The Role of Satellite Cells and Circulating Stem Cells in Regeneration of Skeletal Muscle

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Abstract.

Repair and regeneration of skeletal muscle in the postnatal mammal has long been supposed to be primarily or entirely accomplished by the population of satellite cells that are sequestered between the plasma membane of the muscle fibre and its overlying basement membrane. However it has never been clear whether all satellite cells participate and whether only satellite cells participate in this process. This question has been brought into focus with recent demonstrations that cells originating from the bone marrow occasionally become incorportated into regenerating muscle fibres. This article reviews the evidence for the relative roles of local and circulating cells in this process.

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Introduction

So crucial a tissue as skeletal muscle must have a rapid and efficient repair mechanism to minimize the incapacitating effects of severe damage. There has been, however, much debate as to whether so complex and highly organized an array of syncytial cells could be regenerated and, if so, how, this could occur in postnatal muscle. The matter appeared to be resolved to most peoples' satisfaction by the demonstration that the multinucleate muscle fibre was formed by the fusion of myogenic precursors[15] followed closely by the identification of the satellite cell as the candidate for the myogenic precursor cell of post-natal skeletal muscle [14, 16]. Again at much the same time, evidence of the powerful intrinsic organizational mechanisms residing in the tissue came from the demonstrations by Studitsky [25] and Carlson [4] of the remodeling of functional skeletal muscles from autografts of muscle whose structure had been completely disrupted by mechanical mincing. On these bases, a model of muscle regeneration was built in which the satellite cells were envisaged as the committed muscle-specific stem cell, residing in a quiescent G_O state in normal conditions but becoming activated by local damage into a state of rapid proliferation followed by cell fusion to repair or replaced the damaged syncytial muscle fibres.

This model has persisted largely intact until recent times when it has been brought into a state of some turmoil by demonstrations of apparent versatility of stem cells or precursor cells present in various tissues, whereby they are able to contribute to tissues other than that from which they are derived. In the case of muscle, it was shown that bone marrow derived cells could contribute to regenerating muscle [5] -and, conversely, that cells derived from skeletal muscle were able to fully reconstitute the haematopoietic system [10]. This article attempts to put these two apparently alternative mechanisms of muscle regeneration into perspective and to identify the main questions that arise from the current picture of muscle regeneration.

First, it must be said that the original picture of the satellite cell as the sole source of myogenic cells for muscle regeneration, although it was consistent with the available data, was not critically tested by it. This arises mainly from the difficulty of marrying functional data to the essentially anatomical definition of the satellite cell by its position between the muscle fibre plasmalemma and the overlying basement membrane, for this relationship is usually disturbed during the regenerative process. Early labeling studies, have shown a sequential appearance of thymidine labeled nuclei first in satellite cells and subsequently in myonuclei of the repaired muscle fibres [9, 17, 19, 24] but this again is not a critical test of the relationship since cells other than satellite cells are also labeled and could, in principle, also have contributed to the regenerated muscle.

A major hindrance to any attempt to link the satellite cell to function has been the technical difficulty, until very recently, of identifying it by any means other than electron microscopy, with its inherent sampling problems. To some extent, this has been solved in recent years by the discovery of a number of markers[2, 7, 13,

23], none of which is unique to the satellite cell but which, applied shrewdly to defined systems, is sufficiently discriminating to provide useful quantitative data on numbers, distribution and rates of proliferation.

Standardized highly reproducible models of muscle regeneration have also been, and largely remain, an obstacle to comparative studies of regeneration and of factors that modulate its efficiency. Most experimental models are based on traumatic injury: e.g. by physical crushing, freeze-thaw, heat, by the anoxia induced by grafting or devascularization, or by injection of myotoxic agents such as venoms, local anaesthetics or $BaCl_2$. These are often difficult to set up as highly reproducible techniques, particularly for between-laboratory comparisions and all such large scale models suffer from the fact that they generate an asynchronous process such that the successive stages of necrosis, inflammation, cell proliferation, revascularization, myoblast fusion, re-innervation and fibre maturation occur with different degrees of delay in different parts of the injured tissue so that they overlap one another and to varying extents interfere with one another. Although this may well reflect the mechanisms that occur naturally in injured muscle, this admixture of different processes makes it difficult to analyze their individual contributions to the eventual outcome or to determine the mechanisms by which any given treatment of the system modulates the process. Moreover it is difficult to kill a precise volume of muscle without inflicting damage to other tissues, such as the vascular or neural supply, each of which can exert major impacts on the final outcome.

Tissue culture models of skeletal muscle regeneration have also been much studied, largely because of the compliant nature of myogenic cells in culture. It is possible to routinely derive tissue cultures of skeletal muscle cells from samples of muscle of most species, and a number of cell lines derived from rat and mouse are readily available as laboratory reagents. In general, it is possible to keep these cells in a proliferative phase in a medium rich in serum and growth factors and to stimulate their withdrawal from proliferation and entry into terminal differentiation and cell fusion by substitution of a medium impoverished in growth factors. Although the myofibres produced by such systems remain at an early stage of differentiation, they do signal the achievement of most of the important phases of muscle regeneration. In addition, there is some parallel in culture with the maintenance of a population of reserve cells that do not enter terminal myogenesis but enter a quiescent non differentiated state [2, 22, 28] while retaining the ability to generate further committed myogenic cells to cope with subsequent bouts of muscle damage.

Despite these various cautions, discrepancies between the various studies are not so large as to suggest that radically different mechanisms are operating in different systems, a state of affairs that suggests a highly regulated system of control of the mechanisms of muscle regeneration. Thus far, we do not have sufficient information on these mechanisms to erect testable hypotheses as to how they might account for the degree of accuracy of operation of the system. In particular, we know too little about the frequency and timing of asymmetric divisions, required to retain some cells in the precursor cell compartment, probably as satellite cells, versus symmetrical divisions required for rapid expansion of the myoblast pool.

The Satellite Cell

Most studies of the mechanisms that influence the proliferation, differentiation and fusion of myogenic cells have been conducted on cultures of myogenic cells derived by simple disaggregation of skeletal muscle, commonly from neonatal or foetal animals, sometimes accompanied by some form of enrichment protocol, to eliminate non-myogenic cells. As discussed above few studies can claim to have studied the activities of the satellite cell, as distinct from the myogenic cells that can be cultured from skeletal muscle, In many cases, it is likely that these cells were in fact derived from satellite cells that were present in the original muscle, but it is uncertain whether they give and unbiased representation of these cells. This question is further compounded by an issue that becomes progressively more important with increasing time in culture: namely, whether this experience would predispose to 'culture-friendly' phenotypes in emerging cell populations. For the selective influences on survival and proliferation in tissue culture appear to differ greatly from those that operate in vivo. This is illustrated for example by the finding that when satellite cells are grafted into a recipient muscle, the majority die very rapidly [1, 12, 21] although these same cells had proliferated rapidly and incorporated radiolabelled thymidine in tissue culture. In contrast, the minority that survive and proliferate at the graft site had not incorporated radiolabel in culture and had therefore been cycling only slowly prior to grafting [1]. Nonetheless, study of such myogenic cultures, notably with rodent cell lines such as C2 or L6 have given many valuable insights into the fundamental mechanisms of muscle differentiation and of the various cytokines involved in myogenesis and into the mechanisms via which these operate on the control of cell processes. In consequence, skeletal muscle has been established as one of the archetypes of differentiative processes in vertebrates. The extent to which these developmental processes persist into adult life as control mechanisms for the maintenance as opposed to the initial development of muscle is not known. There appears to be a tacit assumption that muscle regeneration, since it superficially parallels developmental process, is subject to the same mechanisms of control. But this is clearly an oversimplification since the regeneration process is

heavily intermingled with pathological events and is targeted at restitution rather than neogenesis of muscles and must therefore involve some different feedback systems.

Muscle stem cells

The strong, though largely circumstantial evidence that satellite cells are the source of myogenic cells for regeneration of damaged muscle has not been able to address the question as to whether these are the only or even the major source of repair. This question has received fresh stimulus from a number of recent studies showing input into regenerating muscle from cells of bone marrow origin that are presumed to have infiltrated the muscle from the circulation. These findings appear to contradict earlier studies that had failed to find any such contribution from the bone marrow to muscle regeneration [8, 18]. Moreover, local irradiation of the spontaneously degenerative muscle of the mdx mouse had completely blocked local regeneration [20, 26, 27], implying that this muscle could receive no significant input of myogenic cells from elsewhere in the body. However, the positive findings of an input into skeletal muscle have all made use of very sensitive markers that have only recently become available and are able to detect an input into muscle of very small numbers of myogenic cells. Indeed, the amount of muscle reported to be derived from systemically distributed myogenic cells has been well below the levels that would be required to have any functional impact. The nature of a phenomenon that, in itself, is too minor to be selectable by Darwinian mechanisms is a matter of general biological interest but unless it can be raised above these trace levels it is not of therapeutic value [6].

At the same time, we have shown recently, that the grafts of myoblasts, clonally derived in some cases from known satellite cells [3],are able not only to provide cells that will regenerated damaged muscle but are also able to replace satellite cells on these fibres [11]. When single fibres were kept in culture in growth medium, they proliferated rapidly to provide sufficient cells over 3-4 days to totally replace the fibre with which they were initially associated [29]. This period corresponds well to the time during which myoblasts are observed to proliferate after an acute injury to skeletal muscle. This does not prove that the satellite cells are the only source of myogenic cells during differentiation but it does demonstrate that they are fully capable of fulfilling this role.

In short, present evidence suggests that circulating cells, derived in at least some cases from the bone marrow haematopoietic system, has the potential to contribute diffusely to muscle regeneration. Data on this phenomenon are at best semi-quantitative but do not indicate that this occurs with a frequency that could be of physiological relevance to the muscle nor, as things presently stand, of therapeutic value in the treatment of genetic myopathies. A great deal of research effort is currently being directed at gaining an understanding of the mechanisms involved in this systemic contribution to muscle regeneration in the hope that the efficiency of the system can be enhanced to therapeutic levels. Satellite cells on the other hand, can be shown to be a necessary and sufficient source of myoblasts for regeneration of muscle but we do not have more than an outline view of the process and, again, an understanding of the controlling factors might permit us to manipulate it to therapeutic advantage for both inherited and acquired muscle diseases.

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