A cell line (RCDMD), derived from a muscle biopsy taken from a 7-year-old patient with Duchenne muscular dystrophy (DMD), was established in vitro using conditioned media from the UCHT1 thyroid cell line as described elsewhere (*Biochim Biophys Acta* 1992;1134:247–255). Unlike other cell lines established by the same procedure, RCDMD cells were highly refractory to transformation and the resulting cell line grew slowly with a doubling time of approximately 72 h. Further, cells continue to grow after more than 20 doublings and 15 passages. Some of the characteristics of the cell line include lack of reaction with antidystrophin antibodies and the presence of receptors for the dihydropyridine PN200-110 ($K_d$) = 0.3 ± 0.05 nmol/L and $B_{max}$ = 1.06 ± 0.03 pmol/mg protein) and for α-bungarotoxin ($K_d$ = 1.02 ± 0.17 nmol/L and $B_{max}$ = 4.2 ± 0.37 pmol/mg protein). Patch clamped cells in the voltage clamp configuration lack ion currents when growing in complete medium with high serum, but they can be induced to differentiate by serum deprivation and addition of hormones and trace elements. After 5 days in differentiating medium, noninactivating, delayed rectifier potassium currents are seen. At day 12, A-type, inactivating potassium currents as well as transient inward currents are seen. In conditions in which sodium and potassium currents are absent, a very fast activating and fast inactivating calcium current was evident. The cell line offers the possibility of studying cellular mechanisms in the pathophysiology of DMD. 
Key words: Duchenne muscular dystrophy • dystrophin • cell culture • ion channels • muscle differentiation

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# ION CHANNELS IN A SKELETAL MUSCLE CELL LINE FROM A DUCHENNE MUSCULAR DYSTROPHY PATIENT

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The muscular dystrophies comprise a heterogeneous group of heredity myopathies characterized by muscle degeneration followed by replacement of muscle with connective tissue and fat. The most severe and most common form of dystrophy is Duchenne muscular dystrophy (DMD), an X-linked disorder in which progressive proximal weakness starts in childhood and usually leads to death in the second or third decade of life.[34] The severe muscle destruction observed in DMD patients is attributed to the absence of the protein, dystrophin, a 427-kd protein encoded by a 14-kb transcript.[18,29] This deficiency would result in the absence of a structural function played by the protein at the cell membrane level, resulting in thin filament-membrane impairments, increased intracellular $Ca^{2+}$ concentration, and elevated protein degradation.[26,30,34] Because of the complex interaction between tissues, it is difficult to study this problem in animal models for DMD[1,3,9,10,32] in vivo. Therefore, attempts have been made to investigate this question in primary cultures of human DMD muscles.[20,25] However, tissue explant cultures contain, in addition to myogenic cells, a heterogeneous cell

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population, the composition of which might differ in normal and dystrophic muscle cultures (see Witowski[33] for review). It is uncertain in such systems to discriminate between myogenic cell features and effects exerted by other cell types. Furthermore, cultured myoblasts from DMD patients present premature senescence and do not proliferate after a few passages.[2] We therefore tested the feasibility of obtaining cell cultures of an enriched population of myogenic cells dissected out of DMD skeletal muscle patients and tried to immortalize them in culture using a procedure developed in our laboratory.[7,8] The advantage of these cell lines in muscle research is that they can be grown permanently as single cell types, while primary cultures are composed of various kinds of cells (myoblasts, fibroblasts, endothelial cells, etc.). Although a study of the human normal skeletal muscle cell line has been published recently by our group,[8] we are unaware of any literature reports describing immortal DMD muscle cell lines.

## MATERIALS AND METHODS

**Tissue Culture.** The method of immortalization essentially consists in culturing, under standard conditions, DMD skeletal muscle cells in the presence of 10% (v/v) cell-free conditioned medium (UCHT1-CM) obtained from UCHT1 cell monolayers[6] for periods of 3–6 months.[7,8] Procedures to harvest and prepare cell-free UCHT1-CM have been reported previously.[5,8]

A skeletal muscle sample from a 7-year-old patient (A.R.) with a clinical, electromyographic and blood enzymatic pattern of DMD was surgically dissected at the Hospital de Rehabilitación Infantil, Santiago, Chile, with previous family consent. The piece of quadriceps muscle (0.2 cm in diameter) was cleaned of fat and mesenchymal envelopes and finally chopped with a pair of scalpels and digested by collagenase (Sigma C9263, Type V) 0.2 g% in Hank's balanced salt solution ($Ca^{2+}$–$Mg^{2+}$-free) under aseptic conditions. Washed cell pellets were resuspended and seeded into glass Petri dishes, and fed medium consistent of equal volumes of Ham's $F_{12}$ nutrient mixture and Dulbecco's modified Eagle's medium ($F_{12}$/D) supplemented with 10% bovine serum, 2.5% fetal bovine serum, 0.015 mol/L HEPES (*N*-2-hydroxyethylpiperazine-*N*'-2 ethane sulfonic acid) buffer at pH 7.2, 50 mg/L streptomycin sulphate, and 100 mg/L-sodium-penicillin-G, which was used as the growth medium (GM) to which UCHT1-CM was added at 10% final concentration from the onset of muscle cell culture. Cultures were kept at 36°C and 100% humidity in an incubator with a controlled atmosphere of 10% $CO_2$–90% air. Media were renewed completely every 3 days. Differentiation media consisted of $F_{12}$/D enriched with 1% bovine serum and 1% stock supplement as described by Orozco et al.[26] A complete description of all culture procedures mentioned herein have been reported in detail.[5]

For $^3$H-PN200-110 binding studies, cells were cultured for 1 week before the experiment in 15-cm-diameter culture dishes. In order to obtain a large quantity of cells for microsomal preparation, we used at least four of those dishes. To perform some experiments, cells were first cultured in 10-cm glass Petri dishes, then subcultured to plastic Falcon dishes, previously treated with 2 μg/mL polilysine, to achieve a better adhesion of the cells to the dishes (Briefly, the plastic dishes were exposed to 2 mL of 2 μg/mL polilysine sterile solution for 2 h, then they were washed three times with 3 mL PBS solution.) Experiments were performed when confluence was achieved in the dishes.

*$^3$H-PN200-110 Binding Studies.* RCDMD cells were detached from 15-cm confluent glass Petri dishes with a rubber policeman, and placed in 0.25 mol/L sucrose, 1 mmol/L EDTA, and 20 mmol/L Tris-HCl, pH 7.2. The cells were then homogenized in a Polytron apparatus with three bursts of 5 s, separated by intervals of 30 s each. The disrupted cells were centrifuged at 2000 *g* for 10 min, and the pellet was discarded. The supernatant was centrifuged at 100,000 *g* for 1 h, and the pellet was suspended in the sucrose buffer mentioned above. Microsomes (1 mg protein/mL) were incubated during 30 min with increasing concentrations of $^3$H-nitrendipine (0.1 to 2 nmol/L) in the presence or absence of 1 μmol/L nifedipine. The incubation medium contained 50 mmol/L Tris-HCl, pH 7.2, and 1 mmol/L $CaCl_2$ (final concentrations per tube). The reaction was stopped by fast filtration in Whatman GF/B glass fiber filters. The filters were rinsed three times with 3 mL 200 mmol/L choline chloride 20 mmol/L Tris-HCl, pH 7.2. The radioactivity retained in the filters was measured in a Tracor D300 scintillation counter.

Aliquots (50 μL) of the incubation medium were used for total radioactivity measurements. The free ligand concentration was calculated subtracting bound ligand from the total, for each experimental point. The specific $^3$H-binding was defined as a difference between the radioactivity in the absence and in the presence of 1 μmol/L cold nifedipine.

*Patch Clamp Methodologies.* The whole-cell (for macroscopic currents) configuration of the patch clamp technique was used.[15] The cells were visualized in an Olympus CK-2 inverted microscope equipped with phase contrast optics. Glass electrodes (Bluetip, Monoject Scientific, St. Louis, MO) were pulled in several stages using a Sachs-Sutter PC-84 programmable puller (Sutter Instruments Corp., San Francisco, CA), to yield open tip resistances of 1.0–8.0 MΩ. Prior to recording, the cells were rinsed with 1 mL of saline EC1 with the following composition (in millimoles per liter): 135 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES-Na, and 10 dextrose, adjusted with NaOH to pH 7.4. For dissection of $Ca^{2+}$ currents, the extracellular solution EC2 was used, composed of (in millimoles per liter): TEA-Cl 150, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES-KOH 10, and dextrose 10 (pH 7.4). The pipettes were filled with either a normal intracellular solution IC1 consisting of (in millimoles per liter): 140 KCl, 1 $CaCl_2$, 11 EGTA-KOH, 10 HEPES-KOH, 10 dextrose (pH 7.0), or with a low $K^+$ solution IC2 composed of (in millimoles per liter): 140 CsCl, 0.45 $CaCl_2$, 5 EGTA-KOH, 5 HEPES-NaOH, and 10 dextrose. Gigaohm seals (2–10 GΩ) were established between the patch pipette and the cell membrane by applying a soft suction pulse inside the pipette. After canceling the fast capacity current transients using the null-bridge circuitry of our Axopatch-1C patch clamp amplifier (Axon Instruments, Foster City, CA), the whole cell configuration was achieved by applying a brief, stronger pulse of negative pressure. A minimum of 2 min was allowed for exchange of solutions between the pipette and the cell, and to stabilize the membrane resting potential. Command voltage and current steps were generated by an Labmaster digital-to-analog converter board (Axon) controlled by a customized program, Pclamp 5.5 (Axon), installed in a dedicated PC-compatible computer. Cell responses were sampled at rates of 10–50 kHz. All recordings were conducted at room temperature (20°–22°C).

*Current clamp:* Passive properties were studied in the current clamp mode, in response to hyperpolarizing pulses of 0.1- and 0.05-nA amplitude, 200-ms duration. Membrane resistance was estimated as the maximal voltage amplitude in steady state, divided by the applied current pulse amplitude. The membrane time constant (τ) was taken as the point in time corresponding to 0.632 of the maximal voltage amplitude. Cell capacitance ($C$) was calculated as $C = \tau/R$, and was used to estimate cell area (assuming 1 $\mu F/cm^2$) for calculation of ionic current density.

*Voltage clamp:* The cells were clamped at ≤80 mV and depolarizing steps of 70-ms duration with incremented of 10 mV were presented at 0.5 Hz. Capacitative and leak currents were compensated using online subtraction with a conventional P/4 protocol. The records were composed of 1024 sample points, where the first segment (5 ms) was used to form the baseline. The second segment (100 ms) was used to form the nonlinear ion-specific record of the current during the pulse, which is obtained by the addition of the transient during the pulse and the control transient of the current response to the corresponding hyperpolarizing pulses. Positive feedback, series resistance compensation was not used. Voltage errors were calculated assuming a series resistance of two times the open tip resistance of the electrode, multiplied by the peak amplitude of the active membrane current. Only traces with voltage errors less than 3 mV were used. Peak currents were measured and corrected for cell area to obtain current density. External modulators of channel activity (nifedipine, $La^{3+}$) were applied externally with a pipette.

Steady-state inactivation was examined using conventional prepulse protocols.[16] A 1-s conditioning prepulse that held the membrane at potentials between −140 mV and 0 mV (incremented in steps of 10 mV) was followed by a holding potential of −70 mV for 1.4 ms to minimize the effect of tail currents after the prepulse. The test pulse had a duration of 100 ms and drove the membrane potential from −70 mV to 0 mV.

Activation and slow inactivation (now referred to as deactivation) kinetics were studied by fitting the current traces to the classical "$m^3h$" Hodgkin and Huxley[17] model for sodium current activation and deactivation using the least squares fitting method provided by the customized software Pclamp 5.5 (Axon Instruments).[4] In such cases in which two components were evident, a sum of two exponentials was used as model for deactivation.

$$I = [m^3h]I_{max}$$

where $m = 1 - \exp(-t/\tau_a)$, and $h = \exp(-t/\tau_h)$ or $h1 + h2 = [\exp(-t/\tau_{h1})] + [\exp(-t/\tau_{h2})]$.

Function $m$ represents current activation kinetics (with activation time constant $\tau_a$), whereas current deactivation is represented by function $h$ (with deactivation time constant $\tau_h$) or $h1 + h2$ (with deactivation time constants $\tau_{h1}$ and $\tau_{h2}$). The fitting region for activation ranged between the pulse start, immediately after the capacitive spike and the maximal current peak. For deactivation, the maximal current peak and the point at which the

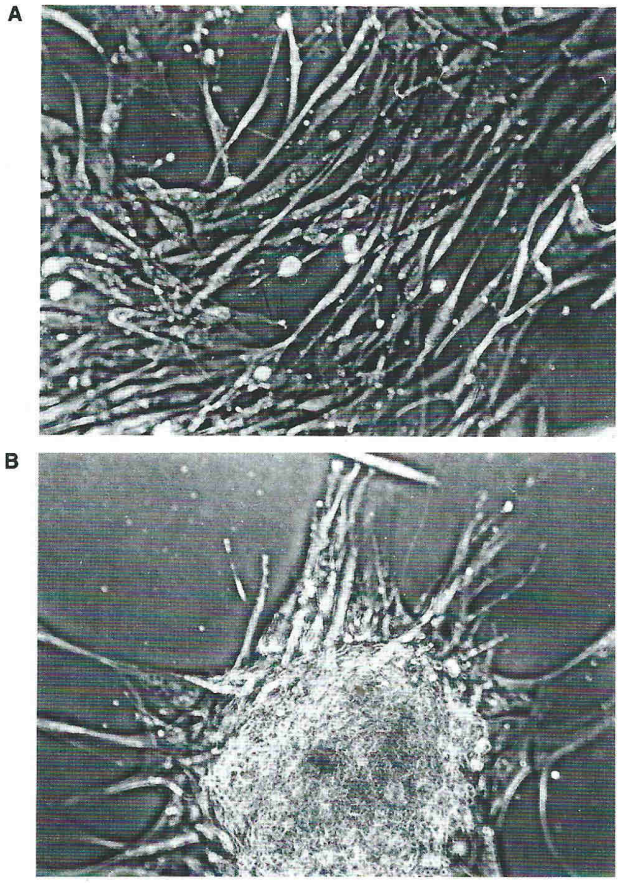



**FIGURE 1.** Phase contrast micrographs of RCDMD cell monolayers cultured in GM at the tenth passage. Some overlapping cells **(A)** and a few piled foci **(B)** were detected. Magnification ×172.

current reached 80% of the steady-state level were used as limits for the fit. Fits were considered adequate for least squares residual values equal or larger than 0.98.

*Other Methods and Materials.* Chromosome studies were performed in cells arrested in metaphase with colchicine, and stained according to standard techniques.[14]

For dystrophin immunofluorescence, cells were cultured in coverslips and fixed in liquid nitrogen. Sheep anti-mouse dystrophin antibodies (1:75 v/v) were generously donated by Dr. E. Hoffman (University of Pittsburgh). Biotinylated donkey anti-sheep IgG serum (Sigma) (1:100 v/v) was used as second antibody. The reaction was revealed by addition of streptavidin conjugated with fluorescein (1:250) for 1 h in the dark. Coverslips were embedded in glycerol and viewed with an inverted microscope equipped with epifluorescence.

[$^3$H] nitrendipine and [$^{45}Ca^{2+}$] were obtained from New England Nuclear (Boston), and nifedipine was obtained from Bayer (Darmstadt, Germany).

## RESULTS

The in vitro immortalization used proved to be successful for the establishment of several animal and human cell lines. From approximately 20 cell lines immortalized with this procedure until now, only human DMD cells proved to be refractory to transformation for a long time and the immortalized cell line finally obtained has growth parame-

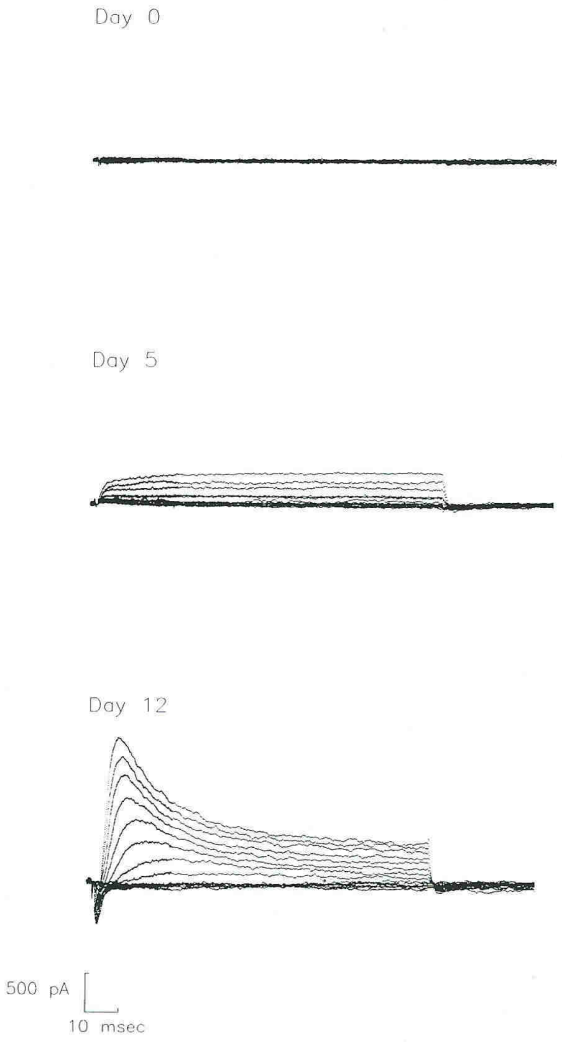
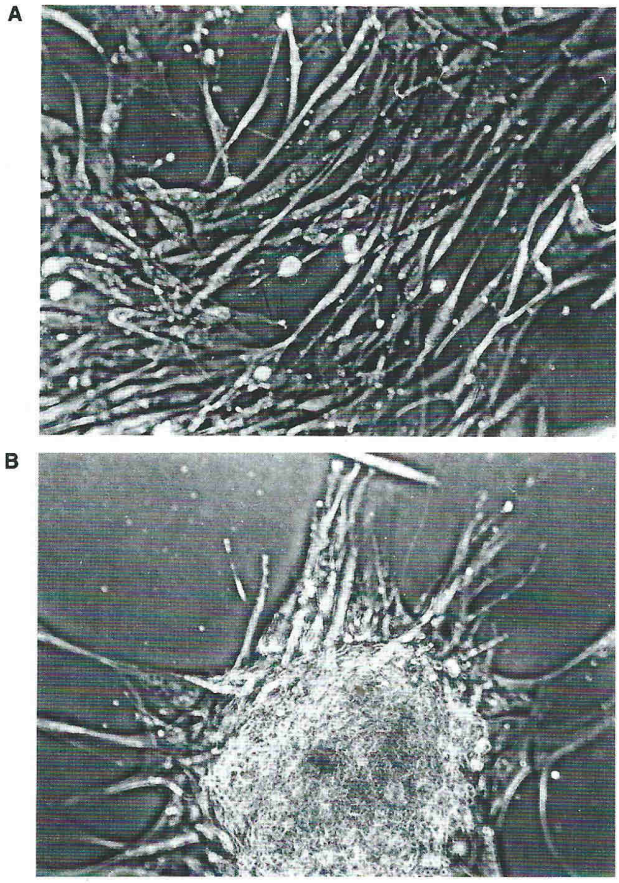



**FIGURE 2.** Current traces obtained using the whole cell configuration of the patch clamp technique under voltage clamp conditions, using normal solutions in the bath and pipette (EC1/Ic1) at various stages of differentiation induced by medium with low serum and hormone supplementation. Note the appearance of a delayed rectifying outward current after 5 days in culture, and the onset of inward current and an A-type outward current at day 12 of differentiation. $V_{holding}$ = −90 mV; responses to 10-mV steps are shown.

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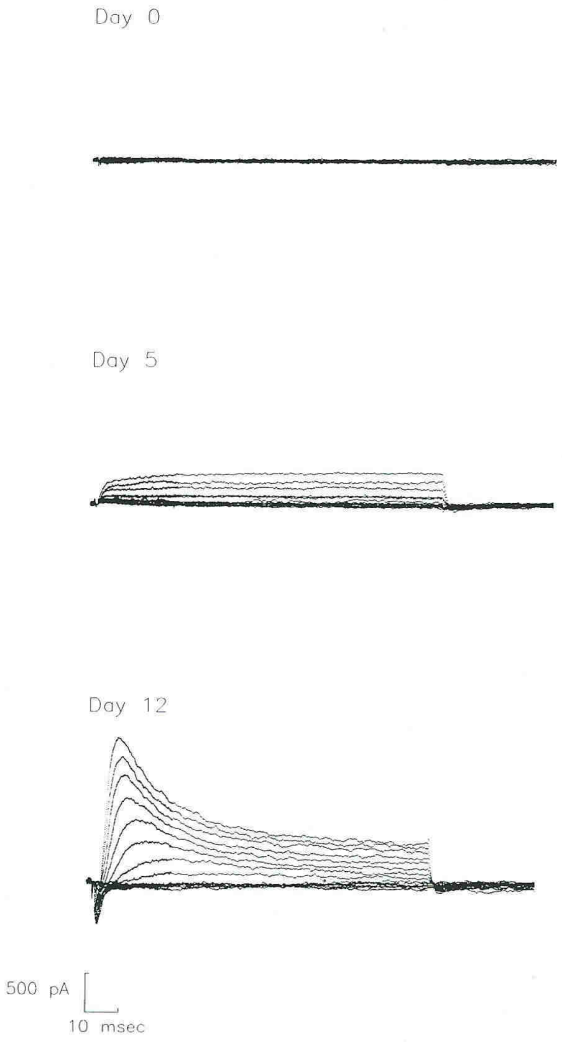


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ters that differ substantially from all other cell lines.

Muscle cultures grown in the presence of 10% UCHT1-CM were enriched in myoblasts using a selective adherence procedure described elsewhere.[35] Following two preplating periods of 6 h each, a uniform monolayer composed of 80% mononuclear, spindle-shaped cells resembling myoblasts bearing a moderate criss-cross pattern were observed (Fig. 1A). Sometimes, piling foci could be observed (Fig. 1B). From the first subculture, cells could be subsequently subcultured by trypsinization but proliferation depended critically on plating at a high cell density (split ratio 1:2). Phase contrast microscopy monitoring of subsequent passages show an enrichment of spindle-shaped mononucleated cells that occasionally fuse into myotube-like structures. By the tenth subculture, a single, homogeneous population of myoblasts was evident and fibroblast-like cells were no longer seen.

After 6 months, RCDMD have undergone as many as 15 successive passages, or an estimated 20 population doublings with a doubling time (DT) of approximately 3 days. Experiments were performed between the tenth and fourteenth passages, and karyotypic analysis revealed a human diploid chromosome number (44 XY), and immunohistochemical staining for dystrophin proved negative.

When cultured in growth medium, no ionic currents are evident (Fig. 2, top traces correspond to day 0). After 5 days in differentiating medium, an outward $K^+$ current resembling a delayed rectifier appears (Fig. 2, center traces). After 12 days of differentiation, a fast activating and inactivating current is evident, along with the onset of an A-type $K^+$ current with a maximal current density of 16 pA/pF and $t_{peak}$ of 8.9 ± 0.52 ms (mean ± SD; $n = 5$) (Fig. 2, lower traces).

When using conditions in which no $Na^+$ or $K^+$ currents are allowed to develop, and $Ca^{2+}$ is the only permeant cation, a fast activating and inactivating current with $t^{peak}$ of 2.2 ± 0.26 ms and maximal current density of 6.5 pA/pF is evident (Fig. 3). The current activates at −40 mV (Fig. 3) and the voltage dependence of inactivation shows a $V_{0.5}$ of −95 mV (Fig. 4A). However, current kinetics appear quite fast, with time constants of 0.68 ± 0.17 ms (mean ± SD; $n = 3$) for activation, and 2.55 ± 0.51 ms (mean ± SD; $n = 3$) for inactivation (values for currents at −20 mV).

The outward A-type $K^+$ current activates at −10 mV (Fig. 4A) and $V_{0.5}$ for inactivation is −30 mV (Fig. 4B). The activation and inactivation time constants at 20 mV are 9.1 and 18.55 ms, respectively.

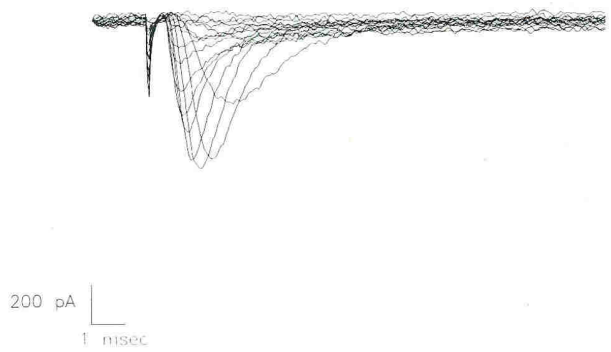


**FIGURE 3.** Current traces using conditions that dissect $Ca^{2+}$ currents (EC2/IC2). A rapidly activating and inactivating inward current is evident after 12 days of differentiation induced in vitro. $V_{holding} = -90$ mV; responses to 10-m V steps are shown.

The binding assay for membrane receptors shows the presence of specific sites for α-bungarotoxin (Fig. 5) and PN200-110 (Fig. 6), blockers of the cholinergic nicotinic ion channels and of the voltage-dependent calcium-selective channels, respectively. In both cases, the possible number of receptor families for α-bungarotoxin and for dihydropyridines can be fitted to a single hyperbola, revealing only one (high affinity) receptor site. Equilibrium binding parameters for α-bungarotoxin were $K_d = 1.02 \pm 0.17$ nmol/L and $B_{max} = 4.2 \pm 0.37$ pmol/mg protein (Fig. 5A). For the dihydropyridine PN200-110, the values obtained were $K_d = 0.3 \pm 0.05$ nmol/L and $B_{max} = 1.06 \pm 0.03$ pmol/mg protein (Fig. 5B).

## DISCUSSION

To obtain a cell line from skeletal muscle in permanent culture may have advantages over other biological material for the study of muscle physiology. Human skeletal muscle may be additionally attractive if we consider the possibilities it offers to research in human muscle pathologies; cell lines, on the other hand, tend not to express many of the features characteristic of differentiated cells from adult tissues and the value, as a research tool, of the cell line we describe here will depend on the markers of differentiated skeletal muscle that it is able to express.

In contrast to rodent cells, the establishment of immortal cell lines that arose spontaneously from normal human cell cultures, without viral, physical, or chemical intervention, has seldom been ob-

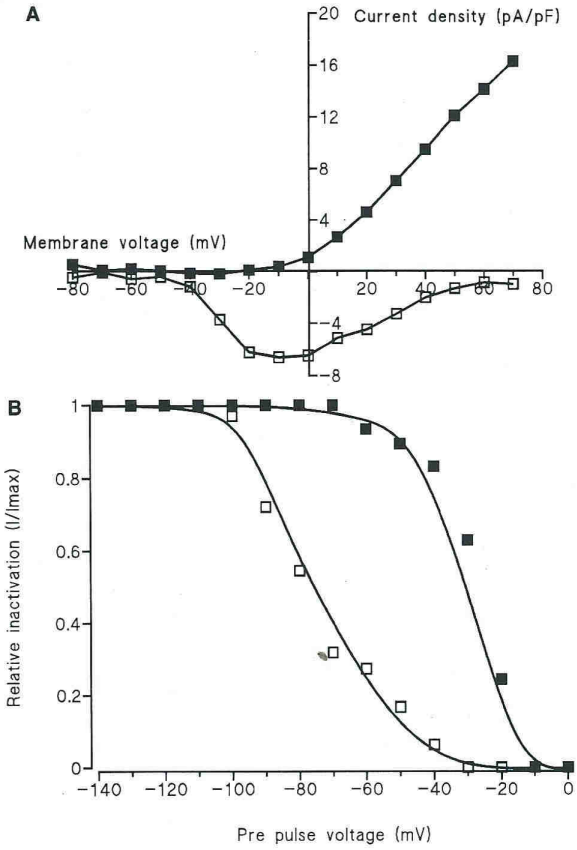


**FIGURE 4. (A)** I–V relationship of inward $Ca^{2+}$ currents (open squares) and A-type $K^+$ currents (solid squares). The inward current activate at −40 mV and peak at −20 to −10 mV (maximal current density = 6.5 pA/pF). A-type current activates at −10 mV. **(B)** Voltage-dependent inactivation of inward $Ca^{2+}$ currents (open squares) and A-type $K^+$ currents (solid squares). A 1-s conditioning pulse was followed after 1.5 ms by a 100-ms test pulse. $V_{0.5}$ = −95 and −30 mV for $Ca^{2+}$ and $K^+$ currents, respectively.

served.[23] However, immortalization and transformation in culture can now be achieved in some normal human cell model systems. Both processes, produced stepwise, require two separate genetic events: one to recover from senescence and another for conversion to the tumorigenic state.[29] Thus, human primary cells immortalized by a variety of means (viruses, chemical, radiation, or spontaneous) could be neoplastically transformed by a carcinogenic agent or a second viral infection.

As our recently reported cell lines derived from normal adult human skeletal muscle[8] and adult rat myocardium,[5] RCDMD cells have been induced to proliferate by unknown factor(s) present in a conditioned medium derived from a thyroid cell line without the concomitant effect of other biological or chemical agents. The rather short time of culture (6 months with 15 passages), slow growth rate (DT of 3 days), and lack of chromosomal alterations are coincident with an immortalized status of the cells which probably have not yet been neoplastically transformed. Myoblasts derived from the muscle of DMD patients have been shown to have dramatically reduced proliferative potential[2]

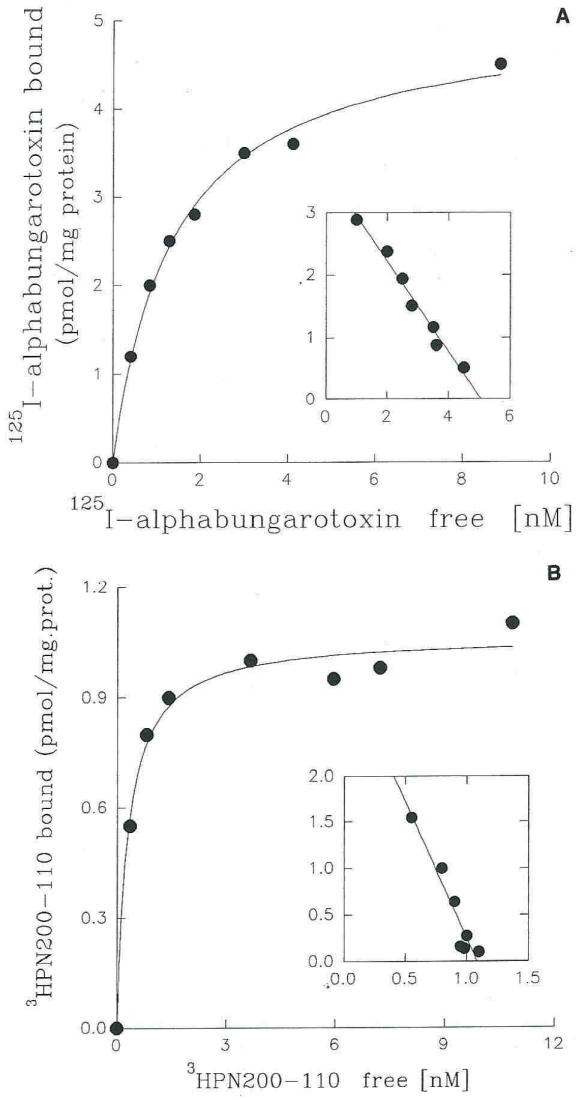


**FIGURE 5. (A)** Specific binding at equilibrium of $^{125}$I-α-bungarotoxin to cell homogenates from the dystrophic cell line cultured in growth medium. Curve fit analysis gave values of $K_d$ = 1.02 ± 0.17 nmol/L and $B_{max}$ = 4.2 ± 0.37 pmol/mg protein. The values are means ± SEM of at least three experiments. **(B)** Specific binding at equilibrium of $^{3}$H-PN200-110 to cell homogenates from the dystrophic cell line cultured in growth medium. Curve fit analysis gave values of $K_d$ = 0.3 ± 0.05 nmol/L and $B_{max}$ = 1.06 ± 0.03 pmol/mg protein. The values are means ± SEM of at least three experiments.

**Table 1.** Nicotinic and DHP receptors in RCMH and RCDMD human skeletal muscle cells lines.

| Ligand | Cell culture | $B_{max}$ (pmol/mg protein) | $K_d$ |
|---|---|---|---|
| $^{125}$I-α-bungarotoxin | RCMH | 1.48 ± 0.32 | 0.69 ± 0.37 nmol/L |
| | | 179 | 1.6 μmol/L |
| | RCDMD | 5.06 ± 0.17 | 1.38 ± 0.09 nmol/L |
| $^{3}$HPN200-110 | RCMH | 0.54 ± 0.22 | 0.28 ± 0.03 nmol/L |
| | | 2.62 ± 0.73 | 12.75 ± 9.52 nmol/L |
| | RCDMD | 1.04 ± 0.03 | 0.29 ± 0.04 nmol/L |

*Values are expressed as the mean ± SD of at least six experiments.*

as well as other abnormalities[11,23] that are probably secondary to dystrophin deficiency.[19,20] Some of these alterations may be responsible for the apparent refractoriness of RCDMD cells to transformation; their immortalization is likely to be a first step in the knowledge of these phenomena.

The skeletal muscle markers we have found include the capacity of cells to express ionic currents characteristic of excitable membranes and the expression of receptors expected to be found in skeletal muscle. The absence of dystrophin defines a marker for gene expression that makes this cell line worthy of further study. Expression of dystrophin in cultured muscle cells has been reported to parallel cell fusion and differentiation.[19,20,24] RCDMD cells differentiate but show little capacity to fuse. Myoblasts from another myoblast cell line, immortalized from normal muscle using the same procedure, display clearly positive immunofluorescence with the antidystrophin antibody used.[8]

Patch clamp studies demonstrated the presence of $Ca^{2+}$ currents and A-type and delayed rectifier $K^+$ currents. The current densities of these currents are roughly four times the values encountered in our cloned cell line, RCMH, derived from normal human muscle.[22] The $Ca^{2+}$ currents have voltage dependence compatible with T-type currents, but the kinetics are greatly accelerated, which could either determine an altered T channel or the presence of a third type of $Ca^{2+}$ channel in these cells.

Functional ion channels were also seen nondirectly through receptors for ion channel blockers; the nicotinic acetylcholine receptor channel, seen as α-bungarotoxin receptor, is present in dystrophic cells with values of $K_d$ comparable to our results in the RCMH line, although the values for $B_{max}$ are approximately three times higher. It is interesting to note that acetylcholine receptor location has been linked to dystrophin location in cultured muscle.[31] A similar situation was found regarding the presence of voltage-gated $Ca^{2+}$ channels (DHP receptors) where $K_d$ values are practically identical (0.28 nmol/L for RCMH, 0.3 mmol/L for dystrophic cells), but $B_{max}$ values in dystrophic cells double those reported in RCMH[8] (see Table 1). These differences could determine a greater amount of receptors, and correlate well with the increased current density observed with the whole-cell patch clamp technique. Ion channel expression increases upon cell differentiation in both skeletal and cardiac muscle cell lines,[5,8,22] a phenomenon that mimics maturation of muscle cell structures during myogenesis[12]; it is possible that, in accordance with the slow growth parameters exhibited by the RCDMD cell line, these cells are more "differentiated" than RCMH cells even when growing in GM. It is also possible that the differences may arise from the fact that RCMH is a cloned cell line, and the dystrophic line reported here has yet to be submitted to cloning procedures.

In contrast to our finding in RCMH, we found only one family of dihydropyridine receptors in these cells, which in turn agrees with what has been found in both frog and mammalian isolated transverse tubule membranes.[13,21] This correlates well with the finding of slow $Ca^{2+}$ currents with the patch clamp technique. Both the density of dihydropyridine receptors and the current density are higher in RCDMD cells when compared to RCMH cells (Table 1), suggesting the possibility of a mechanism at the cell membrane level relating $Ca^{2+}$ influx to the pathogenesis of the disease. Such impairments could determine alterations in intracellular $Ca^{2+}$ concentration and, consequently, in cell homeostasis.

The cell line described here may be useful as a tool both for the study of muscle physiology and in the search for functional abnormalities in dystrophin-defective cells.

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