Stimuli for differentiation of myoblasts in culture vary between cell lines

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Background

The efficient growth of muscle tissue in developing animals is a key aspect of meat production. Muscle cell proliferation and differentiati are complex processes which can be influenced by a wide range of intracellular and extracellular factors (Lasser et al, 1994; Harper et al, 1986). Myoblasts in culture are able to exhibit the processes of cell proliferation, migration, fusion, myobtube formation and developmen of contractile ability which together are the main features of myogenesis. Cell culture studies are thus an important tool in developing our understanding of the factors controlling muscle development. The mouse cell line C2C12 and the rat cell line L6 have been widely used i studies of myoblast differentiation in culture. The culture medium used has often contained foetal serum (calf or horse serum); serum contains hundreds of components including proteins, hormones, enzymes and inhibitors, many of which are related to factors known to influence muscle cell development. Many researchers (eg. Florini and Roberts, 1979, Goto et al, 1999) have therefore turned to serum-fr culture media in order to better define the chemical signals and stimuli given to the cultured cells. However, mammalian myoblasts are diverse in their reactions to environmental conditions during differentiation.

Objective

The aim of this study was to compare the proliferation and differentiation behaviour of 3 separate cell lines (C2C12 cells, L6 cells and a primary culture of myoblasts from chick pectoralis muscle) in serum-free versus serum-containing medium. The purpose of this is to determine whether a pattern of factors influencing differentiation was common to all cell lines, or whether the response of these "model" culture systems was cell-line specific. This study is taken from a larger investigation that also tests the effects of extracellular matrix proteins on myoblast differentiation (Lawson and Purslow, in press).

Methods

L6 and C2C12 cells from ATCC (American Type Culture Collection, Manassus, VA), were maintained under a 5% CO_2 stream at 37°C, plated at 7,500 cells/cm^2 and grown to confluence in DMEM containing 10% foetal calf serum (FCS). Chick myoblasts were isolated fro the pectoralis muscle of embryonic day 10 chicks by incubating minced tissue in Hanks balanced salt solution containing 0.25% trysin at 37°C under 5% CO_2 for 30 minutes, followed by three filtration and centrifugation cycles to remove muscle debris. Cells suspended in DMEM with 10% foetal calf serum (3 x 10^5 cells per ml) were sorted by three repeats of differential adhesion separation; fibroblasts in th culture plate down onto the culture dish more rapidly, so that by removing the supernatant the slowly-adhering myoblasts can be isolated. Final cultures were shown to contain better than 98% myoblasts. Chick myoblasts were kept for a maximum of 5 days after isolation befor use.

Each cell line was grown to confluence in DMEM + 10% FCS and then the culture medium replaced with either (1) fresh DMEM + FCS, (2) DMEM containing 2% horse serum, or (3) AIM-V (Life Technologies, Denmark), a serum-free medium that has been shown to be suitable for various cell lines normally grown in DMEM + serum. Cultures were incubated for up to 10 days.

Cell proliferation in each culture was assayed by counting cell nuclei numbers. Cultures fixed with 4% paraformaldehyde were stained wit DAPI (Yablonka-Reuveni and Rivera, 1994) and viewed by fluorescence microscopy. For each medium condition, the number of nuclei in 10 fields of view from each of 3 dishes was quantified using an Image Pro plus (Image House A/S, Denmark) image analysis system.

The activity of the enzyme creatine phosphokinase (CPK) increases as myoblasts fuse into myotubes. The level of CPK was therefore assayed as a measure of cell differentiation and myotube formation in the different culture conditions, using the CPK assay kit (Sigma, St. Louis, MO), on cell suspensions whose total protein concentration was determined using a BCA kit (Pierce, Rockford, IL), on homogenised cell suspensions.

Results and Discussion

C2C12 cells: Cell proliferation increases steadily in DMEM + 10% FCS, as shown by the number of cell nuclei as a fraction of incubation time (Fig. 1). However, cells in DMEM + 2% horse serum (HS) or serum free medium (AIM-V) stop proliferating, as shown by the constant number of nuclei with time (Fig. 1). In the DMEM + 2% HS and AIM-V medium, the cells differentiate and form myotubes (Fig. 2) After 60 hours incubation, C2C12 cells show a much higher differentiation and myotube formation in AIM-V then DCEM + 2% HS, as shown by the CPK activity (Table I).

L6 cells show a radically different pattern of responses to the different media. Although L6 cells also stop proliferation in DMEM + 2% HS and AIM-V (Fig. 1), the CPK activity per mg protein shows an opposite trend to that of the C2C12 cells (Table I). L6 cells appear to form fewer myotubes in AIM-V serum-free medium than DMEM + 2% HS (Fig. 2). In addition, L6 cells show reduced adhesion to the culture dish and by 6 days begin to lift off the substrate in AIM-V, whereas C2C12 were always strongly adherent.

Chick myoblasts show increased differentiation in both DMEM + 2% HS and AIM-V compared to DMEM + 10% FCS (Table 1). As with L6 cells, but not C2C12 cells, CPK activity was highest in DMEM + 2% HS. However, chick myoblasts, like C2C12 cells, but unlike L6 cells, continued to be adherent to the culture dish in all media. Chick myoblasts also have a higher differentiation (CPK activity) than either C2C12 or L6 in all media tested (Table I).

Conclusions

Choice of a "model system" to study myoblast development is a difficult task, as myoblasts clearly show cell-line specific reactions to chemical changes in the environment designed to switch the cells from their proliferative to differentiation states. The diversity of response between the two rodent-derived cell lines (C2C12 and L6) so often used as models to investigate muscle development is a concern. Chick myoblasts clearly have a greater ability than the transformed mouse and rat cell lines to differentiate in serum-rich conditions. This may imply that the differentiation is sensitive to cell-cell or cell-matrix contacts in the chick cell lines. The effect of cell contact with matrix proteins is the subject of our further studies (Lawson and Purslow, in press.)

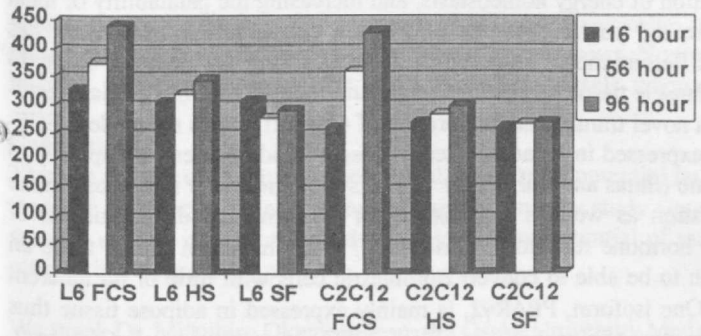

Figure 1. Proliferation rates for L6 and C2C12 cells grown in different media types. Data shown is number of nuclei per field. FCS=fetal calf serum; HS=horse serum; SF=serum free

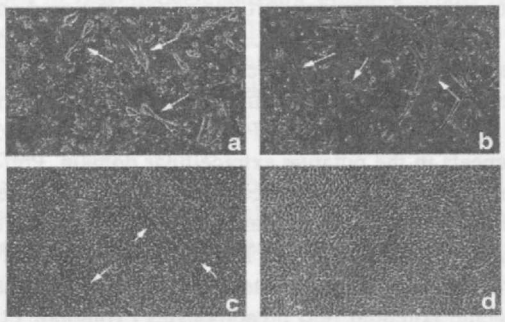

Figure 2. C2C12 and L6 cells differentiated in serum-containing and serum-free media. C2C12 cells (a,b) and L6 cells (c,d) were differentiated in media containing 2% horse serum (a,c) or in serum-free medium (b,d). Several of the resultant myotube structures are indicated with arrows. Myotubes are readily seen in all cells grown and differentiated in serum-containing medium, but L6 cells in serum-free medium do not appear to make visible myotube structures.

	C2C12	L6	Chick
DMEM + 2% Horse Serum	4 ± 2	6 ± 2	41 ± 8
Serum-free	10 ± 2	2 ± 1	26 ± 6

Table 1. Creatine phosphokinase activity per mg. protein of cells grown in serum-containing and serum-free media. C2C12 cells, L6 cells, and chick myoblasts were differentiated in either serum-containing or serum-free media and assayed for creatine phosphokinase. C2C12 cells showed a higher CPK activity when grown in serum free medium, while L6 cells and chick myoblasts showed a higher CPK activity when differentiated in medium containing horse serum. The experiment was repeated on three separate days with different cultures, and a representative data set is shown.

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