

Exogenous Genes Are Expressed in mdx Muscle Fibres Following the Implantation of Primary Mouse Skin Cells

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Abstract

By implanting skin cells carrying exogenously introduced genes into the muscles of the x-linked muscular dystrophic (*mdx*) mouse, an animal model for human Duchenne muscular dystrophy, we show here that the introduced genes are expressed within the host muscle fibres. In one series of experiments the introduced gene was a lacZ reporter gene and in another the full length human dystrophin gene was introduced, via a germ line transgenic route, into the donor skin cells. The results indicate that skin cells could be used to carry genes to muscle fibres and further that the donor cells actually participate in the formation of muscle fibres in the host. Autologous skin cells of a myopathic patient might therefore be of use in designing therapies to alleviate myopathic conditions in cases where the disease is the result of a deficiency in a muscle specific protein.

Key words: exogenous gene delivery, skin cells, muscle fibres.

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In a previous paper we reported the formation of high numbers of newly-formed dystrophin-positive fibres when skin cells from a normal mouse were implanted into the muscles of the dystrophin-deficient *mdx* mouse [8] one of the animal models for the human muscle disorder, Duchenne muscular dystrophy (DMD). Our previous results suggested that the skin cells had contributed to the resultant dystrophin-positive fibres. Characterisation of the implanted cells by immunocytochemistry and clonal analysis revealed them to be of a non-myogenic nature, leading us to suggest that the implanted cells were capable of converting to a myogenic lineage when exposed to the *in vivo mdx* muscle environment. There have been many reports of myogenic differentiation of non-muscle cells when such cells are exposed to myogenic transcription factors [4, 6, 7] and also spontaneous conversion of cells when grown *in vitro* in contact with myogenic cells [2, 23], or when soluble muscle-specific factors induce conversion of the cells [29]. If skin-derived cells have the capability of myogenic conversion they could have potential as a vehicle to carry gene products to myopathic muscle. Furthermore their participation in muscle fibre formation has the added advantage of repopulating a wasting muscle with new muscle fibres.

In our earlier work normal skin cells were able to express the endogenous dystrophin gene within the host muscle environment. In the present paper we demonstrate that

specific genes introduced into the skin cells are expressed within *mdx* muscle fibres formed when the implanted cells participate in fibre formation. In one series of experiments we introduced the lacZ reporter gene, resulting in formation of β -gal positive fibres in *mdx* muscle and in a second series of experiments we implanted skin cells from an *mdx* transgenic mouse carrying the full length human dystrophin cDNA into *mdx* muscle as an initial step to determine if participating skin cells expressed the transgene within host muscle fibres. The transgene was driven by the human skeletal α -actin promoter which restricted expression of the transgene to skeletal muscle [27].

Methods

Preparation of skin fibroblasts from C57Bl/10ScSn or mdx transgenic neonatal mice

The entire skin was removed from the carcasses of either neonatal C57Bl/10ScSn mice or neonatal *mdx* mice transgenic for the full length human dystrophin gene. The hypodermal surface of the donor skin was scraped to ensure it was free from underlying muscular tissue. The skin was finely minced and the resultant tissue fragments transferred to sterile tissue culture flasks and a minimal amount of growth medium consisting of 85% DMEM with L-Glutamine, 10% Fetal Calf Serum, 2% Chick Embryo Extract, 88IU ml⁻¹ Penicillin and 88mg ml⁻¹ Streptomycin, added. After adding the explants and minimal amount of

medium, flasks were placed in an inverted position and incubated in 5% CO₂ for 3 hours to ensure attachment of skin fragments to the substratum, essential for cell outgrowth. After this time flasks were reverted and grown at 37°C in 5% CO₂ until cell outgrowth was established. Following sufficient outgrowth skin fragments were removed and the cells subcultured to establish passage 1 cultures. Cells were passaged several times to generate sufficient cells for implantation. As controls for transgenic experiments, cells were grown from skin harvested from the non-transgenic littermates.

Infection of skin cells with retroviral vector carrying the lacZ gene

Cells derived from C57Bl/10ScSn neonatal skin were grown to passage 6 and infected with culture supernatant containing the ecotropic replication defective retrovirus BAG[17] a Mouse Moloney Leukemia retroviral vector (MoMLV) carrying the lacZ reporter gene coding for p-galactosidase. The vector also carried the Tn-5 derived neomycin phosphotransferase gene (neo^r) which confers resistance to the aminoglycoside G418 in mammalian cells and hence allows subsequent selection of cells that had been retrovirally infected. Cells were plated out at 1.5 x 10⁵ cells / 30 mm tissue culture grade plate and grown overnight in 3 mls of growth medium at 37°C in an atmosphere of 5% CO₂. Retroviral infection was carried out in the presence of 8 µg of Polybrene / ml of supernatant containing the retrovirus. Following infection cells were selected in medium containing 400 µg/ml of the neomycin analogue, G418. Cultures were subsequently maintained in G418-containing medium to ensure only retrovirally infected cells persisted *in vitro*. Samples of cells were assayed for β-gal activity using the X-gal reaction[26]. The number of p-gal positive cells were counted and only cultures where 90% of the cells were P-gal positive were used for implantation. In this series of experiments cells of three different passage numbers - P11, 21 and 32 were implanted into hosts.

Production of mdx transgenic mice carrying the full-length human dystrophin cDNA

Transgenic mice carrying the full length human dystrophin cDNA were produced by microinjection of a dystrophin expression construct utilising the human skeletal muscle α-actin promoter, into the pronuclei of single-cell fertilised ova of C57Bl/10ScSn x CBA/J mice. Transgenic lines were then bred onto the *mdx* background to produce *mdx* animals expressing human dystrophin. Male transgenics were crossed with *mdx* females. The male transgenic offspring from this latter cross which are obligate carriers of the *mdx* mutation were subsequently bred with *mdx* females. In the animals carrying the full length cDNA, expression of human dystrophin was confined mainly to skeletal muscle although it was expressed at very low levels in the heart [27]. Expression of the 427kDa protein-product of the human full length dystrophin cDNA in the

mdx transgenic mouse restores normal muscle phenotype [27].

Screening of mice for presence of the dystrophin transgene

It was necessary to screen transgenic litters as only a proportion of pups were transgenic. The tail tip was removed from each littermate and placed in a 1.5 ml eppendorf tube containing 0.7 ml of buffer consisting of 50mM Tris-HCl pH 8.0; 0.1 M EDTA; 0.1 M NaCl; 1% SDS and 25 µl of 10 mg / ml Proteinase K and incubated overnight at 55°C. Following two phenol / chloroform extractions genomic DNA was spooled on a glass rod after the addition of 0.6 volumes of isopropanol. After washing in 70% ethanol the DNA was dissolved in TE buffer (10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0). Screening was carried out by the polymerase chain reaction (PCR) with 0.5 µg of DNA. The set of primers used; forward 5'-CCTCTGACCCTACACGGAGC-3' and reverse 5'-CAGTTATATCAACATCCAACC-3' produce a specific fragment of 1.4 kb[27]. The PCR was carried out with 5 minutes preincubation of genomic DNA, followed by addition of Taq polymerase, both at 95°C. The reaction was run for 30-35 cycles of 60 seconds denaturing at 95°C, 1 minute annealing at 60°C and 2.5 minutes extension at 72°C. PCR products were run in a 1% analytical agarose gel in 1X TBE buffer at 100V for 2-3 hours after which they were visualised under a short-wave fluorescent lamp.

Characterisation of cells prior to implantation

Each culture was characterised prior to implantation. A sample of each was plated into the wells of a multitest slide and immunocytochemically characterised using antibodies directed against the intermediate filament proteins desmin and vimentin - the former expressed only in myogenic cells, the latter in both fibroblasts and myoblasts[23]; the myogenic transcription factors MyoD and myf-5 [9, 12, 28] and the neuronal cell adhesion molecule (N-CAM) expressed on the surface of myogenic cells [25]. Antibodies used were monoclonal anti-desmin antibody (Sigma cat. no. D1033, 1:10 dilution), monoclonal anti-vimentin (Sigma cat. no. V5255, 1:20 dilution); polyclonal anti-MyoD antisera C-20 (Santa Cruz Biotechnology cat. no. sc-304, 1:50 dilution); polyclonal anti-Myf-5 antisera (Santa Cruz Biotechnology cat. no. sc-302, 1:50 dilution) and polyclonal anti-N-CAM antibody (Santa Cruz Biotechnology cat. no. sc-1508). In addition, all cultures were scored for the presence or absence of multinuclear myotubes which would indicate that cells of a myogenic lineage were present within them.

Preparation of host mdx nude mice and implantation of cells into recipient muscles

All hosts were athymic nude mice bred onto the *mdx* background and maintained over many generations [15]. Skin cells were implanted into previously irradiated host Tibialis Anterior (TA) muscles. Irradiated muscle does not undergo endogenous regeneration, thus constituting a

more suitable model for DMD [24]. Donor cells were implanted into the irradiated TA muscle one week after delivery of 18Gy of X-irradiation to the host muscle [24]. Host mice were anaesthetised by subcutaneous injection of 0.1ml of an anaesthetic cocktail consisting of one part midazolam hydrochloride (5 mg/ml) one part Hypnorm (0.315 mg/ml fentanyl citrate and 10mg/ml fluanisone; Janssen Pharmaceuticals Ltd.) and two parts sterile water. The skin overlying the muscle was incised and 3×10^5 viable cells were implanted in a 10-15 μ l volume, delivered to the muscle through the investing fascia using a finely pulled glass PCR pipette.

Analysis of implanted muscles

Muscles were removed from recipients at 3 or 5 weeks following implantation and immediately frozen in isopentane cooled to -165°C with liquid nitrogen. 8 or 12 μ m thick cryostat sections were cut, the 12 μ m sections being used only for P-gal analysis. Sections at various levels through the muscle were examined histologically and immunocytochemically for the presence of new-formed dystrophin positive fibres. In experiments where donor cells had been infected with the retroviral vector carrying the lacZ gene, serial sections were also analysed for P-gal activity as previously described [26].

Results

Expression of the lacZ reporter gene in C57Bl/10ScSn skin cells

After 2-3 weeks in selection medium donor cells to be implanted into host muscle were assayed for P-gal activity. Cultures showed a wide range of cells positive for P-gal, with some cultures having as few as 10% of cells expressing p-gal. However, only cultures which contained 90-95% of cells which were positive for P-gal were used for implantation experiments (Figure 1). This was the case for all three passage numbers implanted.

Characterisation of implanted cells

Donor cells for implantation were routinely immunocytochemically stained with antibodies directed against vimentin, desmin, MyoD, myf-5 and N-CAM. Donor cells harvested either from C57Bl/10ScSn or *mdx* transgenic mice were positive for vimentin (Figure 2a) an intermediate filament protein present in both myoblasts and fibroblasts but were negative when stained with antibodies directed against desmin, N-CAM, MyoD and myf-5 all of which are markers for myogenic cells (Figure 2b-e). This contrasted with the positive staining observed in cultures of the mouse myogenic C2C12 cell line which were positive for the four myogenic markers used (Figure 2g-j). Vimentin-positivity of both skin cells (Figure 2a) and C2C12 cells (Figure 2f) indicates their common mesenchymal origin. In addition to immunocytochemical analysis, cultures of skin and C2C12 cells were also scored for the presence of multinucleate myotubes. Such struc-

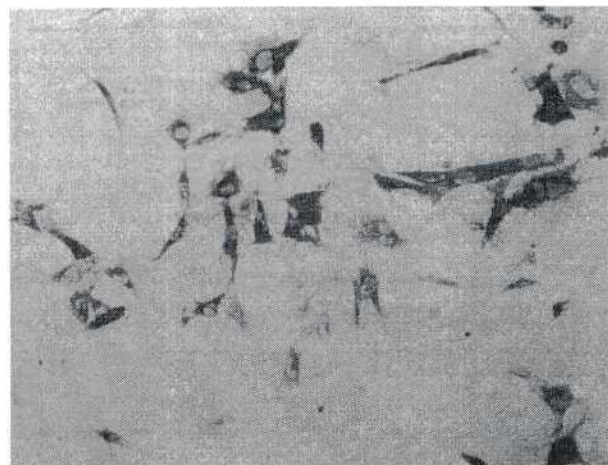


Figure 1. Samples of cultures to be used for implantation into host muscle were stained with the X-gal substrate. Cells which were positive for β -gal, the product of the introduced lacZ reporter gene were blue when stained with X-gal. Only cultures of skin cells which were 90-95% positive for β -gal were used for implantation. Magnification $\times 300$.

tures were never present, even at early passage numbers, in skin cell cultures derived from either C57Bl/10ScSn or *mdx* transgenic skin, although they were present in cultures of C2C12 cells.

Analysis of mdx muscles implanted with lacZ transfected skin cells

P-gal positive C57Bl/10ScSn skin cells were implanted into the right irradiated TA muscle of 13 *mdx* nude mice. Four were implanted with passage 11 cells, 5 with passage 21 and 4 with cells at passage 32. Five weeks later the TA muscles of both legs were removed and 12 μ m thick cryostat sections were cut from the implanted muscles and analysed for P-gal activity. 8 μ m thick sections of the same muscles were also taken for histological and dystrophin analysis. Non-injected muscles of the left leg acted as controls.

Histological analysis

Five weeks after implantation of C57Bl/10ScSn-lacZ skin cells sections cut from the irradiated muscles at the site of injection contained fibres of small cross sectional area in which the nuclei were centrally placed within the muscle fibre. Small diameter fibres with non-peripherally placed nuclei are indicative of fibre regeneration. These fibres were present within the injected muscles regardless of the passage number of the injected cells. In muscles injected with late passage cells, i.e. passage 32, the total number of muscle fibres in these sections were much fewer than in sections of muscles implanted with earlier passage cells. In these passage 32 sections, cellular and connective tissue intervened between the remaining fibres (Figure 3). In addition to the presence of small diameter fibres, sections cut from muscles implanted with all three passage number of cells contained fibres of larger diameter. Some-

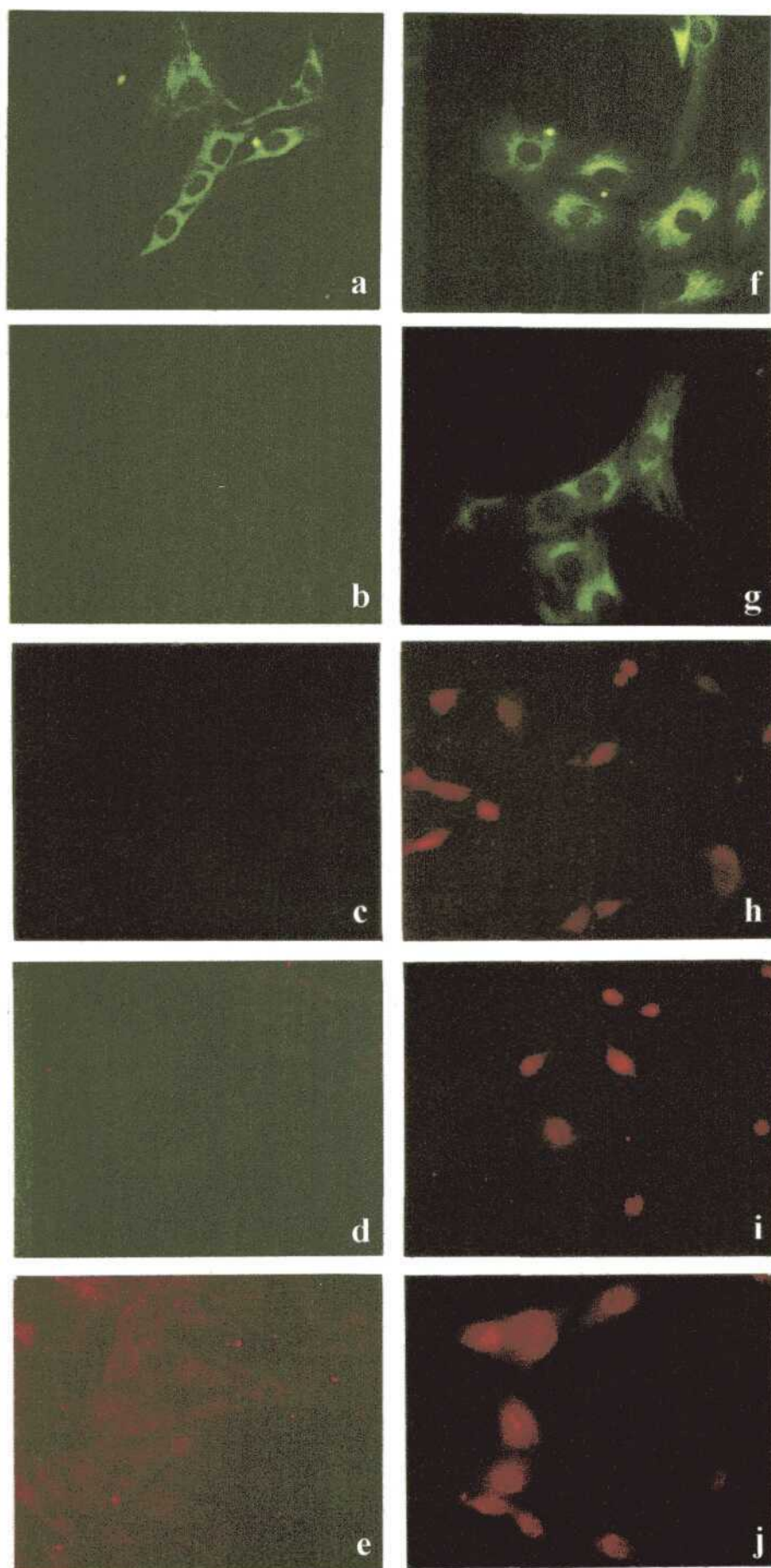


Figure 2. Samples of skin cells to be used for implantation were stained with various markers of myogenesis. All skin cells used for muscle implantation were positive when stained with an antibody directed against the intermediate filament protein vimentin (a: magnification x 600), but were negative for the myogenic markers desmin (b; magnification x 300), N-CAM (c; magnification x 300), MyoD (d; magnification x 300), and myf-5 (e; magnification x 300). In contrast, cells of the mouse myogenic cell line C2C12, used as controls, were positive for desmin (g; magnification x 600), N-CAM (h; magnification x 300), MyoD (i; magnification x 300) and myf-5 (j; magnification x 300). As with the skin cells (a), C2C12 cells were positive for vimentin (f; magnification x 600), indicating that both cell types arose from a mesenchymal origin.

times these larger diameter fibres contained peripheral nuclei, these being fibres which had not undergone regeneration prior to being irradiated [24]. In all sections, the occasional larger diameter fibre with non-peripheral nuclei were observed, these being fibres which had already regenerated prior to the irradiation event.

ft-galactosidase activity

Implanted muscles were sectioned throughout and sections at various different levels were examined histologically to locate the site of injection of the cells. This was apparent by the presence of areas of fibrotic tissue which co-incided with needle track injury. The two levels nearest to the site of injection and separated by some 120 μ m were scored for the number of P-gal positive fibres observed and an average number of β -gal positive fibres for the two sections were recorded (Figure 4). The average percentage of P-gal positive fibres present in the two sections cut near

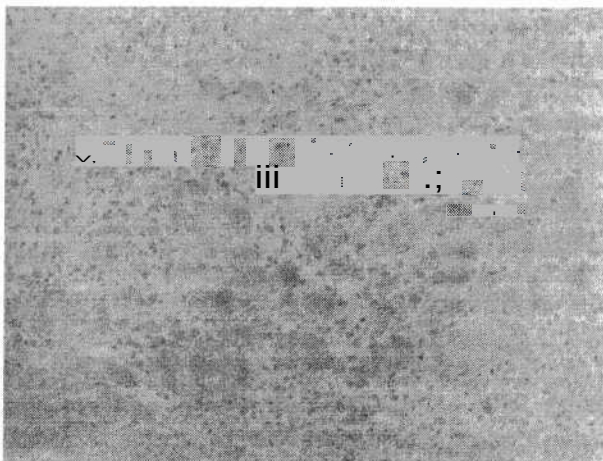


Figure 3. Sections of muscles implanted with passage 32 skin cells were characterised by lower numbers of muscle fibres than observed in muscles implanted with passage 11 or 21 cells. The muscle fibres were replaced by connective tissue and cells. Magnification x 600.

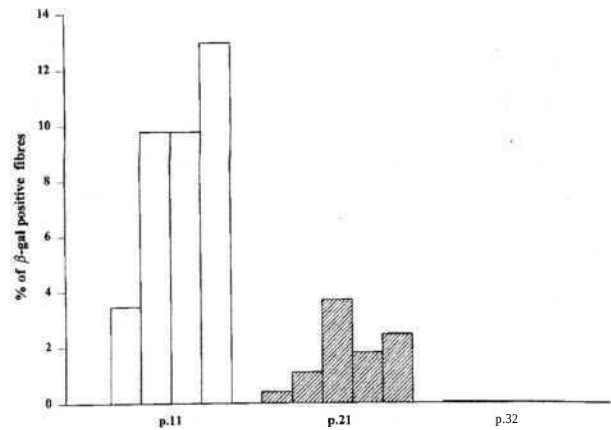


Figure 4. The average percentage of β -gal positive fibres counted in the two step sections of implanted muscle closest to the injection site. The number of β -gal positive fibres was highest following the implantation of passage 11 cells and was considerably lower after implanting passage 21 cells. No β -gal positive fibres were evident in sections of muscle implanted with passage 32 skin cells.

the injection site following the implantation of passage 11 lacZ-C57Bl/10ScSn skin cells ranged from 3.5 to 13%. The fibres nearest to the injection site which were positive for P-gal were often of small cross sectional area (Figure 5). In most of the injected muscles several P-gal positive fibres were also observed in areas removed from the injection site. We noted that the passage number of the implanted cells influenced the number of P-gal positive fibres we observed within the sections. Implantation of passage 21 cells resulted in lower percentages of P-gal positive fibres, 0.5 to 3.7%, when compared with those implanted

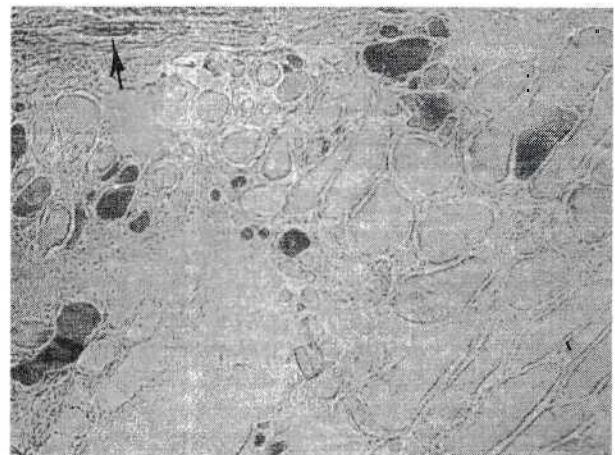


Figure 5. β -gal positive fibres were prominent around the area of the injection site, particularly in muscles implanted with passage 11 skin cells. Such fibres were often of small diameter indicating their recent formation. Mononuclear β -gal positive cells were seen in connective tissue near to the injection site (arrow). Magnification x 1200.

with passage 11 cells. No β -gal positive fibres were found within the muscles implanted with passage 32 cells.

Dystrophin analysis

Sections were stained, as previously detailed [8], with the P6 dystrophin polyclonal antibody which is specific for dystrophin and does not cross react with any other muscle-specific protein [5, 21, 22]. Dystrophin positive fibres were found in muscles implanted with passage 11 and 21 cells. Analysis of serial sections revealed that the majority of fibres which were P-gal positive were also dystrophin positive although there were a few fibres which were P-gal positive but dystrophin negative. A very low number of fibres were P-gal negative but dystrophin positive. Following implantation of passage 32 skin cells, some dystrophin positive fibres were present but in much lower numbers than observed after implanting passage 11 or 21 cells; these dystrophin-positive fibres were P-gal negative. As controls, non-injected irradiated *mdx* muscles were assayed for P-gal activity and dystrophin positivity. No P-gal positive fibres were present in any of the control sections and the numbers of dystrophin positive fibres recorded in uninjected sections were of very low numbers and reflected the low level of revertant fibres previously reported in our colony [10].

Analysis of *mdx* muscles implanted with skin cells derived from transgenic mice

Muscles were examined 3 weeks after implantation of the donor skin cells. Six *mdx* nude hosts were implanted with cells derived from six different transgenic neonates. As controls two hosts were implanted with skin cells derived from non-transgenic littermates.

Histological appearance

Sections cut from the six muscles implanted with transgenic skin cells were characterised by the presence of numerous very small and immature muscle fibres, the majority of which were at the myotube stage of development. These immature fibres were haphazard in their orientation and were not aligned along the long axis of the muscle (Figure 6). They contained centrally located nuclei indicative of their newly regenerated state, although they were present within a muscle where, due to the irradiation treatment, endogenous regeneration could not occur. This would suggest the presence of the new-formed immature fibres was a consequence of the injection of the donor cells. Sections cut from host muscles implanted with non-transgenic skin cells also contained some evidence that regeneration of some fibres had occurred following the implantation of these cells. Such fibres were of small cross-sectional area and contained centrally-located nuclei. However, the sections did not contain the large numbers of very small and disorganised young myofibres characteristic of the muscle implanted with the transgenic skin cells and these control muscles were typical of the histological profile normally seen in non-implanted irradiated *mdx* muscle.



Figure 6. Small immature fibres were a feature of muscle sections following implantation of transgenic skin cells. These newformed myofibres were somewhat haphazard in their orientation. Magnification x 300.

Dystrophin analysis

Muscles examined 3 weeks after the implantation of *mdx* transgenic dermal fibroblasts showed the presence of numerous small diameter fibres which were positive for dystrophin when stained with P6 anti-dystrophin antibody. Four of the muscles contained 10-17% of dystrophin positive fibres and in the remaining two 38 and 40% of fibres were dystrophin-positive (Figure 7). Sections cut from muscles implanted with non-transgenic *mdx* fibroblasts contained exceedingly low numbers of dystrophin positive fibres, reflecting the low levels of revertant fibres we routinely record in our *mdx* colony.

Discussion

In a previous paper we suggested that skin cells harvested from the dermis of a normal mouse are capable of participating in the formation of newly regenerated muscle fibres in *mdx* mouse muscle [8]. In the present work we now confirm this observation by showing the direct participation of skin cells in muscle fibre formation. Prior to implantation the cells were characterised using various markers which are expressed by skeletal muscle cells. Cells for implantation did not express these markers, strongly suggesting they were not of a myogenic lineage. In the first series of experiments reported here implantation of normal mouse skin cells transfected with the lacZ gene, which codes for the bacterial enzyme β -galactosidase resulted in the formation of P-gal positive fibres within the host *mdx* muscle. Our results show that the number of P-gal positive muscle fibres decreased with increasing passage number of the cells implanted and indeed we did not detect any positive fibres following the injection of passage 32 cells. It was interesting to note that these muscles contained fewer fibres as compared to those implanted with passage 11 and 21 cells. Sections cut from muscles implanted with passage 32 cells were characterised by the presence of



Figure 7. Sections cut from muscle implanted with transgenic skin cells contained many fibres which were dystrophin positive when stained with P6 polyclonal antibody. Dystrophin positive fibres were of varied diameter and were not well aligned relative to the long axis of the muscle. Magnification $\times 600$.

connective tissue and unfused mononuclear cells. This indicated to us that the passage 32 cells were less capable of participating in muscle fibre formation as compared with earlier passage cells. This could either reflect the fact that after prolonged times in culture, the cells lose their ability to participate in fibre formation or alternatively, that the number of cells capable of participating in fibre formation is gradually reduced, suggesting that only a subpopulation of the cells we implant are capable of being involved in fibre formation. Our results do not allow us to determine which of these two possibilities is occurring. However, there are several reports in the literature of the existence of a population of mesenchymal stem cells, found within the connective tissues of many organs, including the dermis [30]. Such stem cells are able to differentiate into various mesodermal cell types [3]. Further it has been shown that stem cells obtained from bone marrow and periosteum contribute to myotube formation in the *mdx* mouse [19]. As the number of stem cells have been found to decrease with age, it would be of interest in our work to investigate if skin cells from adult, as opposed to neonatal donors, are less capable of participating in muscle fibre formation.

A low number of mononuclear cells were detected as β -gal positive within the connective tissue surrounding the muscle following the implantation of cells of all passages. As previously stated, all cultures of cells used for implantation were 90-95% positive for P-gal immediately prior to implantation. We therefore find it surprising that we did not detect more P-gal positive mononuclear cells within the sections. The fact that the numbers of p-gal positive fibres decreased with increasing passage number would argue for a loss of "fusibility" of the implanted cells with increasing passage number. This would argue that either the majority of the implanted cells had failed to survive, an event known to occur following the implantation of myo-

genic cells implanted into *mdx* mouse muscle [1] or that after five weeks *in vivo* the cells failed to express the exogenously introduced lacZ gene. Palmer et al., [14] have reported that skin fibroblasts infected with retroviral vectors using different promoters, including the MoMLV LTR, to drive expression of the human protein adenosine deaminase (ADA) show a decrease of expression in the order of 1500 fold after one month of being transplanted into the dermis of Fischer rats. This decrease in ADA expression was only observed in *in vivo* experiments and not in long term cultures of their transfected cells [14].

In our experiments, the majority of p-gal positive fibres were also dystrophin positive indicating that the implanted cells, which were negative for dystrophin when grown *in vitro*, can express dystrophin within *mdx* muscle fibres which is a dystrophin-negative muscle. A low number of fibres were p-gal positive and dystrophin negative. This could be due to the presence of nuclear domains in the muscle fibres where the skin cells have participated in fibre formation. When the skin cells become integrated into the muscle fibre, the p-gal and dystrophin proteins which are encoded only by the donor nuclei, may have a different range of distribution within the fibre. Dystrophin, a component of the cytoskeleton, may not diffuse far from its nucleus of synthesis whereas P-gal, a highly soluble non-sarcomeric protein may diffuse more freely. This view is supported by several reports in which the diffusability of different proteins within the myofibre [13, 16, 18] including dystrophin [11], have been studied. Of the very low number of fibres in our sections which expressed dystrophin but not P-gal we would have to conclude that such a situation reflected the presence of revertant fibres known to be present within *mdx* muscle [10]. However, although the number of such fibres were higher than in sham injected muscles, in the absence of their expressing P-gal we could not include them in the counts of fibres where the skin cells had participated in fibre formation.

Implantation of skin cells from the *mdx* mouse carrying the full length human dystrophin transgene shows that these cells participated in fibre formation and expressed the introduced dystrophin gene. In these experiments the transgene was driven by the skeletal α -actin promoter ensuring that the dystrophin was only expressed within muscle cells. Our observation of high numbers of dystrophin-positive fibres in muscles implanted with the transgenic cells is further evidence that the implanted skin cells have contributed to the formation of the newly-regenerated young muscle fibres. Of particular interest in this experiment is the contrast in the histology of muscle implanted with the control cells (*mdx*) when compared with those implanted with transgenic skin cells. In the former case we did not detect the presence of very immature small calibre fibres present in the muscles implanted with transgenic cells. We have further confirmed this observation in other experiments implanting non-genetically engineered *mdx* skin cells into *mdx* muscle (data not shown). This is an interesting observation that might reflect a difference in

ability of *mdx* skin cells to fuse with *mdx* muscle but this observation most definitely requires further investigation. We are currently in the process of extending this series of experiments to longer time points to evaluate the long-term consequences of implanting such cells into *mdx* muscle and to ascertain if the resultant formed muscle fibres receive an adequate nerve supply. In addition, transgenic skin cells are also being implanted into non-irradiated *mdx* host muscle. In line with our previous results [8] we would expect competition from the endogenous host muscle precursor cells and thus a lower percentage of dystrophin-positive fibres when compared with irradiated muscle. It would also be of value to introduce the transgenic cells into muscles of a non-nude *mdx* host to determine if a competent host immune system results in a lower expression of the transgene, a factor which would also be of significance for potential therapy in humans.

The results reported here show that normal mouse skin cells carrying a reporter gene and those carrying the full length human dystrophin gene are capable of participating in muscle fibre formation and further in expressing the introduced gene in the muscle fibres so formed. These initial observations are encouraging and provide preliminary evidence to show that skin cells can be used as vehicles to express specified genes in muscle fibres. Work is now ongoing to introduce the dystrophin gene under control of the skeletal α -actin promoter into neonatal *mdx* skin cells *ex vivo* and return these cells to the muscles of the same mouse in order to determine the efficacy of autologous skin cell implantation as a route to introducing the dystrophin gene into dystrophin-negative *mdx* muscles.

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