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Regeneration of mamalian striated muscle

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Summary

Six main factors limit the regeneration of mamalian striated muscles: the myogenic cells, the basal membranes, the vascular supply, the innervation, the mechanical forces and the functional utilization. Their rôle is shortly discussed.

Introduction

Muscle remarkably adapts to the many situations which affect the life of any individual : growth during young age, hypertrophy during training, as well as many metabolic perturbations, such as, for instance, hyperkalemia which triggers an increase in Na-K pump density of the muscle membranes. The effects of a lesion on muscle are less well known. Necrotic muscle cells are often replaced by scars of connective tissue. It is argued that this response is unavoidable, since adult muscle is a very differentiated tissue, for that reason, unable to regenerate new muscle cells. Common clinical practice supports this view, since deep burns or lesions to an arterial trunk such as is the case in the Volkmann contracture for example, lead to scarring and not to the regeneration of muscle.

There are, however, experimental situations in which one can observe an extensive regeneration of muscle tissue. One of the most convincing was reported by Studitsky (1), working in Moscow. His experiment was later confirmed by Carlson (2) and by many others, including ourselves.

In the basic experiment of Studitsky, the triceps surae of a rat is excised, minced and grafted back into its original bed, taking care to tuck deep into the graft the extremities of the severed vascular and nervous trunks. If the leg is reopened one month later, one sees that the minced muscle has been replaced by a regenerated muscle, which is contractile, vascularized, innervated and connected to the bones through tendons. The newly formed muscle fibres are parallel to the axis of the leg, and the animal uses the muscle for walking. In the best cases, a nearly complete clinical recovery can occur, and the animal is able to walk and run quasi normally. The only obvious difference with a control animal is that the size of the newly formed muscle is smaller (approximately 25% to 30% of normal).

Many experimental reports have been published on the morphological biochemical and physiological aspects of the regeneration in

Studitsky's model; several other experimental models have been reported, in which muscles subjected to various lesions (ischemia, snake venom toxins, local anaesthetics, local injury, free grafts) also show regeneration; they are criticized in several recent reviews, to which we refer for further information (2, 4 to 9).

We want to focalize this review on a specific question : how far can we now trigger and control the regeneration of striated muscle and what might be the strategy in this respect?

The factors of muscle regeneration

We know that at least six factors are important in the control of regeneration :

1. The myogenic stem cells.

Striated muscles normally contain so-called satellite cells (10). These cells are located in the endomysial tubes, in close apposition to the muscle fibres. They are activated when the parent muscle fibre is injured (11). They proliferate, fuse into myotubes and, if suitably innervated, they differentiate into a normal adult muscle fibre. At the same time, transitions occur in the isomyosins : first, they are embryonic in type, then foetal and finally adult (12). If the myogenic cells of a minced muscle are killed by a freeze and thaw procedure, the graft develops into an adipo-connective tissue which is totally void of any muscle cells (13). But if this devitalized mince is seeded with autologous myogenic cells (i.e. cells obtained from another muscle of the animal) (14), then a few adult muscle fibres are seen in the graft. These muscle cells are normal in their aspects, although somewhat thin (mean diameter 30 µm) and short (2 to 6 mm in length). Their orientation is parallel to the axis of the leg and their tetanic contraction appears to be normal. Unfortunately, their number is quite low, approximately 10 times lower than that observed in a muscle regenerating from a viable mince in Studitsky's experiment. This low efficiency cannot be explained by a low number of seeded cells since the number of cells initially seeded into the devitalized graft is approximately the same than that contained in a Studitsky's mince. It is thus obvious that although myogenic stem cells must be present in order to induce muscle regeneration, other factors are at play that can severely limit their efficiency.

2. The endomysial tubes.

Muscle fibres are encased in so-called endomysial tubes, the structure of which is quite complex (15). The tubes are made of a special type of collagen (collagen type V) which is mainly found in foetal membranes, and also of some collagen type III (typical of blood vessels) and type IV (typical of placenta). They also contain non-collagen proteins (such as

fibronectin) and some granules filled with glycosaminoglycans. Wandering cells can usually cross basal membranes : this occurs when phagocytic cells attack necrotic fibres. Satellite cells and their daughter cells cannot cross it : therefore the myogenic cells are constrained to the inside of the endomysial tubes. If the muscle fibres are destroyed by local anaesthetics (16), snake venom toxins (17) or, more simply, by mechanical compression (11), they die and are phagocytized; the satellite cells proliferate inside the tubes, they fuse and regenerate new muscle fibres. The regeneration in thus fast and morphologically complete. However if the tubes are broken, the myogenic cells spill into the surrounding extracellular spaces, and the regeneration is impaired. This is obviously a major factor which accounts for the relatively poor regeneration in Studitsky's experiment, where endomysial tubes are severed at many places. However, the myogenic cells are still encased in some remnants of the endomysial tubes and thus able to start the regeneration in a favourable environment. The myogenic stem cells that are seeded into a devitalized mince (experiments of Ghins et al, 1985a, 1985b) are in a less favourable position, since they are totally lacking endomysial tubes. This would explain their low capability of regenerating a muscle. This hypothesis predicts that myogenic stem cells added to a Studitsky's mince would add little to the regeneration of minced muscle. This has been effectively observed (18). We come thus to the very important conclusion that regeneration cannot be enhanced by injecting myogenic cells into the muscle that must be restored.

3. The vascular supply.

Ischemia rapidly entails necrosis of muscle cells. Regeneration occurs only if blood supply is restored. Muscle free grafts and minced muscle regenerate to the extent of blood vessels invasion. This factor limits regeneration to grafts smaller than 1 to 2 g. Heavier grafts will regenerate only if the blood supply is restored by vascular microsurgery.

4. The nervous supply.

If the nervous supply has been preserved (as is the case in innervated free grafts), then the neuro-muscular jonctions (which are very sensitive to ischemy) degenerate, but they regenerate quickly, within 8 days (19). If the nervous supply has been severed, (standard free grafts), then the first neuro-muscular junctions are only seen three weeks after the lesion. The extent of the regeneration depends also on the state of the nerve supply. In innervated free grafts, the number of fibres remain unchanged, but they are thinner than normal : the maximal force is thus somewhat weakened (80% of normal) (20). In standard free grafts, the maximal force of the regenerated muscle is lowered to 50% of normal, or less. The loss of force is not so much explained by a loss of intrinsic force of the muscle fibres (the force per unit area of fibres remains normal)

as by the thinness of the fibres, which itself might be explained by the weakness of the nervous supply, since the number of motor units is strikingly decreased by half (19,20). This work, as well as the work of other authors (21 to 23) suggest that the preservation of the original neuro-muscular junctions is important for the rate and the quality of the regeneration. In free grafts, the regenerating nervous fibres are not guided by the endoneural tubes towards the former synaptic regions : therefore the probability is low that they find their way back to their former innervation site. If the neuro-muscular junctions are excised (23) or if the reinnervation is prevented, regeneration aborts.

5. Mechanical forces.

There are very few studies on the action of mechanical forces on muscle regeneration. In Studitsky's model, the newly formed muscle fibres look like tortuous ribbons at the early stages of regeneration, but very rapidly, within 3 to 4 weeks, they align nearly perfectly along the long axis of the leg. The mechanism of this orientation is unknown, but it seems reasonable to assume that the muscle fibres align along the mechanical stresses which occur during passive movements of the leg and the foot. During maturation, there occurs a shift to the left of the force-length diagram of the maximal isometric force (3), a fact which also gives evidence of the occurrence of internal reorganization of the muscle architecture (5). Allbrook (5) has pointed out the importance of ensuring correct alignment and tensions of a repaired muscle belly. The forces developed by the contractile muscle could also play a rôle in the restructuration of the regenerating muscle. A few necrotic muscle fibres are commonly observed in muscle regenerating for 60 days (18). The significance of this fact is unknown : it may be that these fibres were wrongly aligned and were killed by excessive mechanical stresses. Since torn fibres could be replaced by neo-formed fibres, one has here the basis of a mechanism to replace misoriented fibres by correctly oriented fibres.

6. Functional utilisation.

It seems that active mobilization stimulates muscular regeneration (24,8). The importance of this effect was beautifully demonstrated by Smallbruch (25) with the use of the model of compensatory hypertrophy after tenotomy of synergists. He replaced the rat triceps surae by a free graft of soleus : 30 days later, the weight and the number of fibres were 50% higher in the regenerating graft than in a normal soleus. It is thus probable that muscle regeneration in grafts (minced or not) could be improved with a suitable training program.

Conclusion

The information collected up to now on the regeneration of muscle leads already to well accepted principles in the care of muscle lesions. One should spare myogenic stem cells as much as possible, by as small debridment as is compatible with a sound prevention of infection, maintain the connective architecture of the basal membranes, restore blood supply by microvascular anastomoses and the innervation by microsurgical nerve suture, align and stretch properly the muscle to be grafted, and design an early training program for muscle which is compatible with the immobilization required to unite the fractures which are frequently associated with muscle lesions.

It is tempting to speculate whether it is possible to go beyond this. Could it be possible, for instance, to isolate myogenic cells from a patient, grow then in culture in large masses, and embed them in some suitable medium in order to artificially replace a failing muscle in the patient? This aim is not yet possible, mainly because myogenic cells deprived of endomysial tubes regenerate few new muscle cells and are difficult to reinnervate. It may be that in the future some biocompatible support may be discovered which could play the rôle of endomysial tubes and allow a regeneration of a stronger muscle.

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