept d'entre eux. La mise en évidence de l'intégration du provirus utilisé pour le transfert de gène dans des précurseurs hématopoïétiques très immatures laisse espérer une efficacité à long terme de ce traitement.

Les recherches sur les cellules souches de la moelle osseuse sont également à l'origine des premiers résultats suggérant l'existence d'une certaine plasticité des cellules souches adultes. Elles ont montré la capacité des CSH à migrer dans différents tissus et à se transdifférencier dans les lignées spécifiques de l'organe colonisé, myocarde et foie. D'autres études ont suggéré la capacité de lignées de cellules souches neurales à s'engager dans différentes voies de différenciation, notamment hématopoïétique, et celle des cellules souches de la peau à donner naissance à des cellules de la glie, du muscle et des adipocytes.

L'absence de données quant à la fonctionnalité des cellules obtenues par transdifférenciation constitue une première limite dans l'interprétation de ces études. De plus, la plupart de ces résultats sont obtenus après lésion du tissu concerné, et le nombre de cellules matures générées par transdifférenciation est très faible. Enfin, d'après les expériences de Morshead et de McKinney-Freeman, la transdifférenciation des cellules souches neuronales en cellules hématopoïétiques pourrait ne représenter qu'un épiphénomène, et les cellules hématopoïétiques dérivées du muscle pourraient résulter de la différenciation de CSH présentes dans le muscle, plutôt que de cellules souches musculaires. Ces controverses illustrent parfaitement la complexité biologique des cellules souches adultes, qui constituent un domaine d'étude en pleine mutation.

Les cellules souches embryonnaires (ES), isolées à partir de blastocystes, pourraient représenter une alternative intéressante aux cellules souches adultes. Elles présentent in vitro une très forte capacité d'expansion et d'auto-renouvellement ; elles peuvent se différencier, selon le milieu de culture utilisé, en neurones, en cellules musculaires cardiaques ou en cellules secrétant de l'insuline. De plus, elles peuvent être aisément modifiées par recombinaison homologue, transplantation nucléaire et transduction rétrovirale, fournissant un modèle d'étude des voies de différenciation altérées dans différentes maladies héréditaires. Le potentiel des cellules ES a été illustré par les récents travaux de Rideout, qui ont démontré la possibilité de traiter une forme de DICS chez la souris par recombinaison homologue sur des cellules ES obtenues par transfert nucléaire somatique.

Toutefois, dans la perspective d'essais cliniques, il reste à démontrer l'efficacité et l'innocuité à long terme des cellules humaines modifiées. En particulier, les cellules ES indifférenciées présentent un risque de prolifération non régulée, qui pourrait néanmoins être contourné par la caractérisation précise des étapes de différenciation de ces cellules.

1. 'Adult type' bone marrow derived stem cell transplantation

Transplantation of allogeneic bone marrow cells was the first achievement in the field of regenerative medicine. Research in this field was initiated as early as in the 1950s and was reviewed by Van Bekkum [1]. Bone marrow transplantation (BMT) was first performed in the 60s and aimed at replacing the recipient's 'diseased' lymphohematopoietic system with a healthy one from another individual. Since the first successful clinical transplantation was carried out, this approach developed rapidly. The number of patients treated with hematopoietic stem cell transplantation (HSCT) in France, for example, increased from a dozen in 1975, to 415 in 1986 up to 800 in 2001 in the allogeneic setting, while more than 2500 autologous HSCT were performed in the same year. The development of an international registry of volunteer donors explains, at least to some extent, the two-fold increase in the number of allogeneic transplantations performed over the last ten years. Over the sometime period, new HSC sources were defined.

Today, stem cells harvested from the bone marrow or from the peripheral blood after cytokine mobilisation and, finally, stem cells harvested from the cord blood at the time of delivery can be used [2]. This last HSC source presents particular biological characteristics, due to its ontogenic origin; its self-renewal and proliferation capacities are superior to those of adult bone marrow cells [3], allowing them to be ideal target cells when available to correct inherited monogenic diseases by gene transfer. Conversely, their low number is responsible for the slow engraftment and high rejection rate when these cells are used to transplant adult patients [4], thus justifying the efforts aimed at expanding cord blood stem cells without loosing their transplantation capacity.

Although the principal indication of allogeneic HSCT is malignant hemopathies (85%), other diseases, such as inherited immunodeficiencies, metabolic diseases and benign hematopoietic disorders are now currently treated. In the last thirty years, the overall survival and the disease free survival rates have greatly improved all known indications thanks to the progress of supportive care and management of infectious diseases. In addition, the usage of highly purified human HSC, combined with the stem cell dose escalation [5] approach, has permitted to partially by-pass the HLA barrier (even in the case of adults affected by malignancies and vigorously treated by chemotherapy before the transplantation).

2. Recent therapeutical progress in the use of bone marrow derived stem cells

Until recently, the use of stem cells to treat inherited diseases other than those of the lymphohematopoietic system was inconceivable. Two recent clinical trials have in part modified this view.

Horwitz et al. [6] have shown that bone marrow stem cell transplantation can improve a genetic disease of the mesenchyma. Indeed, HLA genoidentical BMT in five children affected by *osteogenesis imperfecta* significantly improved the course of this disease. *Osteogenesis imperfecta* is characterised by the production of abnormal type I collagen, osteopenia, bone fragility, severe bone deformities and failure to thrive. The rationale of BMT was to transplant mesenchymal stem cells able to induce osteoblastic differentiation. In three out of five transplanted children, engraftment of the osteoblastic lineage was shown and, in parallel, their two-year follow-up revealed an improvement in the growth rate, an increase in bone mineral content as well as a significant decrease in the number of fractures.

Similarly, Shapiro et al. have shown that a rapidly progressive neurodegenerative disorder that affects central nervous system (CNS) myelin as well as adrenal cortex can benefit from HSCT, overall when the procedure is performed at an early stage of the disease [7]. X-linked adrenoleukodystrophy is a demyelinating disorder of the central nervous system, leading to a vegetative state and death within 3–5 years, once clinical symptoms are detectable. The rationale for BMT has relied on the hypothesis that functional bone marrow cells from the donor could cross the blood–brain barrier in the recipient and exert a favourable effect on the mechanisms leading to demyelination. The clinical results reported in eighteen transplanted children indeed provide a proof of this concept, since twelve of them survived. Moreover, CNS disease pro-

gression has been halted and a good quality of life maintained. In two out of the 12 long-term surviving children, a complete disappearance of the cerebral lesions was observed on MRI.

Recent advances in gene transfer into human hematopoietic cells combined with a better understanding of the genetic aspects of several immunodeficiencies have recently offered new opportunities in the domain of gene therapy. In this respect, Severe Combined Immunodeficiency (SCID) appeared as a good model for the application of this approach by providing a combination of an expected selective advantage for transduced cells, an absence of immunological response to the vector and/or the therapeutic transgene, together with the accessibility to HSC [8]. A clinical trial based on the ex-vivo retroviral transduction of marrow precursor cells shows that this approach can be particularly effective by leading to long-lasting expression of the transgene in lymphoid precursor cells and thereby correction of the immunodeficiency [9].

So far, nine children with X-linked SCID (γc deficiency) have been treated, including eight 'typical SCID cases'. Among them, a quite normal lymphoid compartment and immunological functions have developed in seven of them.

These results raise several questions, such as the identification of the hematopoietic precursor cells that have been successfully transduced ex-vivo. Provirus integration sites studies suggest that some HSC, able of both self-renewal and differentiation, have been transduced raising hope for sustained efficacy of this treatment (unpublished data, in collaboration with Manfred Schmidt and Christof Von Kalle).

3. Stem cell plasticity: hopes and disillusions for regenerative medicine

In parallel, several recently published data have changed some "dogmas" on boundaries and differentiation capacity of adult stem cells. These data are based on the observation that stem cells particularly those from bone marrow have the capacity of colonising different tissues, and transdifferentiate into cell lineages of the organ [10–14]. It has been reported in experimental settings that bone marrow-derived stem cells can repair an infarcted heart [15] as well as a genetic liver disease [16]. In addition, neural stem cell lines cultured from adult brain tissue may differentiate to form hematopoietic cells [17] or give rise to many different cell types in a chimeric embryo [18]. Finally, skin derived stem cells have been shown to transdifferentiate into nervous glias, muscle, and adipocytes.

All of these data have been used to claim that adults stem cells present in almost all organs could be used to cure a number of genetic or acquired diseases. However, data require a caution in interpretation in order to avoid unfounded hopes. Differentiated cell types, which result from 'plasticity' or transdifferentiation, are usually reported to have the morphological characteristics of the differentiated cells and to display their characteristic surface markers. However, there is limited evidence to date that such adult stem cells can generate mature, fully functional cells or that the cells have restored lost function in vivo, with the only exception of the work reported by Lagasse et al. [16]. Moreover, many experiments involve injury to a particular tissue required to observe transdiffentiation. Finally, the magnitude of this phenomenon is limited to a very low percentage of mature cells detectable, raising the question of the 'clinical' applicability. To these remarks, one should also add recent findings that further cast doubt on the concept of stem-cell plasticity [19, 20]. The results reported by Morshead et al. [21] exclude hematopoietic competence as a consistent property of intravenously infused neural stem cells and suggest that rare transformation events may account for the originally reported neural-to-blood fate switch. Similarly, data reported by McKinney-Freeman et al. show that muscle-derived hematopoietic stem cells are likely derived from the hematopoietic system and thus do not result from a transdifferentiation process of myogenic stem cells [22]. Therefore, therapeutical utilisation of adult stem cells is largely questionable. Unsolved problems, such as proliferation and differentiation capacities of adult stem cells, require fundamental studies. Therefore, studies investigating the functional capacities of embryonic stem cells (ES) are warranted.

4. Embryonic stem cells: properties for regenerative and developmental medicine

ES cells, derived from blastocysts, are capable of long-term self-renewal and differentiation into most tissues while retaining a normal genomic structure. It has been shown that human ES cells can proliferate for two years, including 300 cell cycles, a characteristic that is not shared with any adult stem cell [23].

It must be underlined for instance that after 40 years of intensive research, one is still unable to in vitro expand the bone marrow-derived hematopoietic stem cells without loosing their self-renewal capacity.

Besides their self-renewal capacity, ES cells can be directed towards differentiation pathways by changing growth conditions, leading to development of special-

ised cells such as neurons, heart muscle cells or insulin-secreting cells, opening the way to the development of new therapies. The examples of the functional correction of the neurodegenerative diseases in different animal models by the transplantation of a low member of differentiated or undifferentiated ES cells is particularly encouraging in this direction [24–26]. Moreover, ES cells can been modified by homologous recombination, nuclear transplantation [27] or retroviral transduction, allowing researchers to dispose of in vitro models to study developmental pathways that are mutated in genetic diseases. A better understanding of these cell programs could shed light on the physiopathology of these diseases and enable us to envisage new therapeutic strategies.

The best example of the potentiality of these combined strategies has been recently reported by Rideout et al. [28], whose work provides the proof of principle for nuclear transplantation combined with gene therapy to treat a form of severe combined immune deficiency in mice. Somatic nuclear transfer allowed the isolation of ES cells genetically identical to diseased individuals, thus avoiding rejection, which remains the main obstacle to conventional organ transplantation. These genetically matched ES cells can then be corrected by homologous recombination, as demonstrated recently [28], and induced to differentiate into cells of several different developmental lineages, including neurons and blood, in order to correct human inherited disease.

Nevertheless, in the perspective of clinical trials using human modified ES cells, it is essential to demonstrate that these cell preparations present both relevant biological activity and absence of risk of early or long-term toxicity. To these aims, a critical element is the use of relevant animal models. Rodent animal models could be pertinent, as human disease models, while non-human primates should be useful to assess toxicity. The proliferative capacity of undifferentiated ES is a matter of concern. Undifferentiated ES cells are not suitable for transplantation purpose, due to the risk of unregulated growth. Therefore, the identification of differentiation steps at which the risk of tumour formation is minimised becomes a critical point for further of research.

5. Conclusion

A fruitful research period has begun all around the world focused on the biology of stem cells. Two medical perspectives are around the corner; one deals with developmental medicine. Discoveries in this field can help to understand physiopathology of both infertility and developmental genetic diseases; the second one deals with the so-called ‘regenerative medicine’ that could bypass some therapeutical barriers caused by donor organ scarcity and immunological constraints. Meanwhile, basic research should provide further information of importance for future therapeutic usage of stem cells.

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