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Review articles

Stem cell therapies for neuromuscular diseases

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Abstract

The promise of stem cells for therapy of a variety of disease conditions is one of the most heavily marketed commodities of modern biotechnology. Interest in this prospect has opened up and destabilized a large area of developmental biology, challenging some of its major tenets, and becoming a source of controversy. Much of the disagreement arises from apparently simple conflicts of findings, which may have as much to do with differences of interpretation as of actual data. However, outside a few favoured situations, the practical significance of most of the findings is marginal, with only trivial levels of stem cell participation in tissue reconstruction. Currently, much of the effort in stem cell research is devoted to identifying sources of stem cells and the diversity of fates they can be induced to adopt. Areas that are in need of development are those concerned with delivery to target tissues, improvement of conversion to the desired cell types and effectiveness of functional integration of these cells into the target tissue.

Of these hurdles, perhaps the most important is the efficient delivery of stem cells to the appropriate sites. The most promising results in this respect have been achieved by either targeted injection to discrete sites or, for more diffuse targets, delivery via the intermediary of a bone marrow transplant. For the latter especially, we need a degree of improvement that can only come from a better understanding of the mechanisms involved.

Key words : Stem cell ; bone marrow ; satellite cell ; mesenchymal stem cell ; myogenesis ; neurogenesis.

Introduction

Over the past few years, the ‘stem cell’ concept has become an important theme, or bandwagon, depending on point of view, in the array of strategies that are being considered as potential therapies for genetic or degenerative diseases of various tissues. The attractions of this idea look better in theory than in practice. At first glance it is hard to fault the notion of a cell that continually replaces itself and gives rise to one or more types of functionally committed cell, by virtue of asymmetric divisions that have the capacity to continue substantially undiminished throughout the lifetime of the animal. However, cells that actually live up to this image have been identified in only a limited number of situations, and the concept carries with it a number of innate problems. Up to now, the greatest success of stem cell technology has been achieved in reconstitution of the haematopoietic system. This is not too surprising since in this instance it represents merely a refinement of the long-established practice of bone marrow grafting (Blomberg *et al.*, 1998) that has been applied as a means of replacing faulty blood both in genetic conditions such as immunodeficiencies (Cavazzana-Calvo *et al.*, 2000) and thalassaemias (Antoniou and Grosveld, 1999) and in acquired diseases such as leukaemias (Thomas, 1991). In a more limited way, skin grafting has been similarly revolutionized by use of keratinocyte stem or early precursor cells, either to generate graftable skin-like tissue in vitro or to be directly seeded onto sites of severe burns so as to regenerate an epidermal layer from diffuse sites (Niemann and Watt, 2002). Similarly, grafts of stem cells have proved useful for augmenting bone regeneration over large sites (Yamada *et al.*, 2003).

These have emerged as successful exemplars in large part because they are amenable by nature of their fundamental biological function as tissues that maintain themselves by dynamic processes. Another important factor in all cases, is that appropriate sites are relatively easy to seed with stem cells. In the case of bone marrow, these home efficiently from the circulation to appropriate niches. For skin and bone, the lesions are of limited extent and can be seeded directly. It is instructive to consider the essential biological properties shared by these tissues, since they may provide cues to the successful application of stem cell technology to the reconstruction of other tissues. Their most clearly unifying feature is that all of them, as part of normal life, constantly remodel, via a population of stem cells or early precursors, in response to signals arising from imposed stresses. Thus they possess intrinsic mechanisms that enable them routinely to accommodate input from stem cells in a manner that is coordinated to functional demand on the tissue.

Problems for use of stem cells

Such highly integrated mechanisms are far less conspicuous in many other tissues : notably so in striated muscle and in neural tissue that are major targets for stem cell therapies for neuromuscular diseases. It is true that skeletal muscle possesses an efficient mechanism of regeneration that copes well with acute minor injury but, this appears to be a largely local phenomenon : early muscle transplant and early myoblast transplant data showed some limited spread within, the injected muscle and, at best, traces of movement between adjacent muscles (Grounds *et al.*, 1980 ; Grounds and Partridge, 1983 ; Morgan *et al.*, 1987 ; Partridge *et al.*, 1978), recently confirmed by elegant studies using the GFP marker (Jockusch and Voigt, 2003). In consequence, until quite recently, the endogenous muscle regeneration system was not envisaged as including any means of distributing precursors between or within muscles at a level of efficiency that might have therapeutic value. This, combined with the fact that muscle tissue is extensive and widely distributed, emphasized the difficult nature of skeletal muscle as a target for effective delivery of exogenous stem cells to supplement the endogenous mechanisms in a well integrated and coordinated manner.

The general strategy of developing the use of stem cells for therapeutic application to replacement or supplementation of adult tissues resolves itself into 4 parts. First, it is necessary to identify readily harvestable sources of stem cells. Second it must be possible to convert the stem cell efficiently into the desired cell type. Third, the cells must be efficiently delivered to the target tissue. Last, the cells must become integrated into that tissue in such a way as to exhibit a useful, ideally normal, function.

Sources of stem cells

Endogenous stem cells

At present, considerable resources are being devoted to identifying sources of stem cells and of developing methods of converting them efficiently into various cellular lineages of interest. Despite the obvious difficulties, this has been, until recently, treated as a local mechanism in skeletal muscles largely because of the strong, effective and rapid regeneration and repair of acutely damaged muscle fibres, seemingly largely or entirely from endogenous sources. Thus skeletal muscle has been regarded as the potential source of choice for myogenic stem cells. The muscle satellite cell has long been regarded as the main, perhaps only provenance of these endogenous muscle stem cells and, despite the great deal of effort that has been devoted to determining whether this is so, it has been difficult to establish the matter definitively. In the meantime other classes of stem like cells have been identified within the intrinsic muscle cell population (Qu-Petersen *et al.*, 2002) (Gussoni *et al.*, 1999)

This is in contrast to the CNS and the heart, where damage has commonly been held to elicit at best minor signs of spontaneous renewal of neurons or cardiomyocytes respectively. The seeming absence of endogenous precursor cells has come under challenge in recent years. A long history of failure to find signs of effective regeneration in cardiac muscle, has predisposed to a marked scepticism of a number of claims made in the past few years of the existence of stem cells within the heart (Orlic *et al.*, 2001). This has matured into a dispute on the broad issue of whether damage to the myocardium of adult mammals produces any significant cardiomyogensis (Chien, 2004) (Leri *et al.*, 2004) (Murry *et al.*, 2004), although urodele amphibians seem very competent to regenerate large regions of surgically extirpated ventricular tissue (Brockes *et al.*, 2001).

In the CNS, evidence of the existence of active neurogenesis in the adult is better established and more widely accepted (reviewed in (Kennea and Mehmet, 2002)). Specific zones of proliferating cells have been identified in the hippocampal subgranular zone and subventricular zones of the lateral ventricles of rodents, seemingly giving rise to new neurones throughout adult life. Similar cells been identified in the adult human brain (Eriksson *et al.*, 1998). It has also proven possible to extract neural stem cells from various regions of the adult brain and, in carefully controlled conditions, to propagate them in such a way that they remain undifferentiated and capable of giving rise to all three neural phenotypes, neurones, astrocytes and oligodendrocytes, when transplanted into recipient brains (Kennea and Mehmet, 2002).

In the meantime, perhaps on account of the unsatisfactory outcomes of spontaneous regeneration from endogenous sources in cardiac and neural tissues, there has been a more concerted search for stem cells from sites lying outside of these tissues, and latterly for exogenous sources of skeletal muscle progenitors.

Exogenous stem cells

Despite the highly effective endogenous mechanisms of regeneration in skeletal muscle, increasing attention has been paid recently to external sources of myogenic cells. This is especially important for diseases such as Duchenne muscular dystrophy (DMD) where failure of the endogenous system seems to be an important aspect of the disease (Emery, 2001). In such a situation, an external source of muscle may hold two advantages. First, since it has not been involved in the repair of

diseased muscle, it will not have become exhausted by the process and thus represents a fresh source of autologous myogenic precursors that could be genetically corrected and re-implanted. Second, in some cases, such as bone marrow stem cells or meso-angioblasts, there is the possibility of dispersed delivery of the cells via the blood, thus minimizing the problems of limited migration of myoblasts directly injected into the muscle.

Bone Marrow Stem Cells

Bone marrow as an especially versatile site of mesenchymal stem cell activity, has figured large as a putative source of stem cells with neurogenic and cardiomyogenic potential (Verfaillie *et al.*, 2002 ; Verfaillie *et al.*, 2003). It has the supreme advantage of relatively easy access together with a complete recovery even in the adult donor and the possibility of repeated harvesting. As an experimental system too, it benefits from a number of facilitating features. It is one of the simpler sources of cells, these cells are relatively easy to fractionate by various sorting protocols and they can be routinely established in recipients of a number of species as the main component of bone marrow. This has proven particularly useful where the source can be chosen to carry a genetic marker such as the Y chromosome or an easily identifiable transgene that can be identified in the target tissue. Given such advantages it is probably not coincidental that it is the commonest demonstrated source of multiple stem cell activities. Apart from the virtue of the relative simplicity of experimentation, much of the attraction of bone marrow as a therapeutic source is that it disseminates its stem cells via the blood vascular system, raising the possibility of using this route to distribute the stem cells to the sites where they are required (Ferrari *et al.*, 1998) (Gussoni *et al.*, 1999). This hope seems to derive from a view that, among its functions, bone marrow acts constitutively as a central distribution depot of stem cells to a broad range of tissues (Blau *et al.*, 2001). If it were so, such a mechanism would, almost by definition (having been selected for functional efficacy) deal at least to some extent, with all of the problems of using stem cells for therapeutic reconstitution of adult tissues. That is, there would be an adequate source, effective conversion to the appropriate cell type, well-targeted and efficient delivery to the tissue and good functional integration of these stem cells into that tissue. Up to now, this hope has not been sustained by any results that would indicate that this mechanism operates sufficiently actively to have significant physiological impact in mouse (Ferrari *et al.*, 2001) or in man (Gussoni *et al.*, 2002). Indeed, in dystrophic skeletal muscle that has been been subjected to a high dose of local ionizing radiation to block the endogenous regenerative response, no significant rescue occurs from any circulating cells with myogenic potential (Heslop, 2000).

In a number of cases there is some doubt as to whether the contribution to a particular tissue is indeed a genuine trans-differentiation by a cell with one commitment into an unrelated cell-type since several instances have been found to involve fusion of the supposed stem cell with a cell of the target tissue (Camargo *et al.*, 2003) (Corbel *et al.*, 2003). There do remain however several instances where the trans-differentiation event appears to be genuine. For example, GFP-labelled bone marrow does, in some cases, seem to acquire myogenic markers prior to fusion with the muscle fibre (LaBarge and Blau, 2002) (Dreyfus *et al.*, 2004) (Fukada *et al.*, 2002).

Embryonic Stem Cells

Among the more controversial potential sources of multipotent stem cells are Embryonic Stem (ES) cells derived from the inner cell mass of early embryos at the blastocyst stage. Very similar cells derived from the germinal cells in the later embryo gonad primordia are call Embryonic gonadal (EG) cells. ES cells have proved valuable experimental tools in the mouse where their ability to become fully integrated into other mouse blastocysts into which they are injected has been widely exploited in the generation of gene-targeted mice. On the other hand, they do not spontaneously integrate well into tissues of adult mice, being characterized by the formation of teratomas (Verfaillie *et al.*, 2002). A number of regimes have been developed to adapt embryonic stem cells to appropriate differentiative fates on injection, in particular, into the nervous system (Wernig *et al.*, 2004) but they they have not been applied convincingly to the mass regeneration of major tissues in the adult.

Adult Mesenchymal Stem Cells

Several laboratories have reported the presence in the adult of stem cells with the ability to differentiate into a broad range of mesenchymal tissues and were given the epithet Mesenchymal Stem Cells (MSC). Initially these were isolated by a very stringent tissue culture regime from adult bone marrow (Reyes and Verfaillie, 2001) but cells with similar properties have been described from a number of other mesenchymal tissue sources (Jiang *et al.*, 2002). To general surprise, these cells have shown themselves to be capable of transcending germ layer boundaries and giving rise to tissues of neurectodermal and endodermal origins (Verfaillie, 2002 ; Verfaillie *et al.*, 2002). Although the majority of demonstrations of these capabilities have been performed in tissue culture, adult mesenchymal stem cells are now beginning to be tested *in vivo* for

their ability to reconstitute more than the haematopoietic lineages. Certainly, there have been numerous demonstrations of marked MSCs of various origins contributing to a variety of tissues and acquiring at least some ot the phenotypic markers of those tissues together with several demonstrations of functional improvement of lesioned tissues that have been injected with these cells, but the mechanisms whereby these improvements were mediated has not been fully resolved and the utility of these cells for effective reconstruction of a tissue *in vivo* has yet to be established.

On the other hand, a near relative of the MSC has been isolated from blood vessels of the embryonic and, more recently, the adult mouse. These carry a number of stem cell markers but also antigens characteristic of endothelial cells and have been named Meso-angioblasts (Cossu and Bianco, 2003). Like MSCs they are capable of differentiating into a number of mesenchymal lineages. More interestingly, they have been demonstrated to enter usefully into the reconstruction of genetically defective muscle tissue ; cells obtained from a normal donor have been successfully delivered by intra-arterial injection to muscles of the a-sarcoglycan dystrophic mouse where they impact in the muscle microvasculature, and progressively replace the dystrophic muscle fibres (Sampaolesi *et al.*, 2003).

It has become a question of more than purely academic interest to examine the relationships between the various cells that fall into the mesenchymal stem cell category. While there are several reports, particularly with cells derived from the bone marrow and haematological systems, that they show considerable versatility as to their choice of fates, this very property often carries with it the disadvantages of low conversion rates and poor overall yield. Other preparations, such as the dermal fibroblast (Gibson *et al.*, 1995) have been shown to be convert very efficiently into a single cell type in response to a specific signal (Wise *et al.*, 1996) a property that would seem advantageous for the majority of therapeutic applications.

Delivery

In most tissues, the matter of delivering the stem cells to the correct place is a crucial consideration. Some sites are readily accessible, e.g. the skin or the retina. Others, such as specific and limited sites of lesion in the central nervous system or the heart can similarly be reached by carefully targeted injection. Many tissues however, notably the diffusely distributed mass of skeletal muscle that is subject to degeneration in Duchenne's muscular dystrophy are not practical targets of local injection except for the limited objective of augmenting the strength of a few crucial muscles. Thus the systemic route that is, in principle, feasible with some of the mesenchymal stem cells is a high-priority objective despite its lack of immediate promise.

Conversion to target tissue cell types and integration

As hinted in a number of previous sections, this remains a major question. Is it possible to persuade stem cells to accurately convert to the appropriate cell types and adopt the correct configuration with one another to reconstruct functional tissues ? As illustrated in figure 1, the classic pathway by which stem cells pass through various stages of development is negotiated by virtue of a series of inductive signalling molecules. When we seek to use a stem cell at a particular point in this pathway, we need to accurately simulate or replace those signals in order to put the cell at the optimal developmental stage, in most cases, ideally, as the immediate precursor cell of the target tissue.

Apart from the matter of inducing stem cells to differentiate in a manner appropriate to the target tissue, there is a separate question of persuading them to integrate spatially and functionally into that tissue. In practice, these properties are closely linked because some of the proteins that characterize a given cell type are implicated in its establishment of functional relationships with other cells in the tissue. However, when attempting to generate such relationships in the post-natal individual, further restraints become evident. During embryonic development, most tissues acquire their gross form and structure on a small scale, where cell contact and diffusive signals are able to operate, and subsequently expand. By and large, such developmental mechanisms are not available on the larger postnatal scale and it remains a matter of considerable doubt therefore, whether stem cells can be used to reconstruct large discontinuities in tissues of the adult. For repair of small lesions within tissues, such as those resulting from DMD for instance, there is more hope of useful participation of stem cells, indeed it is largely a question of semantics whether the endogenous tissue precursors such as the satellite cells of skeletal muscle qualify as stem cells in this context.

Practical use of stem cells for therapeutic purposes, raises the same question on a larger scale ; having arrived in the target tissue and having differentiated into the appropriate cell types and formed the correct associations at the local level, can the stem cell progeny integrate into the target tissue in such a way as to restore normal function of the tissue as an integrated part of the body ? This is perhaps the least well established aspect of the technology and presents a particularly formidable challenge for the attempted repair or reconstruction of skeletal muscle and still more for the central nervous system where re-establishment of precise intercellular connections to remote sites is crucial

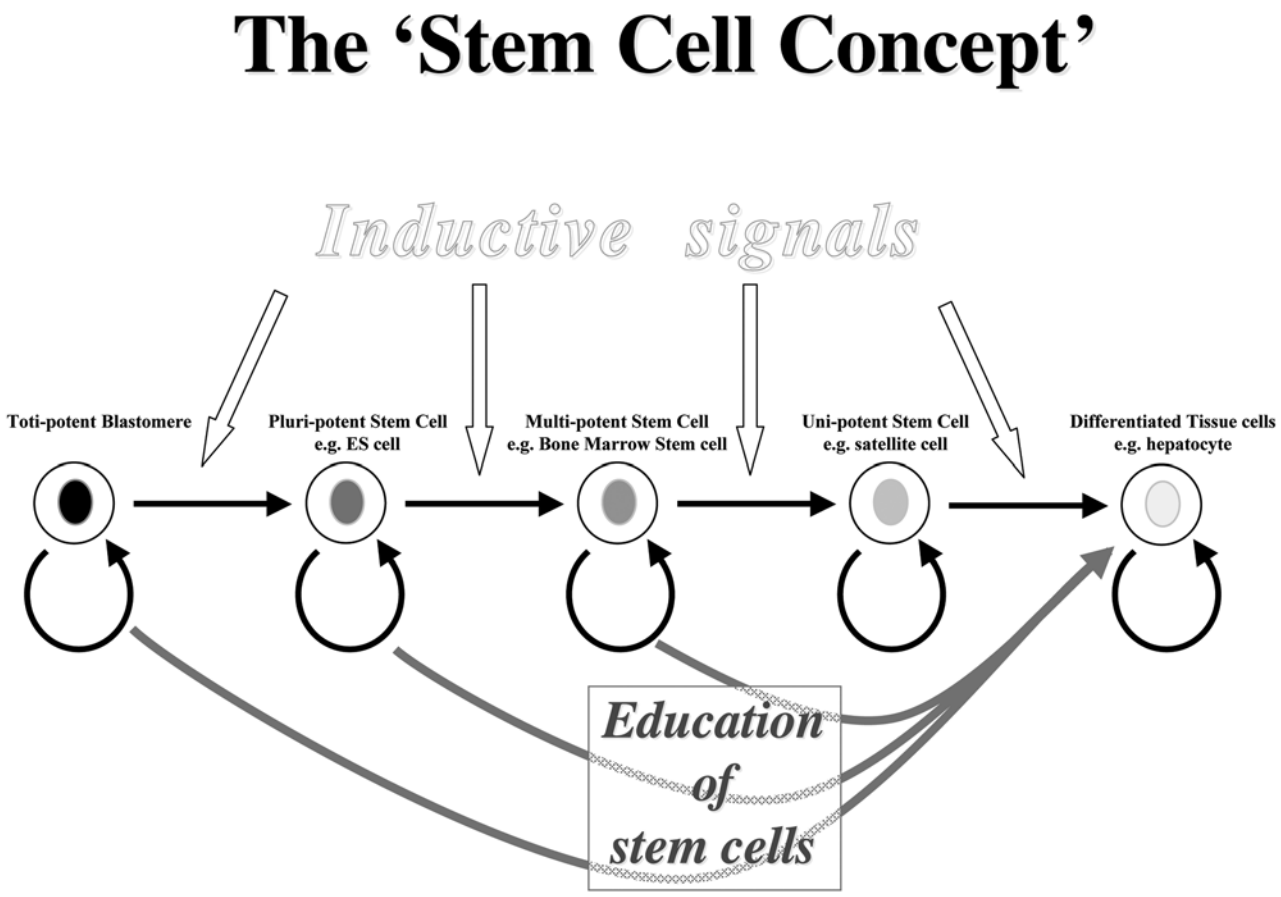

FIG. 1. — A simplified schematic representation of the relationships between various classes of stem cell as envisaged in the standard hierarchical developmental model. This depicts development, as a progressive restriction in the variety of fates open to cells as they descend a hierarchy of 'stem cells'. At each level, cells are capable of asymmetric divisions to replaces themselves and to give rise to cells that are committed to expansion of the next lower level in the hierarchy. One view of the phenomenon of stem cells in adult tissues is that they represent cells within this hierarchy that remain fixed at a particular status and persist quiescently within the adult tissues. To make use of such stem cells, or of embryonic stem cells, we must find a means of replacing or simulating the cascade of inductive signals that normally operates to move these cells down the desired developmental pathway.

Another view would be that these adult, multipotent stem cells re-acquire their potential via a process of de-differentiation to an earlier developmental stage or, alternatively, are able to trans-differentiate by a straightforward switch from one programme to another. In these cases too, there is a need to identify the signals that can efficiently move them into the desired part of the differentiative pathway.

to function. True, in both neural and muscular tissues, it has long been established that a surprising amount of restitution of structure and function is possible at a local level, or where structural cues are maintained, for instance of persistent myofibre basement membranes or of axonal tracts, but it seems unlikely that we will be able to rely on quasi-developmental processes for this purpose on any but the smallest scale.

Conclusion

It is of value to put some perspective on the promise of stem cell therapy for neuromuscular disease, if only to learn lessons from gene therapy that has been a disappointment to many scientists and to the media that inform the public. This is more a reflection of inflated expectations than of poor scientific progress and to avoid a repetition, it is sensible not to underestimate the hurdles to be surmounted in bringing the stem cell to therapy. This brief survey reveals no shortage of sources of supply of stem cells. Nor, in many cases, is there any difficulty in expanding these cells to large numbers without apparent loss of vigour. The main areas in need of development are those concerned with properly accommodating these cells within the target so as to achieve therapeutic function in vivo, i.e. the problems of delivery into the sites they are to repair or replace and of directing them into appropriate paths of differentiation and organization within the target tissue. In the case of both muscle and of neurons, this is a no simple task, because their proper function depends so critically on achieving correct positioning and connections. For glial cell and Schwann cell replacement the presence of an even partially functional neuronal structure would facilitate these requirements.

Since, to achieve these ends, we must rely ultimately on the self-assembly systems that operate to maintain normal tissue structure, it is an important to aim to drive the differentiation of the stem cells to a precursor stage as well as to the mature tissue cell. The success of stem cell strategies will also depend crucially on the extent and degree of loss of structure of the tissue. Scarring of muscle in DMD or gliosis in the CNS, seem likely to be formidable barriers to reconstruction of the original tissue. Such problems would be best avoided by undertaking therapy as early as possible during the disease process, but the hope of some restitution of tissue structure remains an important advantage of early precursor, or stem cells over more straightforward therapies based on gene delivery to the tissue by viral or non-viral vectors. To achieve such aims requires that we gain a better understanding than we presently enjoy of the normal biological control mechanisms that regulate and maintain the structure of muscular and neural tissues.

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