ISOLATION OF SATELLITE CELLS FROM OVINE SKELETAL MUSCLES

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SUMMARY: We describe our protocol for isolating myogenic cells from the semimembranous muscles of lamb. Cultured cells display properties similar to myogenic satellite cells isolated from other species; similar characteristics include the ability to proliferate, differentiate, and fuse to form myotubes in primary cell culture. Using the described procedures, we provide the first documentation of satellite like cells existing in postnatal skeletal muscle of a ruminant animal species.

Key words: primary culture; satellite cells; ovine; surgery.

I. INTRODUCTION

Numerous reports have described proliferative and differentiative properties of embryonic myogenic cells (5,10-12,14-17), neonatal myogenic cells (7), and clonomyogenic cells (8,9) in vitro. However, until recently little was known about the growth dynamics of cultured myogenic cells derived from postnatal skeletal muscle.

Techniques for the isolation and in vitro propagation of postnatal myogenic stem cells (satellite cells) from rats were defined by Bischoff (4) and Allen et al. (1). Indeed, using the latter culture techniques, it was shown that fibroblast growth factor was a potent stimulator of cell proliferation (1). It was later demonstrated that linoleic acid promoted rat satellite cell differentiation when the basal medium contained minimal levels of serum (2). More recent, mechanistically oriented studies revealed that ovine somatomedin, rat insulinlike growth factor-II, and insulin all had the ability to promote rat satellite cell proliferation (6); these studies also provided initial definition of the receptors through which these growth factors induced satellite cells to respond (6).

No report presently exists documenting any growth parameter of primary cultures of myogenic satellite cells derived from the skeletal muscle of any ruminant animal species. Considering that in 1985 over 17 million metric tons of carcass weight were produced from red meat animal species in the United States and that 17 million tons of carcass weight represents 10 to 11 million tons of skeletal muscle, an improvement of a small percentage in muscle growth efficiency would save meat animal producers millions of dollars and benefit the consumers by providing them with high quality meat protein at a more reasonable price. A key to manipulating and improving the efficiency of postnatal skeletal muscle growth in domestic animals is understanding the regulation of satellite cell proliferation and differentiation. This is because satellite cells are responsible for the majority of nuclei that actually reside in adult skeletal muscle fibers (1,2,6) and that muscle protein accretion relies on satellite cell-mediated DNA accretion into individual postnatal myofibers (2). We, therefore, describe the basic methodology needed to isolate the semimembranous muscles from the hind limbs of lambs, and we report the isolation and culture procedures used for the initial characterization of ovine-derived myogenic satellite cells.

II. MATERIALS

A. Equipment

1. Balance, top loading, Ohaus Co.[22], no. 2515, VWR Scientific[33]
2. Balance, top loading, Sartorius Balances[25], no. 3713, Fisher Scientific[9]
3. Centrifuge, table-top, model TJ-6, Beckman, no. 14400, Beckman Instruments, Spinco Div.[4]
4. Filter, Sterivex-GS, 0.22 μm, Cat. no. SVGSB1010, Millipore Corp.[17]
5. Grinder, meat, 1.5-mm plate, Cat. no. 793.83, Sears[27]
6. Hood, laminar flow, no. 3318,076, Baker Co., Inc.[3]
7. Incubator, CO_2, Sheldon Manufact. Inc.[28], Model 6000, no. 35909-750[33]
8. Microscope, Diaphot-TMD, Nikon[19]
9. Mixer, tissue, no. 58220, Scientific Products[26]
10. pH Meter, digital, Altex[4], no. pHI-60[33]
11. pH Electrode combination, Altex[4], no. 34105-509[33]
12. Pipet Aid, Drummond Scientific[7], lot no. 174[33]
13. Refrigerator-Freezer, Gibson[27], model no. RT21T77WSGA

[1] Technical assistance provided.

