Haematopoietic stem cells and mesenchymal stem cells as tools for present and future cellular therapies*

Vincent Kindlera, Domizio Suvab, Caroline Soulasa, Bernard Chapuisb

a Haematology Service, Geneva University Hospital, Geneva, Switzerland
b Orthopaedic Surgery Service, Geneva University Hospital, Geneva, Switzerland

Summary

Postnatal stem cells are present in many adult tissues, and are thought to ensure homoeostasis by replacing functionally declining cells by newly differentiated ones. Postnatal stem cells used as such or after in vitro manipulation hold out strong hopes for reconstructive therapies. For instance, the grafting of native haematopoietic stem cells (HSC) restores haematopoiesis in genetically deficient individuals or in lethally conditioned leukaemic patients, and systemic injection of in vitro amplified mesenchymal stem cells (MSC) induces recovery of bone growth in patients with osteogenesis imperfecta. Moreover, cells differentiated in vitro from postnatal stem cells exhibiting a specific function can also be used for cell therapy. Myeloid dendritic cells (DC) derived from cultures of HSC may induce tumour-specific cytotoxic T lymphocytes to eradicate the tumour via antigen recognition. In addition, long-lived MSC has been engineered to secrete specific proteins coded by a transgene and used as a source of therapeutic molecules in vivo. All these approaches require large quantities of cells that cannot be obtained (with the exception of HSC) directly from the donor. In vitro procedures allowing the production of therapeutic cells from postnatal stem cells are needed and are at present under development. Below we discuss the rationale and methods currently available for generation of therapeutic cells derived from haematopoietic and mesenchymal stem cells.

Key words: haematopoietic stem cells; mesenchymal stem cells; cell therapy; in vitro differentiation

Introduction

Stem cells are able to undertake iterative cell cycling without losing their proliferative and differentiative properties, i.e. they are the only cells that resist differentiation and simultaneously give birth to a numerous progeny [1, 2]. In vivo, two types of stem cell can be distinguished: the zygote, which under physiological conditions produces all embryonic and extraembryonic tissues (such as the placenta and the umbilical cord) which constitute an individual, and the postnatal stem cells that are resident in most of the organism’s tissues after birth and ensure their homoeostasis. Blood, skin, and gut mucosa were the first tissues whose homoeostasis was found to be regulated by stem cells. Now postnatal stem cells have also been identified in muscle, nerve, adipose tissue and bone marrow (BM) stroma [3, 4].

Thanks to their ability to generate large numbers of mature cells, stem cells represent a major hope for reconstructive therapies. While embryonic stem cells (ES) derived from blastocysts [5, 6] could theoretically be used for such applications, their generation and manipulation are beset with moral and ethical concerns. Moreover, these cells frequently generate teratogenic events once injected into a host [6] and their use in human clinical medicine remains to be determined. By contrast, postnatal stem cells are not hampered by such moral and ethical considerations since they are obtained exclusively after the donor’s informed consent, a fact obviously not applicable to ES. Postnatal stem cells are already used as such in some clinical applications, the best example being the immediate use of haematopoietic stem cells (HSC) to reconstitute the haematopoietic system of lethally conditioned leukaemic patients. Moreover, postnatal stem cells amplified in vitro, or their direct progeny, are promising tools.

This review focuses on the feasibility of generating in vitro, from HSC and mesenchymal stem cells (MSC), functional cells that may be used for cell therapies.
Cells derived from HSC

HSC were identified by Till and McCulloch in 1961 [7]. These authors showed that the injection of BM cell suspensions into a lethally irradiated host ensured full recovery of their haematopoiesis. Later on HSC were identified as a subpopulation within CD34+ cells [8, 9]. As proof, the number of CD34+ cells grafted into aplastic patients correlates inversely with the time until haematopoietic restoration [10].

In vitro generation of haematopoietic progenitors

In vitro HSC rapidly lose their ability to self-maintain, as illustrated by their decreased ability to repopulate irradiated hosts after culture [11]. However, culture of CD34+ cells with the combination of growth factors such as FLT3-ligand, thrombopoietin and stem cell factor (FTS) generates large numbers of haematopoietic progenitors. These include monocyte, granulocyte, megakaryocyte, myeloid dendritic cell (DC) and plasmacytoid DC progenitors [12, 13] (Figure 1). Lymphocytes are not generated in these conditions because they require a specific stroma. After amplification with FTS, progenitors can be differentiated into a single lineage in secondary cultures using lineage-specific factors [12] (figure 1). So far, only in vitro generated DC are evaluated as a therapeutic tool.

Differentiation into antigen-presenting cells

DC represent a small proportion of the leukocytes but are major constituents of the immune response. They are antigen-presenting cells (APC) that constantly sample the internal milieu and present processed antigens docked on their major histocompatibility complex (MHC) to T lymphocytes [14]. In the absence of inflammation DC are said to be “immature”, and antigen presentation maintains peripheral tolerance and silences autoimmune reactions. By contrast, within an inflammatory context DC undertake maturation, i.e. they upregulate MHC and T cell costimulatory molecule expression, and stimulate T cells. DC therefore target their action exclusively at T cells specific for the antigen they present, and their state of maturation determines whether they will inhibit or stimulate such T lymphocytes. Consequently, DC represent a powerful tool for piloting the immune response.

DC were obtained for the first time in vitro from CD34+ HSC by Santiago-Schwarz et al. in 1992 by culturing CD34+ cells with GM-CSF and tumour necrosis factor-α (TNF-α) [15]. However, TNF-α which was required for DC differentiation, also induced their maturation and few immature DC were generated in such conditions. This has now been solved using culture systems that uncouple proliferation from differentiation and maturation [16, 17]. The amplification of CD34+ HSC with FTS followed by their differentiation into immature DC with GM-CSF plus IL-4 or into mature DC with GM-CSF, IL-4 and TNF-α generates as many as 1200 DC from one single CD34+ cell [16]. Moreover, CD34+ cells trans-
Cells derived from MSC

MSC were first identified by Friedenstein in 1961 on the basis of their adherence to plastic and their growth in vitro [22]. MSC have been isolated from BM, muscle, synovium, dermis, adipose tissue, cord blood and Wharton’s jelly [23–27]. The pluripotency of MSC was clearly established in 1999, when Pittenger et al. [23] demonstrated that MSC clones differentiated into several distinct mesenchymal lineages (figure 2). In contrast to HSC, MSC have not been, either before or after in vitro amplification, transplanted, isolated and retransplanted into multiple generations of recipients to evaluate their self-renewal capabilities [28]. Moreover, none of the non-specific markers that MSC express after in vitro amplification [29–31] has been prevalent for their enrichment [28], and it is not known whether MSC obtained after selective adherence in vitro are indeed true stem cells. For these reasons several authors suggest that the term “multipotent mesenchymal stromal cell” should be used for these cells obtained after selective adherence, while “mesenchymal stem cell” should be restricted to the true mesenchymal stem cell, which definitely requires further identification [32]. However, irrespective of this issue, the uncertainty concerning the stemness of in vitro amplified MSC should not harness their clinical use as long as they produce a beneficial effect.

MSC as a reconstructive tool

Used immediately after amplification MSC target injured mesenchymal tissues and participate in their regeneration. Allogeneic MSC infused into paediatric patients with osteogenesis imperfecta lacking functional osteoblasts significantly improve bone metabolism [33, 34]. MSC also populate implants and restore bone integrity in a model of acute bone defect [35]. Moreover, MSC play a “bystander” role in HSC grafting by reconstituting the medullar environment facilitating the homing of the grafted cells and consequently haematopoiesis [34, 36].

Figure 2

In vitro differentiation of therapeutic cells from MSC. Cell suspension from BM or various tissues are incubated in flat vessels for 24 hours (here in limiting dilution setting to ensure clonal selection). MSC are selected by washing out non-adherent cells, and are amplified using culture conditions that do not allow leukocyte survival. Clonally amplified MSC can be differentiated into various lineages using specific conditions, or used as such in culture with other cells to evaluate their haematopoietic supporting activity, their immunomodulatory properties and their ability to fuse into myotubules.
MSC as an immunomodulatory tool

MSC are immunotolerising cells. Bulk-amplified MSC block mitogen- and APC-induced T lymphocyte stimulation [30, 37]. Moreover, MSC impede in vitro DC differentiation, suggesting that they also block T cell activation indirectly by altering APC function [38]. The immunosuppression induced by MSC on T cells is clearly MHC- and antigen-independent. Soluble and membrane-bound molecules are involved but these remain to be identified. In some instances in vitro, MSC are stimulatory [39], but the physiological relevance of this observation is not established. By contrast, in vivo MSC are thus far strictly immunosuppressive. Most remarkably, in humans, haploidentical MSC injected into paediatric patients with severe graft versus host disease after reconstitution with allogeneic HSC infusion cured the disease [34, 40], suggesting that in vivo infusion of MSC may be safe even when injected within an inflammatory context. MSC may nevertheless represent a potential danger, because in mice MSC-induced immune depression is associated with uncontrolled growth of tumours which remain under immune control in non-injected animals [41]. Thus, MSC infusion in humans may equally awaken dormant pathogens or malignant cells. This last observation emphasises the importance of embracing the full spectrum of MSC immunoregulatory activities before extending their use in human clinical medicine.

MSC as a privileged vessel of therapeutic factors

Finally, MSC transduced with erythropoietin or factor IX gene constructs and injected subcutaneously into baboons secrete the corresponding gene products for up to 90 days [42], demonstrating that engineered MSC could be used as a source of therapeutic molecules in vivo.

Conclusion

Our understanding of stem cell biology has dramatically increased in the last few years and cell therapies using postnatal stem cells will certainly become more numerous, shortly offering valuable alternatives to classical therapies. MSC will be used, whether associated or not with artificial scaffolds, to achieve bone restoration. They may also be used as immunoregulatory agents to obliterate the deleterious effects of allografting, but careful exploration of their biology must be undertaken beforehand. It is first necessary to establish whether the nonspecific, antigen-independent inhibition of MSC is not a two-edged sword which, though inhibiting graft rejection, may also awaken dormant malignancies. HSC-derived APC may be safer immunoregulatory agents because they mediate their effect via antigen-dependent processes and should therefore hit the chosen target only. Mature DC loaded with a tumour-specific antigen could function as an adjuvant to select and “educate” antigen-specific T cells to eradicate tumour cells, or, conversely, immature DC loaded with a self-antigen should provide powerful inhibition of autoreactive T cells and halt autoimmune processes in susceptible individuals. The danger here does not lie in the specificity of the mechanism but in the state of DC maturation: how sure are we that a mature DC will remain mature long enough to provide a stimulatory signal to T cells in vivo, and how sure are we that an immature tolerising DC generated in vitro will remain so in vivo? All these questions represent an impressive challenge to be met before we can safely extend the clinical use of MSC and HSC. Hopefully, we now have the means to explore these avenues.

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