

MYOBLAST TRANSFER IN THE TREATMENT OF DUCHENNE'S MUSCULAR DYSTROPHY

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Abstract *Background.* Myoblast transfer has been proposed as a technique to replace dystrophin, the skeletal muscle protein that is deficient in Duchenne's muscular dystrophy. Donor myoblasts injected into muscles of affected patients can fuse with host muscle fibers, thus contributing their nuclei, which are potentially capable of replacing deficient gene products. Previous controlled trials involving a single transfer of myoblasts have been unsuccessful.

Methods. We injected donor muscle cells once a month for six months to the biceps brachii muscles of one arm of each of 12 boys with Duchenne's muscular dystrophy. The opposite arms served as sham-injected controls. In each procedure 110 million cells donated by fathers or brothers were transferred. The patients were randomly assigned to receive either cyclosporine or placebo. Strength was measured by quantitative isometric muscle testing. Six months after the final myoblast transfer, the presence

of dystrophin was assessed with the use of peptide antibodies specific to the deleted exons of the dystrophin gene.

Results. There was no significant difference in muscle strength between arms injected with myoblasts and sham-injected arms. In one patient, 10.3 percent of muscle fibers expressed donor-derived dystrophin after myoblast transfer. Three other patients also had a low level of donor dystrophin (<1 percent); eight had none.

Conclusions. Myoblasts transferred once a month for six months failed to improve strength in patients with Duchenne's muscular dystrophy. The value of exon-specific peptide antibodies in the interpretation of myoblast-transfer results was demonstrated in a patient with Duchenne's muscular dystrophy who had a high percentage of donor-derived dystrophin. Specific variables affecting the efficiency of myoblast transfer need to be identified in order to improve upon this technique. (N Engl J Med 1995;333:832-8.)

DUCHENNE'S muscular dystrophy is an X-linked disorder caused by deficiency of the protein dystrophin.^{1,2} In animals, myoblast transfer was shown to be a promising method of dystrophin replacement.³⁻⁵ The technique consists of injecting skeletal muscles with donor cells capable of fusing with host muscle fibers and thus providing the missing gene to replace dystrophin. Encouraging results in murine dystrophies³⁻⁵ led to clinical studies in patients with Duchenne's muscular dystrophy.⁶⁻¹⁰ In one report, donor-derived dystrophin transcripts were demonstrated one month after myoblast injection.⁶ Other investigators failed to identify donor-derived dystrophin or messenger RNA.⁷⁻¹⁰ No improvement in strength was observed in any controlled study of myoblast transfer,^{6,7,9} although uncontrolled trials suggested that muscle strength could be increased.^{8,10} Immunosuppression to prevent rejection of donor cells was used in two controlled trials^{6,7} and one uncontrolled trial.¹⁰ The use of antiinflammatory agents introduces another variable into the interpretation of myoblast transfer, because prednisone¹¹ and cyclosporine¹² have been reported to improve strength in patients with Duchenne's muscular dystrophy.

This study differs from previous controlled investi-

gations in several ways. First, instead of a one-time transfer, myoblasts were transferred from donor to host muscles once a month for six months. One study had suggested that myoblasts could assume a satellite-cell position and be available for fusion at a subsequent time.¹³ Second, dystrophin expression was measured by the use of peptide antibodies specific to the deleted exons of the dystrophin gene. This method allows one to distinguish between dystrophin-positive fibers derived from donor DNA and host fibers that had reverted to normal ("revertant" fibers).^{14,15} Third, patients were randomly assigned to receive cyclosporine or placebo, permitting a direct assessment of the need for antirejection drugs and of the effect of cyclosporine on muscle strength.

METHODS

Patient Population

Twelve boys with Duchenne's muscular dystrophy participated in this one-year randomized, double-blind trial of myoblast transfer (Table 1). Informed consent was obtained from the parents of all subjects; clinical outliers were excluded.¹⁶ To be eligible for the study the boys had to be ambulatory; be 5 to 10 years of age; have an identifiable deletion in the dystrophin gene in peripheral-blood leukocytes^{17,18}; have no dystrophin on Western blot analysis of a muscle-biopsy specimen²; be able to cooperate for efficacy testing; have an available donor free of serologic evidence of the human immunodeficiency virus, human T-cell lymphotropic virus type I, hepatitis B virus, cytomegalovirus, Epstein-Barr virus, or syphilis; and have a haplotype compatible with that of the donor (Table 1).^{19,20}

Myoblast Cultures

Selected heterogeneous populations of normal human myoblasts were isolated without the aid of mechanical sorting, according to established culture techniques.^{21,22} The method yielded approximately 1×10^8 cells by the second passage. Muscle samples of 1 g were re-

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Supported by the Muscular Dystrophy Association, the Muscular Dystrophy Group of Great Britain and Northern Ireland, and a grant from the National Institutes of Health (Mo1-RR-00034).

Table 1. Characteristics of the Patients and Myoblast Donors.

PATIENT No.	AGE (YR)	EXON DELETED	IDENTITY AND AGE (YR) OF DONOR	HLA HAPLOIDENTITY*					ARM INJECTED WITH MYOBLASTS	IMMUNOTHERAPY
1	7	51	Brother, 15	Patient: A25 A28	BW22	BW27	Dr1	Dr6	Left	Cyclosporine
				Donor: A25 A28	BW22	BW27	Dr1	Dr6		
2	6	47-50	Brother, 3	Patient: A3 A11	B7	B45	Dr2	Dr4	Right	None
				Donor: A3 A11	B7	B45	Dr2	Dr4		
3	8	2-20	Father, 33	Patient: A1	AW30	B13	B17	Dr7	Left	Cyclosporine
				Donor: A1		B13	B17	Dr7		
4	6	46-53	Father, 37	Patient: A1 A2	B37	B44	Dr4	Dr10	Right	None
				Donor: A1 A2	B37	B57	Dr7	Dr10		
5	9	45	Brother, 6	Patient: A1 A2	B7	B44	Dr4	Dr13	Left	Cyclosporine
				Donor: A1 A3	B7		Dr4	Dr13		
6	9	43	Father, 40	Patient: A1 A2	B49	B57	Dr4	Dr7	Left	None
				Donor: A28 A2	B49	B57	Dr11	Dr7		
7	7	45, 46	Father, 36	Patient: A1 A11	B8	B51	Dr15	Dr17	Left	Cyclosporine
				Donor: A1	B8	B37	Dr4	Dr17		
8	5	45, 46	Father, 36	Patient: A1 A11	B17	B37	Dr4	D7	Right	Cyclosporine
				Donor: A1	B8	B37	Dr4	Dr17		
9	7	45-52	Father, 35	Patient: A3 A25	B7	B8	Dr3	Dr4	Right	None
				Donor: A3 A2	B7	B62		Dr4		
10	6	3-33	Father, 35	Patient: A2 A10	B44	BW35	Dr4	Dr7	Right	Cyclosporine
				Donor: A2 A3	B44	B7	Dr4	Dr13		
11	8	18-27	Father, 35	Patient: A25 AW30	B8	B49	Dr3	Dr7	Left	None
				Donor: A25 A3	B8	BW35	Dr3	Dr2		
12	5	18-27	Father, 35	Patient: A25 AW30	B8	B49	Dr3	Dr7	Right	None
				Donor: A25 A3	B8	BW35	Dr3	Dr2		

*Mismatches of HLA haplotypes that could be recognized by the patients' cells are in boldface.

moved from the biceps brachii of all donors. Half the sample was minced and dissociated immediately; the other half was maintained at 4°C for seven days before dissociation. The dissociation cocktail consisted of 0.01 percent trypsin (Worthington Biochemical, Freehold, N.J.), collagenases I and II (Sigma, St. Louis), and 0.02 percent EDTA. The delay before isolation provided a greater yield of myoblasts in some but not all cases. After the dissociation step, the muscle cultures were treated identically at every stage. The growth medium for all cultures was Ham's MCDB 121 (Clonetics, San Diego, Calif.) supplemented with 15 percent fetal-calf serum. For each culture we verified that more than 95 percent of cells expressed the myoblast-specific neural-cell adhesion molecule using antibody 5H.11 (courtesy of Frank Walsh, Ph.D.)²³ with a Coulter Elite fluorescence-activated cell sorter.

Before myoblast transfer all donor cultures were tested for fusion competency. Each culture was plated in growth medium at 1×10⁴ per square centimeter, allowed to reach 80 percent confluency, and then incubated for 14 days in a fusion medium (Ham's F10) of which 5 percent consisted of the patient's own serum (previously harvested), with 10 μg of insulin per milliliter.²² After trypsinization, the cells were counted with a phase microscope with a standard hemocytometer. For the first transfer the percentage of cells capable of fusing (the number of multinucleated cells/the total number of cells) was as follows: Donor 1, 58 percent; Donor 2, 48 percent; Donor 3, 53 percent; Donor 4, 67 percent; Donor 5, 70 percent; Donor 6, 37 percent; Donor 7, 50 percent; Donor 8, 50 percent; Donor 9, 62 percent; Donor 10, 74 percent; Donor 11, 74 percent; and Donor 12, 67 percent. We demonstrated the ability of the multinucleated cells (myotubes) to express dystrophin using standard immunostaining methods.^{14,15}

Twenty-four hours before each myoblast transfer, bovine proteins in the growth medium were replaced with the patient's own serum (15 percent). Immediately before injection the myoblasts were harvested, washed in phosphate-buffered saline, and resuspended in phosphate-buffered saline with 1 percent human serum albumin (Baxter Laboratories, Deerfield, Ill.), at a final concentration of 1×10⁶ cells per 100 μl. Two hundred microliters, representing 2 million cells, or an equivalent volume of diluent without cells was loaded into individual 1-ml precoded (myoblast injection or sham injection) sy-

ringes for injection. An aliquot of each prepared suspension of myoblasts demonstrated more than 98 percent viability on the basis of the trypan-blue exclusion method.

Before the myoblasts were transferred, additional tests for adventitious agents (bacteria, fungi, mycoplasma, and virus) and pyrogenicity were performed by the Ohio State University Hospitals Department of Microbiology, in accordance with the Public Health Service Diagnostic Laboratory practices and the guidelines of the American Type Culture Collection for quality control of cell lines.²⁴⁻²⁶

Myoblast-Transfer Procedure

Only the biceps brachii muscles were injected. The patients' arms were randomly assigned to receive either myoblast injections or sham injections (Table 1). Before each transfer procedure, the patients received intramuscular meperidine hydrochloride (2.0 mg per kilogram of body weight) and intravenous lorazepam (0.05 mg per kilogram). Fifty-five sites, each 5 mm apart, distributed in 11 rows and 5 columns, were injected throughout the depth of the muscle with a 1.5-inch, 27-gauge needle. The patients and investigators were unaware of which arm received the myoblasts and which the sham injections.

Immunosuppression

The patients were randomly assigned to receive placebo or oral cyclosporine (5 mg per kilogram per day in divided doses) beginning the day before the initial transplantation and continuing throughout the entire 12 months of the study. On the day before each transplantation and for three days thereafter, all patients (irrespective of whether they had been assigned to placebo or cyclosporine) received prednisone at a dose of 2 mg per kilogram per day to diminish inflammatory infiltration at the injection sites in the peritransplantation period.

Clinical Evaluation

The primary efficacy variable for myoblast transfer was the maximal voluntary isometric strength of elbow flexion (Biomedical Designs, Edmonton, Alberta, Canada). The system uses a force trans-

ducer with a load of 0.5 to 1000 N (approximately 0.05 to 100.0 kg). For each muscle group the final value recorded represented the average of three consecutive trials.

The effects of cyclosporine on muscle strength at sites distant from the site of the myoblast transfers were determined by recording the maximal voluntary isometric strength of elbow extension and by manual muscle testing of 34 muscle groups (excluding the injected muscles) to obtain the average muscle score.^{11,16}

Muscle Biopsies after Myoblast Transfer

Biopsies of bilateral biceps brachii muscle were performed at one year (six months after the final myoblast transfer). Superficial and deep pieces of vertically aligned muscle, each measuring 0.5 by 1 cm, were removed, and the samples were processed for histochemical analysis and immunostaining for dystrophin as previously described.^{14,15,27,28} Dystrophin antibodies included those directed to the carboxy terminal (NCL-DYS2, Novacastra Laboratories, Newcastle upon Tyne, United Kingdom) and polyclonal and monoclonal antibodies (Table 2) raised against exon-specific peptides of the deleted exons of the patients. The polyclonal antibodies included Ab2 and Ab4 for exons 8 and 51, respectively,²⁸ and 9218 for exons 48 through 52.¹⁵ The monoclonal antibodies were as follows: MANDYS1-15 for exons 31 and 32,²⁷ MANDYS17 for exons 26 and 27,²⁷ MANDYS106 for exon 43,²⁷ MANEX45A for exon 45,²⁹ and MANEX50 for exon 50.²⁹

The area of each muscle section was determined with a morphometric system as previously described.³⁰ The number of individual muscle fibers in each section was counted manually in sections stained with modified trichrome. The numbers of dystrophin-positive fibers were counted by two investigators without knowledge of which arms had been injected with myoblasts and which had been sham-injected. The counts did not differ by more than 5 percent between investigators for any patient. The number of dystrophin-positive fibers per square millimeter of area and the percentage of dystrophin-positive fibers per muscle section represent the average of the two counts.

Statistical Analysis

Data were collected with MS Excel and transferred to SAS JMP for analysis. Before conducting the statistical analysis, we divided the study into five periods and calculated aggregate data for each response variable as follows: period 0, mean of two base-line measurements; period 1, mean of months 1 through 3; period 2, mean of months 4 through 6; period 3, mean of months 7 through 9; and period 4, mean of months 10 through 12. After the aggregate data were calculated, a second manipulation was performed. The difference

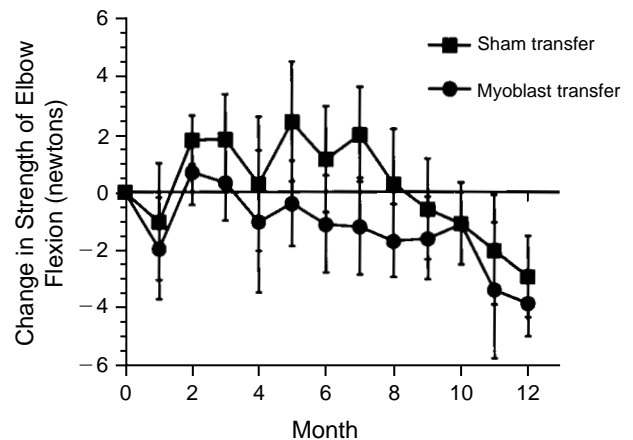


Figure 1. Mean (\pm SD) Change from Base Line (Month 0) in Maximal Voluntary Isometric Strength of Elbow Flexion, According to Whether Myoblast Transfer or Sham Transfer Was Performed.

Myoblast transplantation was performed at base line and months 1, 2, 3, 4, and 5. Strength was measured for 12 months after the initial myoblast or sham transfer. There was no significant difference between arms undergoing myoblast transfer and arms undergoing sham transfer.

from base line was calculated for each period, and the differences from base line for periods 2 through 4 were used in the statistical analysis. All analyses used a repeated-measures analysis of variance model in which the response variable was the calculated difference from base line for each quantitative measure (i.e., quantitative isometric muscle strength of elbow flexor, average muscle score, and so on). In the analysis-of-variance model, the treatment period was included as a nominal factor, immunosuppression (cyclosporine vs. no cyclosporine) was included as a treatment factor, and subjects were included as random factors within each treatment group. An interaction term involving the treatment factor and time factor was also included. A P value of 0.05 or less was considered to indicate statistical significance.

RESULTS

Maximal Voluntary Isometric Strength

Myoblast transfer had no effect on muscle strength (Fig. 1 and 2). Patients underwent myoblast transfer at base line (month 0) and months 1, 2, 3, 4, and 5 for a total of six transfers. In each case, the maximal voluntary isometric strength was measured the day before myoblast transfer. The final test of efficacy was performed at 12 months. Figure 1 shows data from all patients irrespective of whether they received immunosuppressive therapy. At no point was there a statistically significant difference in the degree of change from base line between arms injected with myoblasts and sham-injected arms. Figure 2 shows the results in the two groups according to whether cyclosporine was given. Cyclosporine therapy had no beneficial effect on myoblast transfer.

Expression of Dystrophin

The expression of dystrophin was initially examined after myoblast transfer with a monoclonal antibody to

Table 2. Expression of Donor-Derived Dystrophin after Myoblast Transfer.

PATIENT No.	PEPTIDE ANTIBODIES	EXON IDENTIFIED	PERCENTAGE OF MUSCLE FIBERS RECOGNIZED BY PEPTIDE-SPECIFIC ANTIBODIES
1	Ab4	51	0
	9218	48-52	0
2	MANEX50	50	0
	9218	48-52	0
3	Ab2	8	<1
4	MANEX50	50	0
	9218	48-52	0
5	MANEX45A	45	10.3
6	MANDYS106	43	<1
7	MANEX45A	45	0
8	MANEX45A	45	0
9	MANEX50	50	0
10	MANDYS1-15	31, 32	0
11	MANDYS17	26, 27	0
12	MANDYS17	26, 27	<1

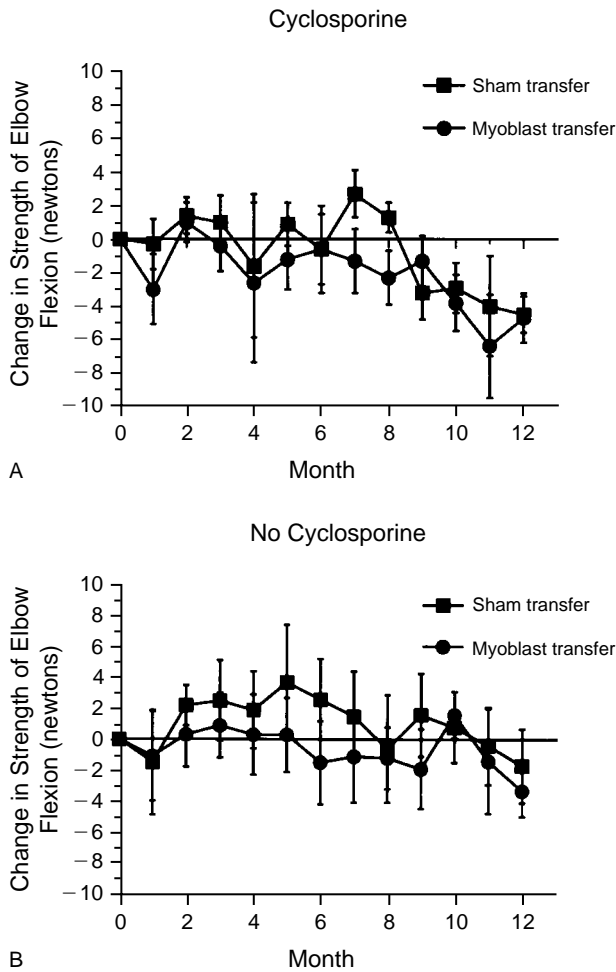


Figure 2. Mean (\pm SD) Change from Base Line (Month 0) in Maximal Voluntary Isometric Strength of Elbow Flexion, According to Whether Myoblast Transfer or Sham Transfer Was Performed and Whether the Patients Received Cyclosporine.

Myoblast transfer accompanied by cyclosporine treatment did not significantly increase strength (Panel A). Myoblast transfer in the absence of cyclosporine treatment also did not significantly increase strength (Panel B).

the carboxy terminal. There were no significant differences in the mean (\pm SD) number of dystrophin-positive fibers between the myoblast-injected arms (superficial region, 2.57 ± 3.18 fibers per square millimeter; deep region, 2.68 ± 2.7 fibers per square millimeter) and sham-injected arms (superficial region, 2.69 ± 3.87 fibers per square millimeter; deep region, 3.96 ± 5.23 fibers per square millimeter). Without the use of exon-specific peptide antibodies for deleted exons it was not possible to determine whether any dystrophin-positive fibers in the arms receiving myoblasts expressed donor-derived dystrophin or represented revertant fibers.^{14,15} A panel of peptide-specific antibodies raised against deleted exons of the patients unequivocally identified muscle fibers expressing donor-derived dystrophin. As shown in Table 2, in 4 of the 12 patients

muscle fibers expressing donor-derived dystrophin were identified. Only a single positive fiber was found in the entire cross section of muscle from Patients 3 and 6, and two positive fibers were identified in muscle from Patient 12. This small number is less than the 1 percent of revertant fibers that express dystrophin that are usually observed in patients with Duchenne's muscular dystrophy.^{14,15} In contrast, Patient 5 had both small and large fibers expressing donor-derived dystrophin (Fig. 3): 10.3 percent of the muscle fibers exhibited dystrophin contributed by the patient's brother (420 dystrophin-positive fibers of a total of 4080 fibers). The number of dystrophin-positive fibers was nearly equally distributed in the superficial and deep regions of the transplanted muscle. Patient 5 and his mother both had the same deletion of exon 45 (data not shown), excluding somatic mosaicism as an explanation for the patient's dystrophin-positive fibers after myoblast transfer.

Effects of Cyclosporine on the Strength of Muscles Not Undergoing Myoblast Transfer

Six boys were treated with 5 mg of cyclosporine per kilogram per day, and six boys received placebo for one year. As shown in Figure 4, there was no significant difference in the average muscle scores between the cyclosporine-treated boys and those given placebo. The loss of muscle strength in both groups was similar to the previously established natural rate of decline.¹⁶ In addition to manual muscle testing, we studied the right and left elbow extensors using maximal voluntary isometric strength as a measure of efficacy in the cyclosporine-treated and placebo groups. This sensitive measure also failed to identify significant differences in strength between the two groups (Fig. 5).

Side Effects

Blood levels of cyclosporine were monitored every month and adjusted to maintain therapeutic levels. Most patients had transient soreness in the injected muscles that typically resolved within three to four days of the procedure. No local or systemic evidence of immune-mediated rejection was seen.

There was no significant difference in the number of side effects between the patients treated with cyclosporine and those given placebo, although excessive hair growth was more common in the cyclosporine-treated group (reported at eight follow-up visits, as compared with one visit in the placebo group) (Table 3). Mild upper respiratory tract infections or otitis media that resolved with antibiotic treatment was reported at six follow-up visits in the cyclosporine group and seven follow-up visits in the placebo group. One patient in each group had transient urticaria lasting two to three weeks that resolved without treatment. In a single patient (treated with cyclosporine) a focal cellulitis developed over the buttocks, requiring drainage

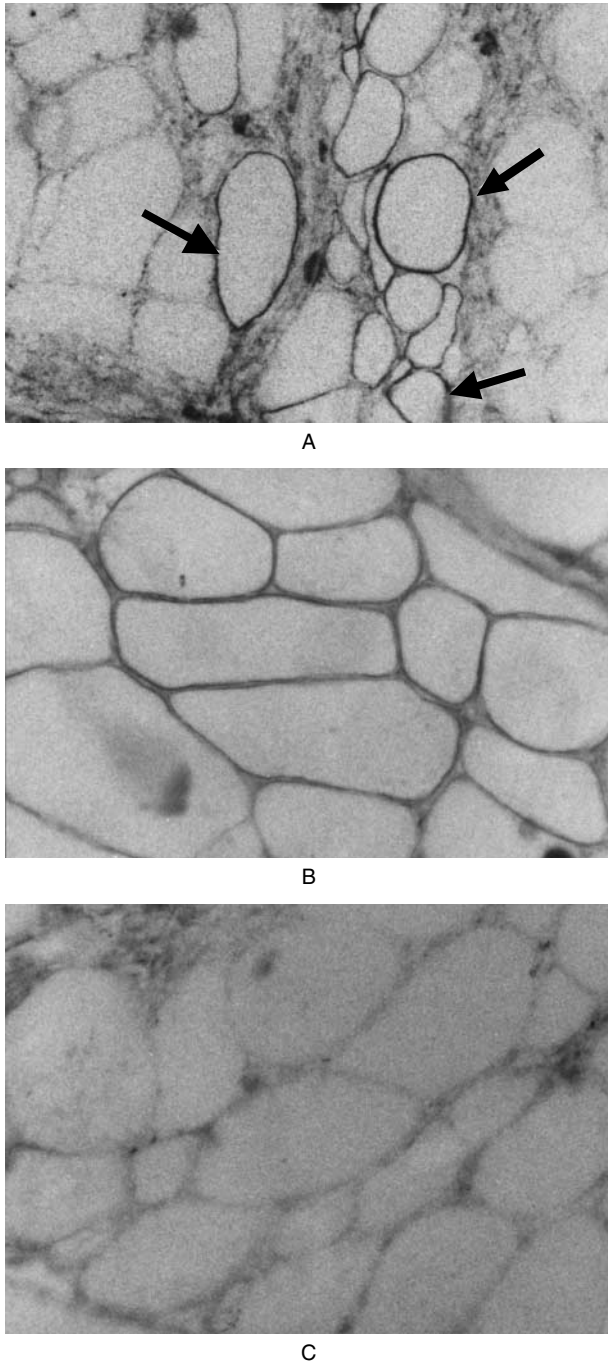


Figure 3. Section of Muscle from Biceps Brachii of Patient 5, in Whom Exon 45 Was Known to Be Deleted.

In Panel A, immunostaining with a monoclonal antibody specific for exon 45 (MANEX45A)²⁹ revealed donor-derived dystrophin on the sarcolemma of the muscle from the arm receiving the myoblast transfers ($\times 270$). The dystrophin-positive fibers (dark membrane) were not contiguous and varied in size (arrows). Muscle fibers showing no dystrophin staining surrounded the cluster of positive fibers. In an adjacent area of the same muscle-biopsy specimen (Panel B), immunostaining with peptide-specific antibody for exon 45 revealed larger, contiguous muscle fibers, most of which expressed donor-derived dystrophin on the sarcolemma ($\times 350$). A section of muscle from the sham-injected arm stained with the exon 45-specific monoclonal antibody showed no dystrophin expression (Panel C, $\times 350$).

and antibiotic treatment. None of the monitored laboratory values differed significantly between the two groups. No patient had to discontinue cyclosporine therapy prematurely, and none had dose-limiting changes in blood pressure or renal function.

DISCUSSION

The present study evaluated myoblast transfer as a treatment for Duchenne's muscular dystrophy.³¹ We used a multiple-injection protocol in which myoblasts were transferred once a month for six months. In a previous report, two transfers of myoblasts to the same muscle in one patient seemed to increase the number of dystrophin-positive fibers.³² Our patients received twice the number of myoblasts per injection site as were given in previous controlled studies.^{6,7,9} A total of 660 million myoblasts were delivered to the biceps brachii muscle of each patient. Law et al. delivered a total of 5 billion cells through 48 injections into the major muscle groups in both legs of patients in an uncontrolled trial.¹⁰ We observed no improvement in muscle strength in any patient, including the boy with 10 percent dystrophin-positive fibers.

An important step in assessing myoblast therapy is determining the extent of expression of donor-derived dystrophin. Gussoni et al. used a method involving the reverse-transcriptase polymerase chain reaction and concluded that 1 percent of host muscle fibers in the transplanted region expressed donor-derived dystrophin.⁶ Although messenger RNA was detected, the levels were low and could have arisen from transcription in myoblasts that had not fused to host muscle fibers. Indeed, these investigators found no difference in the number of dystrophin-positive fibers between muscle injected with myoblasts and sham-injected muscle. The dystrophin-positive fibers on the sham-injected side presumably represented revertant fibers, believed to arise from a second-site mutation in the dystrophin gene that corrects the reading frame.^{14,15} The problems in distinguishing the source of dystrophin in the transplanted region are further illustrated by a patient described by Karpati et al.; in this patient 5 percent of fibers were positive for dystrophin, yet no donor-derived DNA or RNA was found.⁷ In the present study, antibodies were raised to peptides from the deleted DNA exons of each patient. Four patients had donor-derived dystrophin. In three, the proportion of dystrophin-positive fibers was meager, but in a single patient the proportion was considerable (10.3 percent of all fibers). The dystrophin-positive fibers were distributed equally throughout the superficial and deep regions of the biceps muscle, probably reflecting the method of myoblast injection throughout the belly of the muscle. Our observation is a convincing demonstration of the transcription and translation of dystrophin by donor myoblast cells. The 10 percent level of dystrophin expression observed in one patient also has particular importance, since Cox et al. estimated that a 20 percent level of dystrophin expression was potentially protective in experiments with transgenic mice.³³ Unfortu-

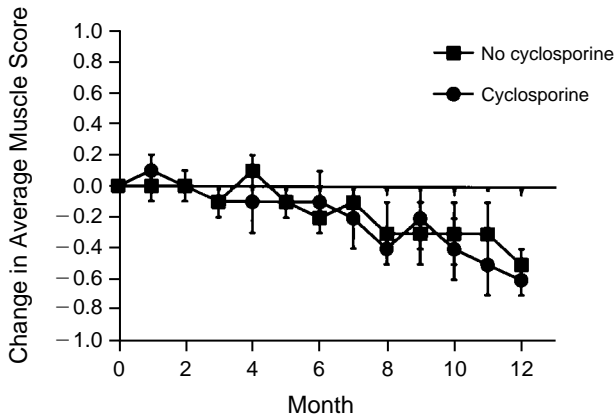


Figure 4. Mean (\pm SD) Change from Base Line (Month 0) in the Average Muscle Score, According to Whether the Patients Received Cyclosporine.

There was no significant difference in the rate of decline in the average muscle score between the two groups. The scores were measured on a 10-point scale in which 10 was the highest possible score.¹¹

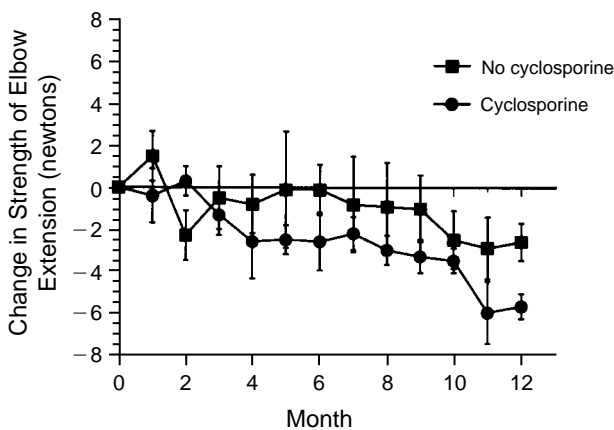


Figure 5. Mean (\pm SD) Change from Base Line (Month 0) in Maximal Voluntary Isometric Strength of Elbow Extension, According to Whether the Patients Received Cyclosporine.

There was no significant difference in the rate of decline in strength between the two groups.

nately, we found no accompanying increase in muscle strength.

It would be of interest to know what factors governed the survival, fusion, and expression of donor myoblasts in our patients.⁷ Connective-tissue barriers alone cannot account for the lack of success, since the patient with the highest level of dystrophin expression was one of the two oldest in the study (age, 9 years) (Table 1). The older age of this patient also tends to negate the possibility that favorable results of myoblast transfer depend on the presence of a greater number of random clusters of regenerating fibers, a finding more prevalent in young patients. The likelihood that the presence of basal lamina hinders myoblast fusion is also not supported by our successful results in the older patient. Immune-mediated rejection probably does have a role, as illustrated in a series of studies by

Huard et al.^{8,13} and Tremblay et al.⁹ In our study the only patient with positive results was taking cyclosporine. Whether this is the predominant factor underlying the relative success of the procedure in a single patient cannot be determined. We did not measure serum antibodies to dystrophin.¹³ Possibly other immunosuppressive regimens could increase the success of myoblast transfer, as indicated by improved results in mdx mice given tacrolimus (FK 506).³⁴

The use of prednisone at the time of myoblast transfer could have potential inhibitory effects on myoblast fusion, but in vivo results might be difficult to predict. In cultures of muscle from patients with Duchenne's or Becker's muscular dystrophy, methylprednisolone inhibits myoblast fusion.³⁵ In contrast, glucocorticoids have no effect on normal cultures³⁵ but enhance the expression of dystrophin in cultures of normal muscle and muscle from patients with Becker's muscular dystrophy in which fusion is arrested.³⁶

A secondary outcome variable of our study concerned the effects of cyclosporine in patients with Duchenne's muscular dystrophy. Sharma et al. reported that treatment with cyclosporine (5 mg per kilogram per day) increased the strength of tibialis anterior muscles, as measured by tetanic force and maximal voluntary isometric contraction.¹² The improvement occurred as early as two weeks after treatment began and persisted for eight weeks. In that open-label study, the response in cyclosporine-treated patients was compared with the natural rate of decline of muscle strength in Duchenne's muscular dystrophy. We were unable to confirm a cyclosporine-induced improvement in strength. We found no difference in strength between cyclosporine-treated patients and patients given placebo by using manual muscle testing¹¹ or maximal voluntary isometric strength of elbow extensors. Although because of our small sample (12 patients) the results of this negative study should be interpreted with caution, the accuracy of the findings is supported by the fact that neither group showed significant deviations from our previously established natural rate of decline in muscle strength in Duchenne's muscular dystrophy.^{11,17,37}

In conclusion, our study indicates that myoblast

Table 3. Side Effects of Cyclosporine and Placebo.*

SIDE EFFECT	CYCLOSPORINE†	PLACEBO
	no. of visits	
Acne	1	0
Hair growth	8	1
Sore throat	1	1
Mouth ulcers	1	2
Fever	0	2
Gum hypertrophy	4	4
Total	15	10

*The values represent the numbers of follow-up visits at which each side effect was reported.

†None of the following side effects of cyclosporine were reported: chills, hand tremor, numb hands or feet, skin changes, or fever.

transfer in its current state of development does not improve strength in patients with Duchenne's muscular dystrophy despite the transfer of a large number of cells. However, we also demonstrated that myoblast transfer can be used to deliver cells capable of fusing with host muscle fibers and expressing donor-derived dystrophin. Only through further work will it be possible to identify the important variables affecting the efficiency of transfer and thus making the use of myoblasts a potential form of cell therapy for muscle diseases.

We are indebted to the patients and their families; tremendous demands were placed on them in terms of time, effort, and expense in complying with all the complexities of the study.

REFERENCES

- Hoffman EP, Brown RH Jr, Kunkel LM. Dystrophin: the protein product of the Duchenne muscular dystrophy locus. *Cell* 1987;51:919-28.
- Hoffman EP, Fischbeck KH, Brown RH, et al. Characterization of dystrophin in muscle-biopsy specimens from patients with Duchenne's or Becker's muscular dystrophy. *N Engl J Med* 1988;318:1363-8.
- Law PK, Goodwin TG, Wang MG. Normal myoblast injections provide genetic treatment for murine dystrophy. *Muscle Nerve* 1988;11:525-33.
- Partridge TA, Morgan JE, Coulton GR, Hoffman EP, Kunkel LM. Conversion of mdx myofibers from dystrophin-negative to -positive by injection of normal myoblasts. *Nature* 1989;337:176-9.
- Karpati G, Pouliot Y, Zubrzycka-Gaarn E, et al. Dystrophin is expressed in mdx skeletal muscle fibers after normal myoblast implantation. *Am J Pathol* 1989;135:27-32.
- Gussoni E, Pavlath G, Lanctot AM, et al. Normal dystrophin transcripts detected in Duchenne muscular dystrophy patients after myoblast transplantation. *Nature* 1992;356:435-8.
- Karpati G, Ajdukovic D, Arnold D, et al. Myoblast transfer in Duchenne muscular dystrophy. *Ann Neurol* 1993;34:8-17.
- Huard J, Bouchard JP, Roy R, et al. Human myoblast transplantation: preliminary results of 4 cases. *Muscle Nerve* 1992;15:550-60.
- Tremblay JP, Malouin F, Roy R, et al. Results of a triple blind clinical study of myoblast transplantations without immunosuppressive treatment in young boys with Duchenne muscular dystrophy. *Cell Transplant* 1993;2:99-112.
- Law PK, Goodwin TG, Fang Q, et al. Feasibility, safety, and efficacy of myoblast transfer therapy on Duchenne muscular dystrophy boys. *Cell Transplant* 1992;1:235-44.
- Mendell JR, Moxley RT, Griggs RC, et al. Randomized, double-blind six-month trial of prednisone in Duchenne's muscular dystrophy. *N Engl J Med* 1989;320:1592-7.
- Sharma KR, Mynhier MA, Miller RG. Cyclosporine increases muscular force generation in Duchenne muscular dystrophy. *Neurology* 1993;43:527-32.
- Huard J, Roy R, Bouchard JP, Malouin F, Richards CL, Tremblay JP. Human myoblast transplantation between immunohistocompatible donors and recipients produces immune reactions. *Transplant Proc* 1992;24:3049-51.
- Burrow KL, Covert DD, Klein CJ, et al. Dystrophin expression and somatic reversion in prednisone-treated and untreated Duchenne dystrophy. *Neurology* 1991;41:661-6.
- Klein CJ, Covert DD, Bulman DE, Ray PN, Mendell UR, Burghes AH. Somatic reversion/suppression in Duchenne muscular dystrophy (DMD): evidence supporting a frame-restoring mechanism in rare dystrophin-positive fibers. *Am J Hum Genet* 1992;50:950-9.
- Brooke MH, Fenichel GM, Griggs RC, et al. Clinical investigation in Duchenne dystrophy. 2. Determination of the "power" of therapeutic trials based on the natural history. *Muscle Nerve* 1983;6:91-103.
- Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT. Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acids Res* 1988;16:11141-56.
- Beggs AH, Koenig M, Boyce FM, Kunkel LM. Detection of 98% of DMD/BMD gene deletions by polymerase chain reaction. *Hum Genet* 1990;86:45-8.
- van Rood JJ, van Leeuwen A. Major and minor histocompatibility systems in man and their importance in bone marrow transplantation. *Transplant Proc* 1976;8:429-36.
- Bodmer WF, Albert E, Bodmer JG, et al. Nomenclature for factors of the HLA system, 1987. In: Dupont B, ed. *Immunobiology of HLA: histocompatibility testing 1987*. Vol. I. New York: Springer-Verlag, 1989:72-9.
- Blau HM, Webster C, Pavlath GK. Purification and proliferation of human myoblasts isolated with fluorescence activated cell sorting. *Adv Exp Med Biol* 1990;280:97-100.
- Blau HM, Webster C. Isolation and characterization of human muscle cells. *Proc Natl Acad Sci U S A* 1981;78:5623-7.
- Walsh FS. N-CAM is a target cell surface antigen for the purification of muscle cells for myoblast transfer therapy. *Adv Exp Med Biol* 1990;280:41-6.
- Cour I, Maxwell G, Hay RJ. Tests for bacterial and fungal contaminants in cell cultures as applied at the American Type Culture Collection (ATCC). *Tissue Cult Assoc Man* 1979;5:1157-60.
- Macy ML. Tests for mycoplasma contamination of cultured cells as applied at the American Type Culture Collection (ATCC). *Tissue Cult Assoc Man* 1979;5:1151-5.
- Aldrich CD, Macic P. Reverse transcriptase assay for detection of oncogenic RNA viruses in cultured cells. *Tissue Cult Assoc Man* 1979;5:1147-50.
- Nguyen TM, Morris GE. Use of epitope libraries to identify exon-specific monoclonal antibodies for characterization of altered dystrophins in muscular dystrophy. *Am J Hum Genet* 1993;52:1057-66.
- Winnard AV, Mendell JR, Prior TW, Florence J, Burghes AHM. Frameshift deletions of exons 3-7 and revertant fibers in Duchenne muscular dystrophy: mechanisms of dystrophin production. *Am J Hum Genet* 1995;56:158-66.
- Le Thiet T, Nguyen TM, Hori S, Sewry CA, Dubowitz V, Morris GE. Characterization of genetic deletions in Becker muscular dystrophy using monoclonal antibodies against a deletion-prone region of dystrophin. *Am J Med Genet* (in press).
- Mendell JR, Sahenk Z, Gales T, Paul L. Amyloid filaments in inclusion body myositis: novel findings provide insight into nature of filaments. *Arch Neurol* 1991;48:1229-34.
- Karpati G, Johnston W, Ajdukovic G, et al. Myoblast transfer (MT) in McArdle's disease (McD). *Neurology* 1992;42:Suppl 3:387. abstract.
- Huard J, Bouchard JP, Roy R, et al. Myoblast transplantation produced dystrophin-positive muscle fibres in a 16-year-old patient with Duchenne muscular dystrophy. *Clin Sci* 1991;81:287-8.
- Cox GA, Cole NM, Matsumura K, et al. Overexpression of dystrophin in transgenic mdx mice eliminates dystrophic symptoms without toxicity. *Nature* 1993;364:725-9.
- Kinoshita I, Vilquin JT, Guerette B, Asselin I, Roy R, Tremblay JP. Very efficient myoblast allotransplantation in mice under FK506 immunosuppression. *Muscle Nerve* 1994;17:1407-15.
- Hardiman O, Brown RH Jr, Beggs AH, Specht L, Sklar RM. Differential glucocorticoid effects on the fusion of Duchenne/Becker and control muscle cultures: pharmacologic detection of accelerated aging in dystrophic muscle. *Neurology* 1992;42:1085-91.
- Hardiman O, Sklar RM, Brown RH Jr. Methylprednisolone selectively affects dystrophin expression in human muscle cultures. *Neurology* 1993;43:423-5.
- Brooke MH, Fenichel GM, Griggs RC, et al. Clinical investigation of Duchenne muscular dystrophy: interesting results in a trial of prednisone. *Arch Neurol* 1987;44:812-7.