# Defective myoblasts identified in Duchenne muscular dystrophy

(satellite muscle cells/clonal analysis)

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**ABSTRACT**    A defect in the proliferative capacity of satellite cells, mononucleated precursors of mature muscle fibers, was found in clonal analyses of cells cultured from Duchenne muscular dystrophy (DMD) patients. The total yield of myoblasts per gram of muscle biopsy was decreased to 5% of normal. Of the DMD myoblast clones obtained, a large proportion contained a morphological class of flat distended cells that had an increased generation time and ceased to proliferate beyond 100–1,000 cells but could be induced to fuse and form myotubes. The altered muscle phenotype was detected in all cultures from DMD patients but was rarely found among myoblasts of controls. By age 14 yr, it comprised as many as 90% of DMD myoblasts. The remaining DMD myoblast clones, which initially grew well, had severely impaired proliferative capacity upon passage and further cultivation. Eventually all myoblasts from DMD muscle tissue exhibited defective growth potential. In contrast, the fibroblast yield and proliferative capacity from DMD samples did not differ from normal. Based on these findings, we propose a hypothesis for the etiology of DMD: Dividing myoblasts are required for muscle growth and maintenance, and the limited capacity of DMD myoblasts to grow is directly related to the progressive muscle degeneration characteristic of the disease.

Duchenne muscular dystrophy (DMD) is a chronic, lethal X-linked disease that affects 1 in 4,800 males (1). Although DMD is well defined clinically, the cellular and biochemical basis for the disease is unknown. A major problem in the biological study of muscular dystrophy has been the heterogeneous nature of muscle tissue. Muscle is composed of a mixture of cell types present in variable ratios, especially in disease states. When muscle is dissociated and the cells plated in culture, both myoblasts and fibroblasts are obtained and the rate of proliferation of the fibroblasts frequently exceeds that of the myoblasts. DMD muscle cultures have been compared with normal muscle cultures and a number of differences reported, including increased collagen synthesis (2), decreased specific activity of creatine kinase (3), increased ratio of nonmuscle to muscle creatine kinase isozymes (3–5), altered lipid biosynthesis (6), and clustered myotube morphology (5, 7, 8). However, it is unclear whether these differences are intrinsic to the DMD myoblasts and related to the disease or a reflection of the proportion of fibroblasts present in the mixed cultures analyzed.

We have developed methods for obtaining pure populations of human myoblasts that can be studied under standardized conditions (9). Our methods use postnatal muscle as a source. Consequently, the myoblasts obtained in culture are muscle satellite cells, the mononucleated myoblasts of mature muscle fibers, positioned between the basement membrane and the sarcolemma (10). It is these satellite cells that are capable of proliferating *in vitro* (11, 12) and are probably responsible for postnatal muscle regeneration and fiber growth *in vivo*. We have

previously used pure populations of myoblasts obtained by these methods to study differentiated muscle properties and observed no differences between normal and DMD myotube morphology or function (13, 14).

We report here evidence of a cellular abnormality in the morphology and proliferative capacity of undifferentiated muscle satellite cells from patients with DMD. The yield of myoblasts per gram of dystrophic biopsy was decreased by 95% and the growth potential of the remaining myoblasts was greatly diminished. These results lead us to postulate that the limited proliferative capacity of DMD satellite cells has profound effects on postnatal muscle development and results in the progressive muscle degeneration characteristic of the disease.

## MATERIALS AND METHODS

**Muscle Samples.** Cultured cells obtained from the muscle biopsies of 9 males with diagnosed DMD and 1 obligate carrier were compared with those of 14 age-matched normal controls. All dystrophic and normal samples analyzed were from the vastus lateralis or medialis muscles of the thigh, with the exceptions noted in Table 1. All patients were diagnosed prior to reaching 5 yr of age and exhibited classical clinical symptoms of the disease: proximal weakness, pseudohypertrophy, serum creatine kinase activity 100-fold greater than the laboratory range for normal individuals, and histological evidence of myopathy typical of DMD. The carrier (B.L.), the mother of two sons with DMD (J.L. and S.L.), had normal serum creatine kinase activity, no clinical symptoms of the disease, and a normal biopsy of the vastus lateralis muscle. Dystrophic muscle samples were obtained from volunteers or as a consequence of orthopedic procedures or diagnostic biopsy, in accordance with the guidelines of the Stanford Human Subjects Committee. Control samples, uncomplicated by nerve or muscle disease, were obtained as a consequence of orthopedic procedures, frequently correction of congenital dislocation of the hip, or at autopsy (P.Z. and A.S.).

**Clonal Analysis.** To isolate cells from normal and DMD samples, muscle was dissected to remove connective tissue and treated with trypsin, and the dissociated cells were plated on collagen at low density (5–50 clones per 60-mm dish), as described (9). After 3–6 days in culture, the medium containing the debris of dissociated myofibers was removed, revealing the presence of single cells. These were primarily fibroblasts and muscle satellite cells. The cell yield was determined as the number of cells per 0.1 g of biopsy that attached to the culture dish. For clonal analysis, individual cells were circled and the clones to which they gave rise were analyzed. Clonal growth kinetics were determined by daily counting of the number of cells in each clone. As previously documented (9), a lag of 2–5 days was typically observed before satellite cells from dystrophic or normal muscle tissue began to divide in culture, in

Abbreviation: DMD, Duchenne muscular dystrophy.

good agreement with the findings of others (15, 16). The number of clones monitored per sample varied, primarily due to the amount of tissue obtained. To examine cell morphology, clones were visualized by using an inverted Leitz microscope with phase-contrast optics.

Myoblast and fibroblast clones that proliferated well were individually passaged by using cloning wells. Some cells of each clone were plated in microwells to test their myogenic properties or ability to fuse and form multinucleated myotubes. The remainder of the cells were grown to high density as mass cultures and then either were stored frozen or immediately used in experiments of muscle differentiated properties (9, 13, 14). The proliferation or fusion of cells at both clonal and high density was manipulated by changes in the composition of the culture medium, as described (9).

**Statistical Analysis.** From the growth curves, the average doubling time for each normal and DMD muscle clone was determined. The distribution of doubling times for normal and DMD clones was compared as a function of age by rank order analysis using the Mann–Whitney $U$ test (17, 18).

## RESULTS

The total cell yield from normal and dystrophic muscle was compared. For each of the nine DMD biopsies analyzed, the number of cells per 0.1 g of tissue that attached to culture dishes was diminished by an order of magnitude relative to normal controls. As previously reported (9), an average yield of $5 \pm 1 \times 10^3$ cells per 0.1 g of tissue was obtained from controls.

The proportions of the cell types present in the cultures from DMD and normal samples differed. Human fibroblasts could be easily distinguished from human myoblasts by phase-contrast microscopy, as shown in Fig. 1. We extensively tested the reliability of this morphological mode of identification of cell types by exposing cells to fusion medium and then scoring those clones that developed multinucleated myotubes. Of 559 clones of cells of fibroblastic morphology, none fused in fusion medium. Of 406 clones of cells of myoblast morphology, 98% fused to form myotubes. Using these morphological criteria, we determined the proportion of cell types obtained as a function of age of muscle donor, as shown in Table 1. Cells isolated from control samples, regardless of donor age, were 96 ± 2% myoblasts and 4 ± 2% fibroblasts, in good agreement with the findings of others (19). By contrast, the proportion of cells isolated from DMD muscle that were myoblasts decreased with increasing age of patient, from approximately 90% to 30% of total clones. Concurrently, the proportion of fibroblasts increased from 10% to 70%.

The overall reduction in cell yield and the shift in the proportion of cell types obtained from DMD samples were due to a deficit of myoblasts. This is clear from an analysis of the absolute yield of the two cell types, myoblasts and fibroblasts (Table
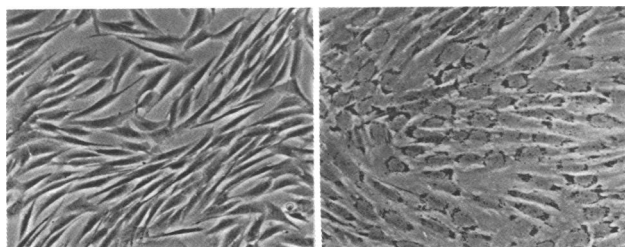


FIG. 1. Morphology of myoblast and fibroblast cell types. Myoblasts are small refractile, triangular cells (*Left*) that are easily distinguished from broad, flat fibroblasts with abundant dark perinuclear granules (*Right*). Photographed *in vivo* with phase-contrast optics. (×70.)

Table 1. Clonal analysis of cell type by morphology

| Donor* | Age, yr | $n^†$ | M, %‡ | F, %§ | D/M, %¶ | Yield per 0.1 g of tissue‖ | |
|---|---|---|---|---|---|---|---|
| | | | | | | M | F |
| **Normal** | | | | | | | |
| L.S. | 2 | 167 | 100 | 0 | 0.6 | 3,095 | 0 |
| B.N. | 2 | 170 | 96 | 4 | 0 | 13,370 | 575 |
| K.D. | 2 | 24 | 75 | 25 | 0 | 690 | 230 |
| P.M. | 2 | 229 | 85 | 15 | 0 | 15,000 | 2,615 |
| M.C. | 4 | 98 | 92 | 6 | 0 | 1,800 | 160 |
| B.F. | 5 | 73 | 86 | 14 | 0 | 1,210 | 190 |
| B.M. | 6 | 57 | 98 | 2 | 0 | 9,335 | 165 |
| H.B. | 7 | 384 | 100 | 0 | 0 | 1,485 | 5 |
| S.W.** | 8 | 329 | 100 | 0 | 0.3 | 3,705 | 0 |
| A.B. (L)** | 8 | 142 | 100 | 0 | 0 | 2,535 | 0 |
| A.B. (R)** | 8 | 533 | 100 | 0 | 0 | 5,860 | 0 |
| C.B. | 12 | 40 | 98 | 2 | 0 | 4,755 | 120 |
| K.Z.** | 19 | 20 | 100 | 0 | 0 | — | — |
| Mean | | | 96 | 4 | <1 | 4,715 | 144 |
| ± SEM†† | | | ± 2 | ± 2 | | ± 1,234 | ± 56 |
| **DMD** | | | | | | | |
| P.D. | 2 | 55 | 80 | 20 | 2 | 150 | 35 |
| J.G.** | 3 | 136 | 90 | 10 | 22 | 650 | 75 |
| W.M.** | 5 | 112 | 95 | 5 | 24 | 3,925 | 220 |
| M.A. | 6 | 26 | 39 | 61 | 70 | 60 | 95 |
| J.L.** | 8 | 247 | 62 | 38 | 67 | 215 | 130 |
| P.A. | 9 | 24 | 54 | 46 | 67 | 75 | 65 |
| R.B. | 12 | 133 | 27 | 73 | 56 | 250 | 675 |
| S.L.** | 14 | 273 | 27 | 73 | 77 | 105 | 290 |
| M.B. | 14 | 262 | 29 | 71 | 93 | 56 | 128 |
| Mean | | | NA‡‡ | NA‡‡ | NA‡‡ | 215 | 143 |
| ± SEM†† | | | | | | ± 77 | ± 31 |
| **Other** | | | | | | | |
| R.B. (S) | 12 | 184 | 72 | 28 | 41 | 2,290 | 875 |
| B.L. | 31 | 111 | 95 | 5 | 5 | 1,505 | 75 |
| P.Z. | 63 | 136 | 54 | 46 | 1 | — | — |
| A.S. | 77 | 10 | 40 | 50 | 0 | — | — |

\* Samples were from the vastus lateralis muscle, with the exception of S.W. and K.Z., from whom the biceps femoris and thoracis spinales muscles were obtained, respectively. From R.B. both semimembranosus (S) and vastus lateralis muscles were obtained. From A.B., vastus lateralis muscles of left (L) and right (R) legs were analyzed. J.L. and S.L. as well as M.A. and P.A. are pairs of brothers. B.L. is an obligate carrier, the mother of J.L. and S.L. Donors P.Z. and A.S. were normal individuals from whom samples were obtained at autopsy following cardiac arrest. Normal donors consisted of four males and eight females; DMD donors were all males; and other donors were all male, with the exception of B.L.

† $n$ is the number of total clones analyzed for each sample.

‡ M is the % of total clones that were muscle (including D) (see text).

§ F is the % of total clones that were fibroblasts.

¶ D/M is the % of total muscle clones that had a diseased phenotype.

‖ Yield of myoblasts (M) and fibroblasts (F) per 0.1 g of tissue was extrapolated from the number of clones obtained per size of tissue dissociated.

** Samples used in the analysis presented in Fig. 4.

†† Trimmed means were calculated by eliminating the highest and lowest values in each case.

‡‡ NA, not applicable because values changed with patient age.

1). The range in the values obtained for individual samples is broad due to the heterogeneity of the biological material analyzed, yet the patterns for control and DMD groups are distinct. Mean values were compared, because the yield did not vary with age. From normal controls, an average of 144 ± 56
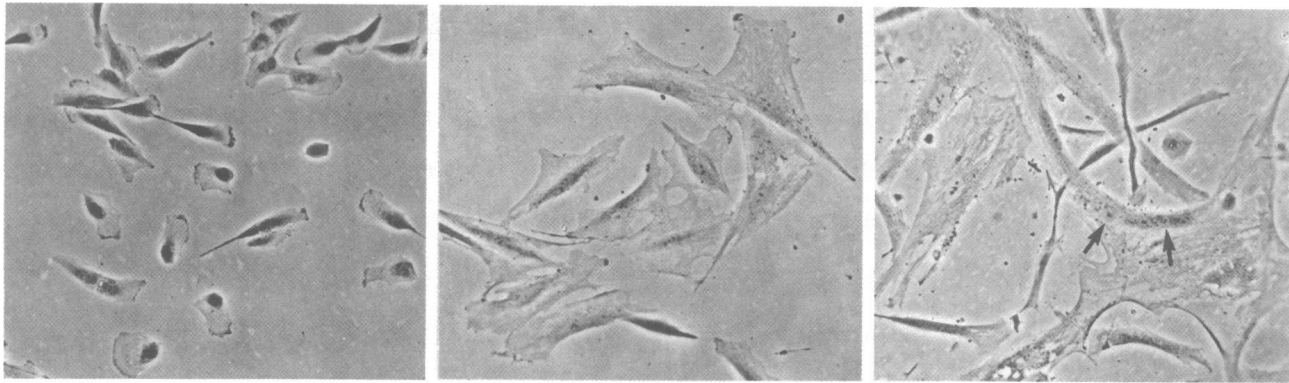
FIG. 2. Morphology of DMD myoblast cell types. Proliferating myoblasts at clonal density are typically small, refractile, triangular cells that are actively dividing (*Left*). The altered myoblast phenotype is characterized by large, flat, distended cells (*Center*). These cells have limited proliferative capacity but can be induced to fuse and form multinucleated myotubes indicated by arrows (*Right*). Photographed *in vivo* with phase-contrast optics. (×120.)

fibroblasts and 4,715 ± 1,234 myoblasts was obtained per 0.1 g of muscle biopsy. However, from similar biopsies of dystrophic patients, an average of 143 ± 31 fibroblasts and 215 ± 77 myoblasts was obtained. Thus, the expected number of fibroblasts, but only 5% of the expected number of myoblasts, were isolated from DMD muscle. This 95% reduction in myoblast yield is not likely to be due to increased sensitivity of the cells to the dissociating agent, trypsin, because no significant decrease in cell number was observed when DMD myoblasts obtained in culture were trypsin-passaged and replated.

We have identified a third morphological cell type, designated D, present among clones of DMD muscle origin (Fig. 2). These cells are large, flat, and distended and are clearly distinguished from normal myoblasts, which are smaller, refractile, and actively dividing. Clones of these cells, which typically reach a few hundred cells in size and then cease dividing, fuse to form multinucleated myotubes when exposed to fusion medium. Thus, they appear to be myoblasts with an altered morphology and defective proliferative capacity. The proportion of total DMD myoblast clones exhibiting this morphology increased from 2% to 93% over a 12-yr age span (Table 1, see D/
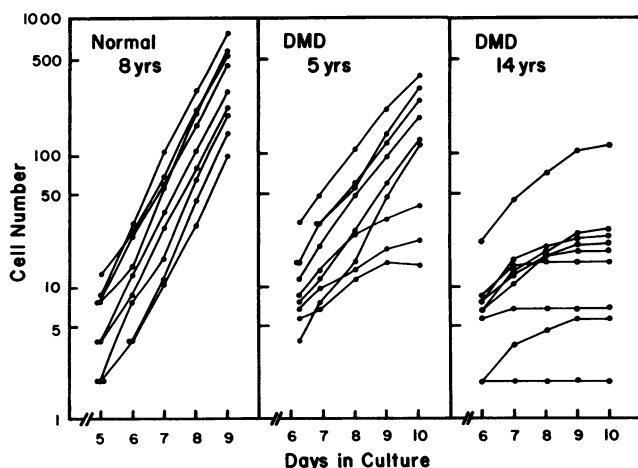


FIG. 3. Clonal analysis of myoblast growth kinetics. Growth kinetics for representative myoblast clones of a normal (*Left*) and two DMD samples (*Center* and *Right*) are shown. Growth of normal clones is highly uniform and typically exponential. Growth of DMD clones from a young patient is heterogeneous and often is not exponential. In older DMD patients, almost all clones exhibit defective growth kinetics. The normal clones were from S.W. and the DMD clones were from W.M. and M.B. of Table 1.

M). By contrast, only 2 clones of D cells were observed among the 2,266 myoblast clones of normal muscle analyzed (L.S. and S.W.).

The growth kinetics of clones derived from the myoblasts of normal and DMD muscle biopsies also differed. Representative growth curves of clones from biopsies of a typical normal individual (S.W.), a 5-yr-old DMD patient (W.M.), and a 14-yr-old DMD patient (M.B.) are shown in Fig. 3. The slopes of the growth curves for the myoblasts of a given normal sample are very similar and typically exponential, whereas those for the myoblasts of a dystrophic sample from a young patient are highly heterogeneous and often are not exponential. This is due to the presence of D cells. By 14 yr of age almost no clones exhibit normal growth kinetics.

We determined the average doubling times for 142 muscle clones from four normal and four DMD samples. The doubling time for the normal myoblasts was 16.3 ± 1.6 hr, as previously observed (9), whereas the doubling time for the DMD myoblasts was increased and highly variable. In contrast, the fibroblast clones from eight DMD patients had normal growth kinetics and a doubling time of 17.6 ± 0.4 hr. We then scored the proportion of muscle clones with doubling times >25 hr for donors of different ages (Fig. 4 *Left*). The distribution of doubling times for DMD and normal clones were significantly different by the Mann–Whitney U test (P < 0.01). The proportion of DMD muscle clones with long doubling times increased to nearly 80% as the age of the muscle donor increased, whereas only one normal clone had a doubling time >25 hr. Thus, DMD myoblasts, but not fibroblasts, have prolonged generation times in culture. A similar age-related increase in the proportion of muscle clones with altered morphology was apparent for the same group of DMD patients (Fig. 4 *Right*). At 3 yr of age, the difference between control and DMD muscle clones was evident. By age 14, as many as 80% of the DMD muscle clones, but <1% of normal clones, had an altered morphology. Thus, both altered morphology and prolonged doubling times characterize the diseased myoblast phenotype.

Even the DMD myoblasts that initially exhibit exponential growth kinetics and morphology typical of normal myoblasts eventually give rise to D cells. When myoblast clones were trypsin-treated, replated, and allowed to grow to high density, a large proportion of the clones from nine DMD patients did not proliferate beyond 10–12 doublings in culture (Table 2). In contrast, control myoblasts rarely had decreased proliferative capacity and, like other human primary cells (20), typically underwent 35–45 doublings in culture. Again, the proportion of DMD myoblast clones with decreased proliferative capacity

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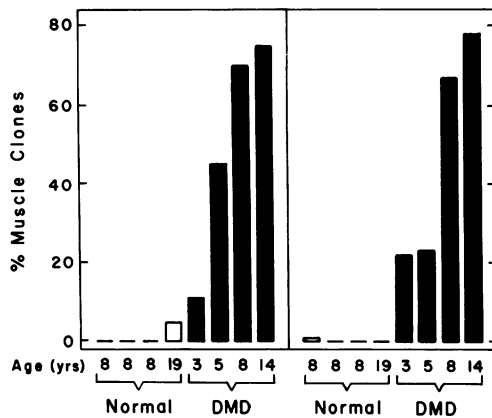*Proc. Natl. Acad. Sci. USA 80 (1983)* 4859



FIG. 4. Clonal analysis of myoblast growth kinetics and cell morphology. The proportion of myoblast clones that had an altered phenotype was identified for normal (open bars) and DMD (closed bars) samples. As a function of age of donor, the proportion of clones with a doubling time >25 hr (*Left*) and the proportion of clones with altered cell morphology (*Right*) are shown for each sample. The samples used in this analysis are indicated by ** in Table 1. Growth kinetics were monitored for 59 normal and 83 DMD myoblast clones. Morphology was determined for 1,024 normal and 1,006 DMD myoblast clones. The difference between normal and DMD clonal growth kinetics was also examined by rank order analysis of doubling times and was found to be significant by the Mann–Whitney $U$ test ($P < 0.01$).

increased markedly with patient age. By age 6, most myoblast clones were growth-impaired.

The individuals termed other donors in Table 1 represent single cases of particular interest. B.L., an asymptomatic obligate carrier of DMD, had a normal myoblast yield but a significant proportion (5%) of clones with the diseased myoblast phenotype. This increase was not due to her advanced age (31 yr), because the proportion of myoblasts with an altered phenotype obtained from P.Z. (63 yr) and A.S. (77 yr) was ≈1%, in good agreement with the other 12 normal samples studied. For the DMD patient R.B., biopsies from the vastus lateralis and the semimembranosus were obtained. The vastus lateralis is one of the first muscles to exhibit clinical weakness, whereas the semimembranosus is affected later. An analysis of 317 clones from these two muscles revealed that the ratio of myoblasts to fibroblasts (3:7 in vastus lateralis; 7:3 in semimembranosus) and the myoblast yield per 0.1 g of tissue (250 from vastus lateralis; 2,290 from semimembranosus) differed. In these parameters, the clinically less-affected semimembranosus more closely approximated normal muscle samples. However, the proportion

Table 2. Decreased proliferative capacity of DMD muscle clones

| Donor | Age, yr | Muscle clones analyzed, no.* | Clones with limited growth capacity, %† |
|-------|---------|------------------------------|------------------------------------------|
| P.D. | 2 | 22 | 0 |
| J.G. | 3 | 36 | 17 |
| W.M. | 5 | 70 | 7 |
| M.A. | 6 | 3 | 100 |
| J.L. | 8 | 36 | 86 |
| P.A. | 9 | 4 | 100 |
| R.B. | 12 | 3 | 100 |
| S.L. | 14 | 14 | 86 |
| M.B. | 14 | 9 | 89 |

* The number of clones analyzed was determined by the size of the tissue sample, proportion of myoblast clones obtained, and access to clones on the dish. Only myoblast clones with normal cell morphology and growth kinetics were passaged for analysis of proliferative capacity.
† Clones with limited growth capacity underwent <12 cell doublings. Control myoblast clones routinely underwent 35–45 doublings.

of myoblast clones with a diseased phenotype did not differ markedly for these two muscles (56% and 41%). These two cases show that D cells can constitute a significant proportion of myoblasts in muscles that clinically are relatively unaffected.

## DISCUSSION

We were able to monitor the progeny of individual cells using the methods we developed for culturing human muscle (9). This approach, or clonal analysis, revealed that the yield and proliferative capacity of satellite myoblast cells, mononucleated precursors of mature muscle fibers, but not fibroblasts, were markedly decreased in DMD. Only 5% of the normal number of myoblasts was obtained per gram of dystrophic muscle biopsy and a large proportion of these cells gave rise to clones with altered cell morphology, increased doubling times, and poor growth potential. Those DMD myoblasts, which as clones were characterized by normal growth kinetics and morphology, exhibited decreased growth potential upon passage and further cultivation. Thus, all myoblasts from DMD tissues eventually manifested the defect. These results constitute evidence of a cellular abnormality in the undifferentiated myoblast in DMD.

Several hypotheses could explain the observed defect in satellite muscle cell proliferation and suggest either a primary or secondary role for these cells in the etiology of DMD. The decreased proliferative capacity of DMD myoblasts could be secondary to muscle fiber degeneration and due to the demands upon the muscle to regenerate muscle fibers. In this case, the appearance of the altered muscle phenotype *in vitro* would merely be the consequence of the multiple cell divisions already required *in vivo* to replenish damaged muscle fibers and a reflection of early myoblast senescence. This hypothesis is supported by the observed age-related increase in the proportion of myoblasts with an altered phenotype and agrees with the general assumption that the primary role of satellite cells in DMD is to replace degenerating myotubes (21). It does not explain the markedly decreased yield of myoblasts from very young patients or the significant proportion of cells with the diseased phenotype present in the relatively unaffected semimembranosus muscle of the patient R.B. or the muscle of the asymptomatic obligate carrier B.L. An alternate hypothesis is that the diseased myoblast phenotype is of primary importance to the development of the disease and reflects an intrinsic defect in the muscle satellite cells. This defect results in the diminished proliferative capacity observed in culture. If satellite cells are not only required for regeneration but also contribute to the mass of growing myofibers in early postnatal development, the 95% deficit in these cells and the decreased growth potential characteristic of the remaining 5% could severely impair muscle growth.

Several lines of evidence indicate that muscle satellite cells are capable of division and contribute substantially to the growth of postnatal muscle. Certainly, the satellite cells have the potential to divide numerous times. Satellite cells can fully regenerate chemically destroyed rat myofibers at least 12 times in succession *in vivo* (22). We have found that each human myoblast obtained from a normal individual is capable of yielding an average of $10^8$ progeny *in vitro* (9). It seems reasonable that this proliferative potential is not just reserved for muscle regeneration but is also utilized for muscle growth. During the first 13 yr of human life, a 6-fold increase in muscle fiber diameter occurs (23), which is paralleled by an increase in the number of nuclei per unit fiber (21). In the mouse and rat, similar increases in the size and nuclear number of muscle fibers accompany postnatal development (24–26). Because no nuclear division occurs within differentiated multinucleated myotubes

(27, 28), these additional nuclei must be exogenous in origin and contributed by the division and fusion of adjacent satellite cells.

Our results provide evidence in support of a primary defect in the undifferentiated myoblasts of DMD muscle. First, the yield of myoblasts, but not fibroblasts, from DMD biopsies of the vastus lateralis muscle is decreased by 95%, irrespective of the age of the donor. In addition, in two samples (B.L. and R.B., Table 1, other donors) in which muscle regeneration was minimal, a significant proportion of myoblasts with the altered phenotype was obtained. B.L. was an obligate carrier of DMD and among the one-third of carriers whose muscle biopsy and serum creatine kinase activity were normal and therefore not diagnosable by current methods (1). Accordingly, the total cell yield per 0.1 g of biopsy and the ratio of myoblast to fibroblast cells obtained from B.L. were within the normal range. However, 5% of her myoblasts had an altered phenotype, a proportion significantly greater than normal. This observation presents the possibility of a method of detection for this group of carriers. From the DMD patient R.B., two muscles were biopsied, the semimembranosus, which was only moderately affected, and the vastus lateralis, which was severely affected both clinically and histologically. Accordingly, the total cell yield and myoblast to fibroblast ratio for the semimembranosus closely approximated those values observed for the cells of normal samples and differed markedly from the vastus lateralis. However, the proportion of muscle cells with a diseased phenotype was similar (56% and 41%) for these two muscles. In both of these cases, a significant proportion of defective myoblasts was obtained from muscles that were not undergoing extensive myofiber degeneration. Thus, their appearance is not likely to be secondary to the proliferative demands upon the myoblasts during muscle regeneration.

These results lead us to conclude that there is a basic defect in the undifferentiated satellite myoblasts of muscle tissue from individuals with the gene for DMD. The proliferative capacity of these cells is severely impaired and the resulting decrease in myogenic cell yield limits muscle fiber growth. Prenatal muscle development appears to be minimally affected, because the histology of muscle and serum creatine kinase levels of second trimester fetuses at risk for DMD were found to be indistinguishable from those of normal controls (29). As a result, DMD can presently only be definitively diagnosed after birth. These observations suggest a developmental basis for the disease. In view of our findings, this could be an inability of DMD myoblasts to mature in response to a specific developmental signal. Alternatively, like erythropoietic cells (30–33), there may be subsets of muscle precursor cells, or myoblasts, with different roles at different stages of development. Individuals with DMD may lack or have a defective myoblast subset. These myoblasts may be essential to postnatal development and a deficit could result in impaired muscle growth leading to the pathogenesis characteristic of the disease.

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