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The involvement of galectin-1 in skeletal muscle determination, differentiation and regeneration

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The dogma that a cell is rigidly committed to one tissue type has been heavily challenged over the past few years with numerous reports of transdifferentiation of cells between different lineages. Cells capable of entering lineages other than that of their tissue of origin have been identified in several diverse tissues. Recently we have focussed on a non-committed myogenic cell within the dermis that is capable, under certain conditions, of expressing muscle specific markers and even fusing to the terminally differentiated stage of muscle cell development. We have identified galectin-1 as being a potent factor implicated in this process. In this review we discuss our findings and consider the involvement of galectin-1 in muscle determination, differentiation and regeneration.
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A wealth of recent literature has questioned the belief that once a cell is committed to a particular differentiation pathway its fate is sealed and it cannot change its developmental course. However, many tissues have now been shown to harbour cells capable of differentiating into a lineage, which differs from that of the tissue of origin (for reviews see *Journal of Pathology*, Volume 197). In this respect we have been studying a cell resident in the dermis that is capable of entering the myogenic lineage [1–5]. As yet we have not fully characterised this cell, but it would appear that there are only a limited number of them within the dermis. This became evident when we cultured mouse dermal fibroblasts in medium in which muscle cells had previously been grown. Under such conditions, only a small percentage of the original culture expressed the myogenic marker MyoD and fused to the multinucleate state [2]. Such expression of myogenicity was not observed in cells cultured in control medium. An intriguing observation from this work was that contact between the dermal fibroblast and muscle cells was not required for this shift of dermal cells towards the myogenic lineage, thus suggesting the presence of a soluble factor within the muscle conditioned medium. This was supported by the work of Breton *et al.* [6] who also proposed the involvement of a soluble factor in causing fibroblasts to become myoblasts. Moreover, by using the lacZ reporter gene driven by a muscle specific promoter, they noted that conversion occurred prior to fusion. Our results, and those of Breton *et al.* led us to search for a candidate soluble factor.

Galectin-1 is present in striated and smooth muscle although its expression depends on the physiological or developmental stage of the tissue [7]. In relation to skeletal muscle, it is present in the cytoplasm of myoblasts, but is released into the extracellular compartment upon fusion of myoblasts to the terminally differentiated myotube stage of development [8,9]. Several authors have reported varying effects of galectin-1 on growth and proliferation of muscle cell lines *in vitro* [10–15]. We therefore queried whether galectin-1 might be the factor within conditioned medium responsible for the conversion of dermal fibroblasts.

Growing of murine dermal fibroblasts in medium harvested from COS-1 cells transfected with plasmid containing a galectin-1 construct (COS-1 cells had previously been shown to secrete galectin-1 into the extracellular environment [16]) resulted in up to 30% of cultured fibroblasts expressing muscle specific markers [3]. This is a much greater percentage than had been observed when culturing these cells in medium conditioned by muscle cells, where, in our hands, following growth in medium harvested from the murine C2C12 mouse cell line, only

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5% of cells in culture were capable of expressing MyoD [2]. Western blot analysis of the muscle cell conditioned medium, and that derived from COS-1 cells, confirmed both media contained galectin-1. A much higher level of expression in the COS-1 transfected medium *i.e.* of the order of 18.5 ug/ml as compared with 9–10 ug/ml within the muscle cell conditioned medium could possibly account for the higher level of conversion when culturing cells in COS-1 transfected as opposed to muscle cell conditioned medium [4]. Cloning of the dermal fibroblasts resulted in even more cells converting. We found no evidence of conversion of clones grown in control medium whereas 100% of cells converted into the myogenic lineage in three dermal fibroblast clones that had been grown in COS-1 transfected medium. Exposure to this medium also resulted in the expression of muscle specific markers in cultures of human dermal fibroblasts [5]. One further interesting observation was the absolute inability of fibroblasts derived from muscle to enter the myogenic lineage [4].

The ultimate aim of our work is to develop autologous cell based therapies for neuromuscular diseases such as Duchenne muscular dystrophy. With this in mind we are searching for a suitable, but readily available and accessible cell derived from the patient into which a functional copy of the gene defective in the myopathy can be inserted. Cells containing the introduced 'normal' gene could then be used to repopulate the patient's muscle, where their incorporation could result in expression of the introduced gene product within the newly formed muscle fibres [1]. A cell derived from the skin that can be manipulated to form muscle, under the influence of galectin-1, could be of great benefit. If this becomes therapeutically viable, it would be important to understand how galectin-1 interacts not only with the dermal fibroblast but also with the converted cell as it is introduced into the host muscle. Two studies have reported an increase in fusion to the terminally differentiated state of rat L6 and L8 myoblasts in the presence of galectin-1 [11,12]. However, a further group [17] described that in the presence of a 14 kD lectin derived from chick, fusion was inhibition in primary chick myoblasts and also in a variety of rat cell lines. Following on from these studies we researched the effect of galectin-1 on murine myoblasts. The number of cells entering the terminally differentiated multinucleate stage was increased when primary myoblasts and cells derived from the C2C12 mouse muscle cell line were grown in galectin-1 [4]. These results, supporting those of Den and Malinzak [11] and Gartner and Podleski [12], confirm that galectin-1 enhances the fusion of myoblasts to myotubes and would explain why galectin-1 is expressed at high levels when maximum cell fusion is occurring during muscle development [7]. It has also been reported that myoblasts release galectin-1 while undergoing differentiation, but not while proliferating [8,9].

To further elucidate the involvement of galectin-1 in muscle determination and differentiation, we have initiated studies using cells derived from the galectin-1 null mouse [18]. Although the gross morphology of muscle derived from this mutant was reported as normal, the details of myoblast fusion and muscle differentiation had not been investigated in this animal. When grown in culture, in comparison to normal myoblasts, myoblasts from the galectin-1 mutant show reduced fusion to the multinucleate state, as indicated both visually (Figure 1) and by determining the fusion index of the cells. There is however, an increase in fusion of these cells towards levels observed in wild type myoblast cultures when galectin-1 is added to the growth medium [19]. This provides further evidence for the involvement of galectin-1 in promoting the terminal differentiation of myoblasts. We have no evidence as yet to suggest the involvement of different signalling pathways and cell surface receptors for the effect of galectin-1 in inducing myogenic determination in non-committed myogenic cells as opposed to its effect on inducing terminal differentiation in those cells already committed to the myogenic pathway. However, signalling pathways and receptor activation in the two cell types warrants further investigation.

Although at a very early stage in our investigations, we queried whether there is a delay in muscle development at

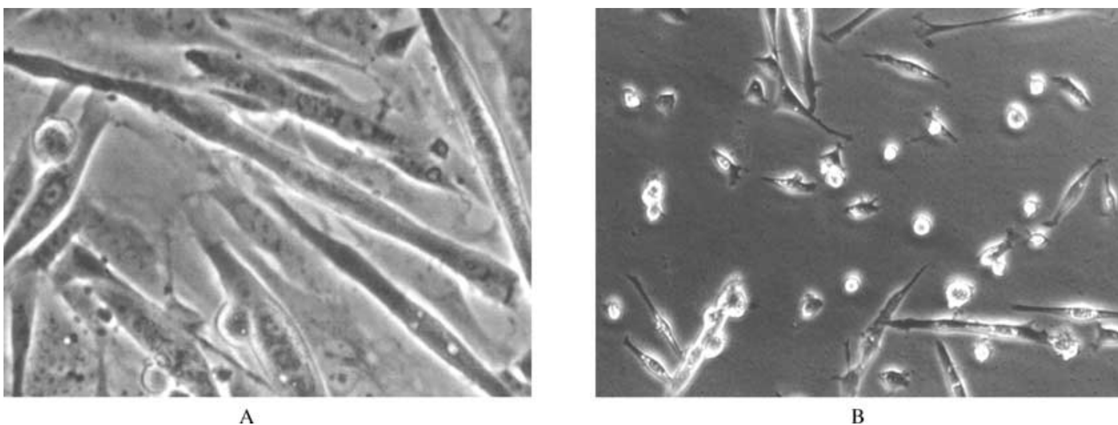

Figure 1. Primary myoblasts derived from the (A) normal mouse and (B) galectin-1 null mouse muscle. After 8 days in culture more mononuclear myoblasts had fused to the terminally differentiated state in control muscle than in galectin-1 null cultures.

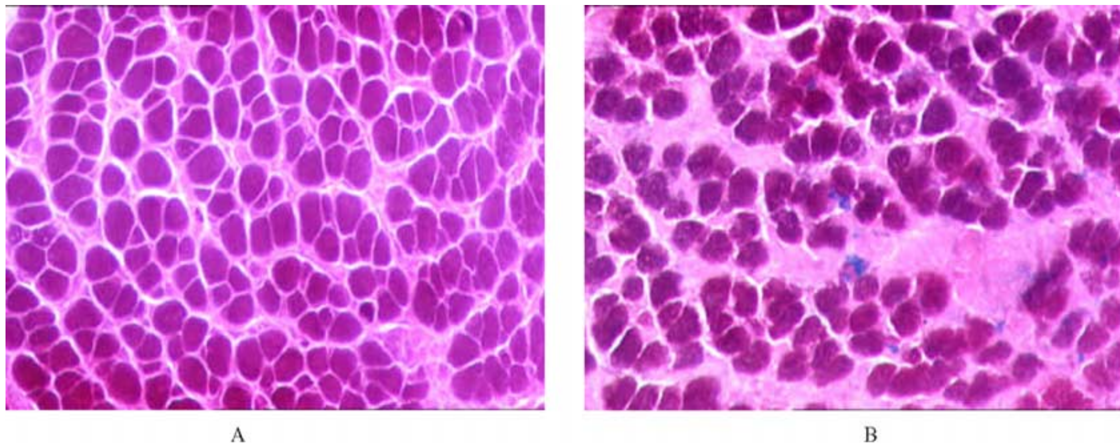

Figure 2. Cryostat sections cut from leg muscle of postnatal day 4 (A) control and (B) galectin-1 null mutant mouse. Control muscle forms well defined fascicles with very little intervening connective tissue, in contrast to the galectin-1 null muscle of comparable age. More connective tissue appears to be interspersed between the fibres of galectin-1 null muscle. Magnification ×200.

early post-natal stages in the galectin-1 null mouse, compared to normal muscle [19]. Between 1 to 4 days postnatally, muscle fascicle formation is less well defined, fibrous connective tissue elements more numerous, and an increased amount of adipose tissue is observed in mutant *versus* wild type muscle (Figure 2). By three weeks of age there is very little morphological difference discernible between wild type muscle and that of the galectin-1 null mutant, although there is some evidence of variation in fibre diameter size in the mutant *versus* normal muscle at this stage. This would suggest that after the initial delay observed during the early stages of muscle formation, skeletal muscle lacking galectin-1 has the ability to recover and form functional muscle. The delay in development may be the consequence of an inability of mononuclear myoblasts to fuse to the terminally differentiated state in the absence of galectin-1. Subsequent recovery of the muscle as growth ensues could implicate the presence of some other, as yet unidentified factor, capable of compensating for the lack of galectin-1. Further insight into the involvement of galectin-1 in muscle fibre formation could be gleaned by exploring the ability of galectin-1 null muscle to regenerate after the induction of trauma. Injection of 1.2% barium chloride into muscle causes widespread degeneration followed by an extensive regeneration of the fibres [20]. Five days after barium chloride exposure, the presence in normal muscle of numerous small diameter fibres with centrally placed nuclei, is an indicator of recent regeneration. However, this is not the case with galectin-1 null muscle where sections display far more connective tissue and a diminished number of regenerated fibres, in comparison to the control (Figure 3). Again this is suggestive of a delay in the formation of new muscle fibres

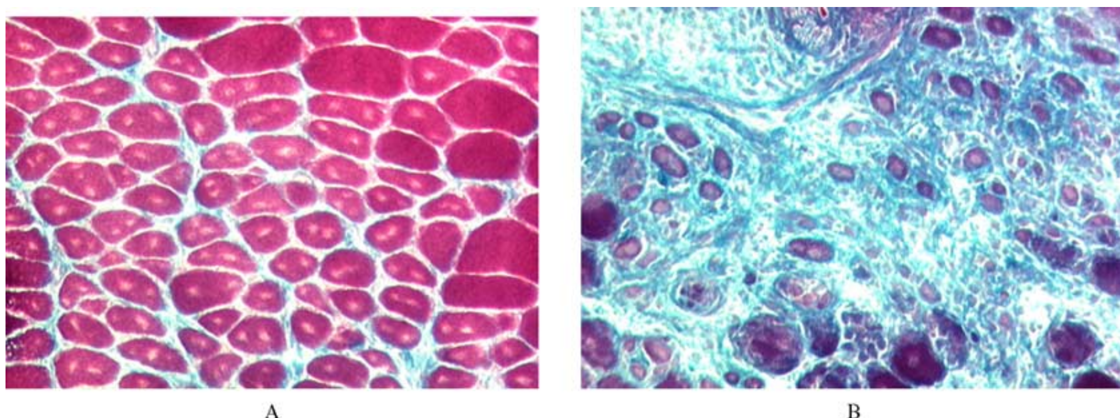

Figure 3. Cryostat section of tibialis anterior muscle from (A) normal and (B) galectin-1 null mouse 5 days after injection of barium chloride. The majority of muscle fibres in the normal mouse have undergone regeneration as can be seen from the presence of non-peripherally placed nuclei. A few fibres have not been affected by the barium chloride treatment and are of larger diameter than those that have regenerated. Much fewer fibres have undergone regeneration in the galectin-1 null mouse muscle and the section contains a much higher proportion of connective tissue than observed in control muscle. Those fibres that have regenerated in (B) are very small in diameter compared with regenerated control fibres (A). Magnification ×100.

in the absence of galectin-1, an interesting observation given that mice deficient in the muscle transcription factor MyoD, although developing apparently normally, exhibit a delay or abnormality in the ability of their muscle fibres to regenerate [21,22].

The foregoing account of our recent work highlights two intriguing aspects of galectin-1. First, it indicates the importance of this lectin in inducing an, as yet, unidentified cell in the dermis to enter the myogenic lineage. It is perhaps not surprising that the dermis should yield a cell with myogenic ability given that both dermal fibroblasts and myoblasts originate from the dermomyotome component of the somite [23]. This brings into question whether some cells in the dermis are more prone to entering a myogenic fate because of their developmental origins and it is therefore imperative to characterise the converting cell much more thoroughly. The environment into which the cells are introduced also warrants investigation. Our immunocytochemical studies, currently being confirming by western blot analysis (personal communication), show increased levels of galectin-1 expression in muscle undergoing regeneration, as compared to non-regenerating muscle. These increased levels of galectin-1 as muscle regenerates, may well explain why dermal fibroblasts implanted into regenerating host muscle can participate in the formation of new muscle fibres [1]. Second, our work underlines the crucial role of galectin-1 in promoting fusion of myogenic cells. The effect of galectin-1 on myoblast fusion, may best be explained by reports in various cell types, describing its role in both promoting and inhibiting cell-cell and cell-matrix adhesion [24–26]. In relation to myoblasts, their adherence to the extracellular component laminin is disrupted in the presence of galectin-1 [10]. In this situation, galectin-1 interacts with laminin, freeing the myoblasts from the extracellular matrix and perhaps accounting for increased myoblast fusion in the presence of the galectin. Our work with myoblasts derived from the galectin-1 null mouse further supports this. Such myoblasts exhibit a reduced fusion *in vitro* in comparison with control muscle cells as the laminin-myoblast interaction is maintained in the absence of galectin-1. In contrast, we have found that both dermal and muscle fibroblasts derived from the galectin-1 null mouse show a reduced adhesion to the substratum, as compared to wild type cells. Such reduction in adhesion can be eliminated by coating of the tissue culture flask even with the non-extracellular matrix substance, gelatin. This however is contrary to that reported by Yamaoka *et al*. [27], where there was a loss of anchorage dependence in 3T3 fibroblasts overexpressing galectin-1. Of course it is possible that there may be a dose dependent effect, supported by the varied descriptions of both inhibition or enhancement of cell-cell and cell-substrate adhesion in the presence of galectin-1 [10,24–26,28].

In conclusion it would appear that galectin-1 induces a non-committed myogenic cell within the dermis to express myogenic markers, increases the terminal differentiation of committed myogenic cells and has a role to play in the development and regenerative capacity of muscle. This raises many more questions than answers and leaves much to be elucidated. As more knowledge is being accumulated about galectin-1, the receptors it acts through [29–33] and the signalling pathways involved [34–36] the exact role of galectin-1 in muscle determination, differentiation and regeneration may further unfold.

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