INTRODUCTION

We have shown that implanting a cell, which does not appear to be a committed myogenic cell, into the muscles of the X-linked muscular dystrophic (mdx) mouse results in the formation of dystrophin-positive fibers (11,25). Dystrophin, a cytoskeletal protein, is not present in either the muscles of the mdx mouse or in Duchenne muscular dystrophy (DMD) patients. The ability of these cells, which are derived from the skin, to convert to muscle and produce dystrophin-positive fibers in the mdx mouse could provide a useful therapeutic tool in the treatment of DMD. We have also reported that the skin fibroblasts we had used for implantation into the mdx mouse muscle would also, under certain conditions, convert to myogenesis in vitro (31). A study by Salvatori et al. (26) also indicated that, when grown in contact with muscle cells, 1-10% of 10T/12 cells (a nonmuscle cell line) converted to muscle cells. Breton et al. (3) cocultured dermal fibroblasts infected with the LacZ gene linked to the muscle-specific desmin promoter with myotubes derived from the muscular dysgenic mouse. LacZ-positive mononuclear cells and myotubes were observed in such cultures, indicating that the fibroblasts had switched on muscle-specific genes and converted to myoblasts as well as contributing to the formation of myotubes. In our in vitro study (31) we described the conversion of up to 10% of fibroblasts when these cells were cultured in medium in which myoblasts had been previously grown. That the converting cells did not require to be in contact with the muscle cells implied that a soluble factor may be present within the myoblast conditioned medium that caused the fibroblasts to become myogenic. The presence of a soluble factor was also suggested by Breton et al. (3), who found that conversion of fibroblasts to myoblasts could occur at some distance from the inducing myotube. Very little about the nature of conversion is known, but earlier studies (31) showed that the 10% conversion rate observed only occurs in a muscle cell environment. In the present study, therefore, two specific questions were addressed: 1) If only 10% of dermal cells convert, is there a subpopulation of cells present within the mouse skin more capable of converting than the other cells? 2)
Is there a factor within conditioned medium that causes conversion?

In order to answer the first question we investigated whether cloning the cells would produce individual colonies that were all fibroblasts, all myoblasts, or a mixture of the two cell types. Clones did not convert when grown in normal tissue culture growth medium, with all cells remaining fibroblastic. Conversion was only observed when clones were grown in conditioned medium. Under these conditions a mixture of the two cell types was observed in each clonally derived population.

With regards to the second question, there are a number of growth factors that act on muscle tissue. Some of these factors, such as fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF), have been shown to have a mitogenic effect on muscle satellite cells (5,6). It has further been suggested that such factors could be released from damaged muscle, thereby promoting such mitogenic effects. In 1992 Harrison and Wilson (12) reported that a 14-15-kDa lectin called β-galactoside binding protein (BGBP) was secreted by myoblasts and myotubes. BGBP has also been found to be a cell growth regulator of fibroblasts acting via a specific cell surface receptor to inhibit the MAP kinase cascade, a mitogenic pathway normally activated by growth factors binding to tyrosine kinase receptors (29). These observations led us to question whether this lectin could be the factor within myoblast-conditioned medium that caused a proportion of the dermal fibroblasts in our previous experiments to convert to myogenesis. Accordingly, in the present work we cultured the fibroblasts in medium enhanced by BGBP. Preliminary observations indicate that its presence in the medium does indeed cause up to 30% of the fibroblasts to convert.

MATERIALS AND METHODS

Preparation and Maintenance of Cultures of Myoblasts and Dermal Fibroblasts

C2C12 mouse myoblasts were obtained from the American Type Culture Collection (ATCC) and routinely maintained in gelatin-coated flasks in Dulbecco’s minimal essential medium (DMEM) (Biowhittaker, Ltd) supplemented with 10% (v/v) fetal calf serum (PAA Laboratories), 100 IU/ml penicillin/100 mg/ml streptomycin, and 1% (w/v) L-glutamine (Life Technologies). Propagation of cultures followed routine practice, using trypsin/EDTA dissociation (a 0.25% and 0.025% mixture, respectively, in Hanks basal salt solution) and plating densities of 1 x 10^5 cells/ml of medium.

Primary dermal fibroblast cultures were obtained from explant cultures of neonatal skin from the C57Bl/10 ScSn strain of mouse. The entire skin was removed and the hypodermal surface scraped to ensure the removal of any adherent underlying muscular tissue before finely mincing and transferring the tissue fragments to a 35-cm² growth area tissue culture flask. To encourage attachment of the skin fragments to the tissue culture substratum the flasks were inverted and transferred for 2 h to an incubator set at 37°C and delivering 5% CO₂. Flasks were reverted and normal growth medium added. Cultures were maintained for 4 days after which time substantial outgrowth of skin cells had occurred to enable subculturing of the cells. Successive passages of cells were maintained in gelatin-coated 75-cm² growth area tissue culture flasks. Propagation of cultures followed routine practice using trypsin/EDTA dissociation and plating densities of 1 x 10^5 cells/ml. Aliquots of passage cells were regularly plated out at a cell density of 1 x 10^5 cells/ml onto the gelatin-coated wells of Multitest slides (ICN-Flow). To detect any possible contamination from myoblasts, these cells were stained for the muscle-specific intermediate protein filament desmin (27) according to previous methodology (16,21). While occasional desmin-positive cells were observed at very early passage, later cultures were observed to be myoblast free and were therefore the cultures used in all subsequent assays.

Dermal fibroblasts were cultured in different types of medium in order to determine the conditions for their conversion to myoblasts. Normal growth medium (control) consisted of DMEM supplemented with 20% (v/v) fetal calf serum, 2% (v/v) chick embryo extract (Imperial Laboratories), 100 IU/ml penicillin/100 mg/ml streptomycin, and 1% (w/v) L-glutamine (w/v). Medium was also harvested from C2C12 cells after at least 2 days in culture, thus constituting C2C12 conditioned medium that was either diluted with normal growth medium 1:1 (CM 1:1), 1:4 (CM 1:4), or diluted 1:1 and spun at 350 x g (CMS) prior to addition to cultures. In each case the conditioned medium was filtered through a 0.22-μm filter (Sartorius Ltd) prior to use to ensure there were no cells present. In addition, conditioned medium was regularly placed in tissue culture flasks and monitored for up to 10 days to ensure that it did not contain any cells. Dermal fibroblasts were maintained in the various types of culture medium from passage 2 onwards and following each passage samples of cells were incubated overnight on the wells of Multitest slides at a density of 1 x 10^5 cells/ml and used the next day for immunocytochemical analysis.

Cell Cloning

For experiments on cloned cells, dermal fibroblasts were used at passage 3, 4, or 5. Dermal fibroblasts were plated out at 100 cells per 100-mm gelatin-coated tissue culture grade petri dish and allowed to adhere overnight, after which the positions of the individual cells were marked and the cells allowed to grow for approximately
1 week. After this time sterile cloning discs (Sigma Chemical Co., Ltd), which had been soaked in trypsin/EDTA, were placed on each single colony until the cells detached, and the disc with attached cells was placed in individual wells of a gelatin-coated 24-well tissue culture plate. The colonies were grown to subconfluence and transferred to the wells of gelatin-coated Multiwell slides for characterization by immunocytochemistry.

**Immunocytochemistry**

Cells were stained with antibodies directed against the intermediate filament proteins desmin and vimentin, the former being present in myoblasts and absent in fibroblasts, the latter being present in all cells of a mesenchymal origin and therefore present in both myoblasts and fibroblasts. After overnight adherence to Multiwell wells, cells were fixed in 1:1 methanol/acetic acid for 10 min and air dried. Primary antibody (50 μl), either 1:10 dilution of anti-desmin (Sigma Chemical Co., D1033) or 1:20 dilution of anti-vimentin (Sigma Chemical Co., V5235), was added to each well and incubated at room temperature for 1 h in PBS containing 1% (w/v) bovine serum albumin (BSA) and 0.01% sodium azide (w/v) (Sigma Chemical Co.). Wells were washed three times in PBS prior to the addition of a 1:100 dilution of FITC-conjugated goat-anti-mouse IgG (Sigma Chemical Co., F0257) for 1 h. The slides were then washed three times in PBS followed by a 1-min addition of 0.02% (w/v) 4',6-diamidino-2-phenylindole (DAPI) in PBS to enable visualization of the nuclei of all marked cells. Slides were washed in PBS and mounted in glycerin aqueous mountant. The slides were viewed on a Zeiss Axioskop fluorescent microscope and photographs taken using a Zeiss MC100 camera and Kodak Ektachrome 160T film.

**Analysis of Converted Cells Within Cultures**

At each passage the number of desmin-positive cells relative to the total number of cells present was counted. These counts were carried out for cultures grown in conditioned and nonconditioned medium. The results were expressed as a percentage of desmin-positive cells based on counting three separate areas of four separate wells for each culture.

**Investigating the Effects of βGBP on Dermal Fibroblasts**

A cDNA clone of βGBP in the CDMS plasmid (29,30) was a kind gift of Dr. V. Wells (King’s College, London). The plasmid was grown up in E. coli and selected using the p3 selection plasmid and banded on a caesium chloride gradient. The plasmid was then used to transfect COS-1 cells, which were previously shown to secrete expressed βGBP protein into the media (30). COS-1 cells were plated out at 1 x 10⁴, 1 x 10⁵, and 2 x 10⁵/ml, and 1.5 μg of plasmid was used for each transfection. The plasmid was incubated with Lipofectamine (Life Technologies) for 45 min prior to addition to the cells for 24 h, after which time the cells were returned to normal growth medium. Three days after transfection, growth media was collected, filtered, and diluted 1:1, 1:5, 1:10, 1:50, or 1:100 with normal growth medium before addition to cultures (noncloned) of dermal fibroblasts. Dermal fibroblasts were incubated with the transfection medium overnight before replacing with normal growth medium (ON) or left on continuously for the full duration of the experiment (FD). When sufficient cell growth had been achieved, cells were transferred to the wells of Multiwell slides for characterization by immunocytochemical methods as previously described.

**Purity of CDMS Plasmid**

In order to ensure that the CDMS plasmid was pure, it was cut with the restriction enzymes EcoRI and XhoI and run on a 1% agarose gel. DNA (1 μg) was added to 1 μl of either XhoI, EcoRI, or 1 μl of each restriction enzyme, in the appropriate enzyme buffer. The reaction mixture was incubated at 37°C for 45 min after which 4 μl of loading buffer (0.25% (w/v) xylene cyanol FF,

<table>
<thead>
<tr>
<th>Passage Number</th>
<th>Control</th>
<th>CM (1:1)</th>
<th>CM (1:4)</th>
<th>CMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>p1</td>
<td>0.4 ± 0.5 (n = 2)</td>
<td>—</td>
<td>—</td>
<td>0 ± 0 (n = 1)</td>
</tr>
<tr>
<td>p2</td>
<td>0 ± 0 (n = 1)</td>
<td>0.4 ± 0.2 (n = 4)</td>
<td>0 ± 0 (n = 1)</td>
<td>0 ± 0 (n = 1)</td>
</tr>
<tr>
<td>p3</td>
<td>0 ± 0 (n = 1)</td>
<td>0 ± 0 (n = 1)</td>
<td>0.3 ± 0.3 (n = 1)</td>
<td>0 ± 0 (n = 1)</td>
</tr>
<tr>
<td>p4</td>
<td>0 ± 0 (n = 1)</td>
<td>0.4 ± 0.4 (n = 3)</td>
<td>0 ± 0 (n = 1)</td>
<td>0 ± 0 (n = 1)</td>
</tr>
<tr>
<td>p5</td>
<td>0 ± 0 (n = 1)</td>
<td>22.4 ± 9.3 (n = 2)</td>
<td>3.4 ± 1.8 (n = 1)</td>
<td>20.1 ± 6.1 (n = 1)</td>
</tr>
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</table>

Few desmin-positive cells were present in cells grown in conditioned medium at early passage numbers. The highest number of desmin-positive cells were present in cultures grown in CM 1:1 and CMS.
0.25% (w/v) bromophenol blue, 30% (v/v) glycerol in sterile distilled water) was added. The samples were run on a 1% (w/v) agarose gel in TAE buffer containing 1 μl of ethidium bromide. The DNA bands were visualized by UV irradiation of the gel. This procedure was carried out in order to confirm that the correct sized fragments had been generated with respect to those present on the plasmid map.

Transfection Efficiency

As the βGBP plasmid does not contain a selectable marker or a reporter gene, COS-1 cells were separately transfected with a plasmid containing the LacZ reporter gene under the same conditions as that used for βGBP. In this way we could gain an estimate of the transfection efficiency by staining of the LacZ transfected cells using the X-gal colorimetric reaction (7).

Staining for β-gal Activity

Cells were washed in warm PBS and fixed in 0.5% (v/v) gluteraldehyde in PBS for 10 min on ice. Slides were washed briefly for 30 s in ice-cold 2 mM magnesium chloride in PBS followed by a further 10-min wash. Cells were then incubated in ice-cold detergent solution (2 mM magnesium chloride, 0.01% sodium deoxycholate, 0.02% NP40 in PBS) for 10 min prior to being incubated in the dark in X-gal solution at 37°C overnight. X-gal solution was prepared by diluting stock X-gal to a final concentration of 1 mg/ml in diluent consisting of 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM magnesium chloride, 0.01% (w/v) sodium deoxycholate, and 0.02% (v/v) NP40 in PBS, pH 7.4. Slides were washed in PBS, dehydrated, and mounted.

Figure 1. (A–C) Dermal fibroblasts stained for the muscle-specific marker desmin (green) and with the fluorescent nuclear marker DAPI (blue). Scale bar: 50 μm. (A) Whole population dermal fibroblasts grown in control growth medium. The cells are negative for desmin, showing they are nonmyogenic. The positive DAPI stain marks each nucleus. (B) Whole population dermal fibroblasts grown in CM 1:1. (C) Whole population dermal fibroblasts grown in muscle cell conditioned growth medium CM 1:1. In both (B) and (C) some cells are positive for desmin and all are positive for DAPI. (D) Dermal fibroblasts that have been stained with vinculin (green), a marker of all cells of a mesenchymal origin, and DAPI. All cells are positive for both these markers.
**Polyacrylamide Gel Electrophoresis (PAGE)**

SDS-PAGE was used to investigate the presence of βGBP protein in the medium harvested from COS-1 cells transfected with the βGBP plasmid. Samples of media were diluted 1:1 in sample buffer consisting of 65 mM Tris, pH 6.8, 3% (w/v) SDS, 20% (v/v) glycerol, 0.7 M β-mercaptoethanol, and 0.025% (w/v) bromophenol blue and boiled for 4 min. They were then run on 12.5% SDS-polyacrylamide gels (acrylamide/bisacrylamide, 37.5:1, 30% T, 2.57% C, Sigma Chemical Co.) with a 4% stacking gel under standard conditions (18) and the proteins visualized by staining with 0.1% (w/v) Coomassie blue.

**RESULTS**

**Effect of Conditioned Medium on Whole Population Dermal Fibroblasts**

Dermal fibroblasts, generated from explant cultures, were grown up to passage 5 in culture. These cells did not undergo any cloning procedure, and are designated whole population dermal fibroblasts. At each passage, the number of cells staining positively for the muscle-specific marker desmin were counted relative to the total number of cells in culture (Table 1). When dermal fibroblasts were grown in nonconditioned medium we very rarely observed any desmin-positive cells within these cultures. Two cultures at passage 1 were examined. One such culture contained less than 1% of desmin-positive cells, whereas the second passage 1 culture examined did not contain a single desmin-positive cell (Fig. 1A). The passage 1 culture that did contain a few positive cells was followed through to passage 2, 3, and 4 and in these succeeding passages no desmin-positive cells were evident. Of the remaining 8 cultures examined (Table 1) desmin-positive cells were never observed.

Cells grown in CM 1:1 or CM 1:4 produced very few desmin-positive cells in cultures of passages 2–4. This was also true for cells grown in CMS medium. Passage 5 cultures yielded very different results. When grown in CM 1:4 medium, over 3% of the cells were desmin positive (Fig. 1B). Cells grown in the higher concentration of conditioned medium (i.e., CM 1:1) showed greater than 20% desmin-positive cells (Fig. 1C). This percentage was also seen when cells were grown in CMS medium.

CM 1:4 was used because previously we detected up to 5% of conversion of dermal fibroblasts to myoblasts when grown in this concentration of C2C12 conditioned medium (11). This level of conversion was therefore verified in the present work using such a concentration of conditioned medium. Increasing the concentration of the conditioned medium increased the proportion of converted cells approximately fourfold. A 20% conversion

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Table 2: Percentage of Desmin-Positive Cells Present Within Cloned Dermal Fibroblast Cultures When Treated With Control Medium or CM 1:1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CM 1:1:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desmin-Positive Cells</td>
<td>0 ± 0 (n = 2)</td>
<td>37.2 ± 3.9 (n = 3)</td>
</tr>
</tbody>
</table>

Desmin-positive cells were not observed in control medium. An average of 37% of cells were desmin positive following growth of cultures in CM 1:1.
level was also seen in passage 5 cells treated with CMS medium (i.e., medium that was also diluted 1:1 but was also centrifuged prior to use in culture). We are confident that these positive cells are not the result of the presence of C2C12 cells remaining in the conditioned medium and being carried over into the fibroblast cultures as we routinely cultured samples of filtered conditioned medium and never observed any cells growing in such medium.

**Effect of Conditioned Medium on Cloned Cells**

In order to investigate whether there was a subpopulation of cells present within the skin that readily converted to myogenesis, clones from single skin cells were grown in normal and conditioned media in order to isolate these converting cells. When such clones were grown in normal medium, no conversion to myogenesis was detected (Fig. 2A, Table 2). This result did not therefore differ from the result obtained from the whole population of skin cells and it appeared that, when grown in normal medium, cloning of the cells had no effect on conversion. Culturing clones in conditioned medium gave a very different result. When grown in CM 1:1, up to 40% of the cells of a clone expressed myogenic markers (Fig. 2B, Table 2). This was in comparison with up to 20% when the whole population of skin cells was cultured in CM 1:1. As the number of cells expressing myogenic markers is higher in the clones than in the whole population, it is possible that we could be isolating a subpopulation of cells during the cloning process that is more likely to convert.

**Effect of βGBP on Whole Populations of Dermal Fibroblasts**

That conversion of cells to myogenesis was not detected in normal growth medium, but only when either whole-populations of skin cells or clones derived from such populations were cultured in conditioned medium, supports the hypothesis that there is a factor within the conditioned medium responsible for this conversion. As βGBP was a possible candidate for this factor, a plasmid containing a βGBP construct (Fig. 3) was obtained. The plasmid was grown in E. coli (29,30) and purified. When the purified plasmid was cut with the restriction enzymes EcoRI and Xhol it yielded fragments of 342 and 166 bp and with Xhol alone a fragment of 551 bp was produced (Fig. 4). These results indicate that pure plasmid had been produced. In order to obtain βGBP protein, which has been shown to be secreted into tissue culture media (29,30), COS-1 cells were transiently transfected with βGBP plasmid. It was not possible to calculate the exact transfection efficiency of the CDMS plasmid as it does not contain a reporter gene. Therefore, to gain some estimate of transfection efficiency under the conditions used, COS-1 cells were separately transfected, with a plasmid containing the LacZ reporter gene. This latter gave a transfection efficiency of up to 70% (Fig. 5). Media harvested from the COS-1 cells transfected with βGBP plasmid was run out on a SDS-PAGE gel. In transfected cells (Fig. 6, lanes 2–5) a band is evident, as indicated by the arrows at just below 15 kDa. This band is more intense than that observed at a

![Figure 3. Plasmid map for CDMS plasmid containing the βGBP construct.](image-url)
similar molecular weight in untransfected cells (Fig. 6, lane 1). This result, together with work of Wells and Mulluci (30), who used a similar technique to produce βGBP, was taken as evidence that βGBP was being secreted into the medium.

Medium from βGBP-transfected COS-1 cells was added to cultures of whole populations of skin cells. Medium was diluted 1:1, 1:3, 1:10, 1:50, and 1:100 with normal growth medium before addition to the cells. In one of the cultures cells were only grown in diluted βGBP medium overnight (ON), whereas in others the cells were grown in this medium for the full duration of the culture (FD). Growth of cells in βGBP medium overnight was sufficient to induce conversion of cells. The use of the more concentrated βGBP medium (1:1, 1:3, and 1:10) was detrimental to growth of some of the cultures. Few cells remained in these cultures and those present exhibited a stressed morphology by extending their cytoskeletons (Fig. 7). In other cultures, however, 14% of cells grown in 1:1 medium (FD), 11% of cells in 1:10 medium (ON), and 31% of cells grown in 1:100 medium (FD) expressed the muscle-specific marker desmin (Fig. 8).

**DISCUSSION**

Introducing donor cells into diseased muscle is an important concept in the treatment of muscle diseases. The object is to take normal donor muscle cells or the patient's own muscle cells, carrying normal genes, and to implant them into the muscles of dystrophic patients (14,15,16,17). Although this type of approach, termed myoblast transfer therapy, is currently undergoing clini-
Figure 5. Phase contrast of COS-1 cells analyzed for β-gal activity following transfection with plasmid containing the LacZ reporter gene. β-gal-positive cells appear black, and gray areas depict nontransfected cells. The transfection efficiency was up to 70%.

cal trials, the use of donor cells may present problems of immune rejection (15,22). However, implantation of the patient's own myoblasts with the dystrophin gene inserted (10) may not be appropriate due to the involvement of dystrophic myoblasts in the disease process (28). Consequently, other candidate cells that can contribute to muscle fiber formation and can express gene products deficient in dystrophic muscle may be more suitable. As dermal fibroblasts can convert to myogenic cells under certain conditions (3,11,13,25,26,31), they

Figure 7. Dermal fibroblast cultured in 1:1 BGBP conditioned medium. The cell is vimentin (green) and DAPI (blue) positive. Spreading of the cell cytoskeleton indicates the level of BGBP was detrimental to normal growth of the cell. Scale bar: 50 µm.

Figure 6. SDS-PAGE gel stained with Coomassie blue. Lane 1: medium from untransfected COS-1 cells. Lane 2: medium removed after 10 days from transfected COS cells plated out at 3 x 10^6 cells/ml. Lanes 3-5: medium removed after 5 days from transfected COS-1 cells plated at 1 x 10^6 cells, 1 x 10^7, and 3 x 10^7, respectively. Lane 6: molecular weight markers. Arrows indicate a band of approximately 15 kDa at the appropriate molecular weight for BGBP. Although there is a faint band present in untransfected cells (lane 1), indicating the presence of a protein at this molecular weight, the band intensity is increased following transfection of these cells with plasmid CMDS. This result shows that, following transfection, more protein of this molecular weight is being produced. Taken together with the results of Wells and Meltz (28) this implies that BGBP is being secreted into the medium following transfection with CMDS.
warrant further investigation for use in autologous cell transplantation strategies. For this approach to advance, however, it is important to discover the nature of the converting cell and the factors responsible for such conversion.

The results of the current experiments indicate that neither whole population dermal fibroblasts nor clones express myogenic markers when grown in normal control medium. However, when cultured in muscle cell conditioned medium (CM 1:1), cells expressing myogenic markers were present in both whole population and cloned cultures. The number of myogenic cells in clones was substantially higher (40%) than in whole population cultures (20%), suggesting that cloned cells are more susceptible to conversion. The increased number of myogenic cells following the cloning procedure may reflect a greater survival potential of the cells that convert, given that they have to survive for long periods of time at a low cell density. Although these experiments do not prove the presence of a subpopulation of converting cells in the skin, it suggests some may more readily convert than others. This is an interesting observation as there have been reports of multipotent cells or stem cells, within various tissue that retain the ability to differentiate along several lineages. For example, mesenchymal stem cells can differentiate into four different lineages: muscle, fat, cartilage, and bone (4,32). They have also been identified in various human tissues, including the dermis (9,32). Interestingly, evidence exists for a cell exhibiting stem cell properties in myoblast cultures, which is responsible for fiber formation in mouse myoblast transfer studies (11). Further, it has been reported that the injection of bone marrow stem cells via the mouse tail vein results in some of these cells migrating to muscle and participating in fiber formation (8).

Similarly, bone marrow transplantation studies have shown that bone marrow cells are capable of undergoing myogenic differentiation into both skeletal and cardiac muscle (2). Some form of stem cell, capable of myogenic conversion, may therefore reside in the skin. This question is currently being further investigated. The area of stem cell research is an expanding field and the understanding of these cells could prove to be very beneficial in relation to cell-based therapies for muscle diseases.

Our experiments indicate the presence of a factor within muscle cell conditioned medium, necessary for myogenic conversion of dermal fibroblasts. We are interested in determining the identity of this factor. It is a prerequisite that the factor has to be released by myoblasts, act on fibroblasts, and promote their differentiation to a myogenic cell type. Although studies have reported a number of growth factors that have mitogenic effects on muscle cells, these have not been shown to affect dermal fibroblasts (5,6). However the lectin, βGBP, is released from myoblasts and myotubes (12) and is a cell growth regulator of fibroblasts (29). Consequently, we investigated whether βGBP could be the agent in muscle cell conditioned medium, responsible for dermal fibroblast conversion. Our experiments indicate that treating cultures of dermal fibroblasts with βGBP results in cells expressing myogenic markers. This suggests the identification of a factor responsible for switching skin cells to a myogenic pathway. An intriguing observation is that populations of hematopoietic stem cells, which produce multiple lineages, have a lect-
tin binding site (24). This could be very relevant if converting cells in the skin are indeed stem cells. βGBP could be capable of binding to such a site on stem cells and cause them to enter a specific differentiation pathway. It is of interest to note that we have evidence that the dermal fibroblasts we use for implantation into host muscle can also be driven, in vitro, along an osteogenic pathway (20), although we have not yet substantiated this in vivo.

It is also interesting to note that there is an unidentified factor present in crushed muscle extracts that is mitogenic to myoblasts (5,6). We intend to investigate the effects of βGBP on muscle cells, particularly as we have preliminary evidence indicating its presence in regenerating muscle. It is interesting to speculate that βGBP could be released from regenerating muscle and act as a mechanism for recruitment of satellite cells to new fiber formation.

Although there are still many areas to investigate, our experiments have advanced our understanding of the conversion of skin cells to muscle, enabling us to further explore the potential of autologous-based cell therapy for muscle disorders using cells derived from the skin. It is also of interest to determine if dermal fibroblasts treated with βGBP will terminally differentiate into myotubes in vitro and myofibers in vivo. This work is currently under investigation.

The experiments reported in this article were carried out using mouse cells. Ultimately we have to ensure the same effects using human skin cells. The murine βGBP protein has 89% homology to the human version of βGBP. We have preliminary evidence indicating the conversion of human dermal fibroblasts to myogenesis using murine βGBP. This finding suggests our approach may be applicable to human cells.

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