Characteristics of Ovine Skeletal Muscle Satellite Cell Strains in a Defined Culture Medium Formulated to Enhance Differentiation: Fusion and the IGF-I System

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Abstract

Previous data from our laboratory suggested that sheep satellite cell strains possess unique patterns of proliferation, differentiation, and myosin heavy chain isoform expression. These data, however, were based on cultures possessing marginal levels of morphological differentiation. Therefore, the goals of the present project were to formulate a defined medium which would support greater differentiation and to compare proliferation, differentiation and production of IGF-I and IGF-I binding proteins between the ovine satellite cell strains. Analysis of four defined fusion media yielded one medium which promoted moderately high fusion levels (84%) in one ovine satellite cell strain and 10 to 75% in other ovine satellite cell strains. Two strains which did not previously differentiate with exposure to 1% horse serum-containing medium fused 10% and 64% when treated with the defined fusion medium. In order to define cell lineage differences, media conditioned by the three most divergent satellite cell strains were assessed for IGF-I and IGF-I binding proteins (IGFBPs) at day one and day five following fusion induction. Negligible levels of IGF-I were expressed by all satellite cell strains tested. Differences were detected, however, in the amount and type of IGFBP present. After 24 hours of fusion induction, the nonfusing strain 10A displayed measurable IGFBP. Alternatively, ligand analysis of conditioned media from fusing strains O_1 and G_1 revealed IGFBP at 36.0 kDa and 28.2 kDa. After 120 hours, minimal IGFBP expression persisted with the O_1 strain, while only the 28.2 kDa IGFBP was detected in the G_1 or 10A strains. These data demonstrate the effectiveness of the defined fusion medium for inducing differentiation of sheep satellite cells and suggest that developmental patterns of IGF-I binding protein expression may be different between nontransformed sheep satellite cell strains.

Key words: defined media, fusion, satellite cell, IGF-I, binding proteins.

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Following Alexander Mauro's report of skeletal muscle satellite cells [41], considerable attention has been directed to identifying intrinsic and extrinsic factors responsible for satellite cell activation, proliferation and differentiation [2, 20, 42]. An applied focus of contemporary research is the definition of the involvement of satellite cells in the growth and regeneration of normal and diseased adult human myofibers. Cells derived from laboratory animals [4, 46, 51] and a limited number of humans [7, 56] are available as models for analyzing variables of myogenesis, but only recently have satellite cells from domestic animals been isolated and studied *in vitro* [14, 16, 27, 44].

Little is known about the mechanisms by which satellite cells direct normal muscle growth [7] or influence muscle composition in meat-producing animals [1]. At the same time, increased public awareness of health issues has brought a demand for leaner meat products for human consumption. Therefore, just as satellite cell research directly benefits humans through emerging gene therapy and other biomedical applications, utilization of this research might also indirectly affect human health by improving the composition and quality of meat from meat-producing animals.

An understanding of the regulation of myogenesis requires knowledge of the history or origin of the myogenic precursor cells of the muscle fibers [10, 42, 49]. The concept of heterogeneity has been applied to both the multiple types of myoblasts found within the developing

animal embryo [21, 43, 55] and the multiple types of muscle fibers [20, 42, 49] observed in an animal. Evidence suggest that myoblasts are committed during early development to produce different adult fiber types, and that myoblasts exist as distinct populations which have different developmental potentials [10, 43]. Particular groups of myoblasts have been designated (embryonic, fetal, and adult) [43, 55] which produce different types of myotubes, which in turn express a distinctive class or classes of myosin heavy chain (MHC) for the lifetime of the muscle fiber [8, 49]. The term *adult myoblast* has been equated with the satellite cell of skeletal muscle [21, 29, 57]. Both human and mouse satellite cells were found to be resistant to TPA (phorbol ester tetradecanoyl-phorbol 13-acetate), whereas human and mouse embryonic myoblasts were not [10]. Differences were also found in the expression of desmin by adult and embryonic myoblasts [57] and in the type of fast myosin expressed by fetal myoblasts and satellite cells [29].

Less evidence is available to support the idea that heterogeneity also exists within satellite cell populations. Early observations suggested that satellite cell numbers differed in fibers within the same muscle [52]. Moreover, human satellite cell clones derived from a single muscle displayed differences in fusion potential [58]. Two types of chicken satellite cells were identified: a fast myogenic lineage and a fast/slow myogenic lineage [55], which were not distributed evenly between muscles but rather were restricted to specific muscles [20]. In addition, at least two satellite cell populations were identified in isolates from adult rat muscles which expressed differential patterns of MHC isoforms [6, 18]. Thus a rather convincing argument could be made that satellite cells not only are different from other myoblasts but may also exist as distinct populations with different myogenic potentials.

Insulin-like growth factor-I (IGF-I) stimulates mitosis and differentiation of myogenic cells [2, 22, 34]. Cultures of satellite cells respond to exogenous IGF-I [17, 19, 48] and also synthesize their own IGF-I [31, 33]. In the circulation, IGF-I is bound to binding proteins (IGFBPs), which have been designated as six distinct types [54]. The majority of circulating IGF-I and II is bound to two predominant binding proteins, IGFBP-2 and IGFBP-3 [3]. Although most of the serum IGFBPs are derived from the liver, many individual tissues also produce IGFBPs, which are believed to play a local regulatory role, modulating IGF actions in an autocrine or paracrine fashion [9]. McCusker and Clemmons [38] demonstrated that rat L6 myoblasts secrete IGFBP-4 and -5, mouse BC_3H-1 myocytes secrete IGFBP-4, and porcine vascular smooth muscle cells secrete IGFBP-2. An earlier report by McCusker et al [37] showed that differentiating L6 myoblasts secreted more IGFBP-5 during differentiation. Thus, muscle cells appear to have distinct patterns of IGFBP secretion which vary with cell type and developmental stage.

We have previously shown that ovine satellite cells may be isolated, clonal populations propagated, and non-transformed strains established [45]. These strains possess a finite life span of 8 to 20 passages, express different forms of myosin heavy chain, and exhibit divergent proliferation and fusion potentials [45]. Therefore, the present project was designed to further define characteristics of these ovine satellite cell strains. We report differences among ovine satellite cell strains in their potential to fuse to form multinucleated myotubes when exposed to a chemically defined fusion medium. Further, little IGF-I is synthesized and secreted by ovine satellite cell strains *in vitro*, whereas different patterns of IGF-I binding proteins are produced by different strains of these cells. In general these data support the concept that heterologous satellite cell populations exist in the skeletal muscle of sheep.

Materials and Methods

Materials

The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): pig skin gelatin, pantothenate, triiodothyronine, fibronectin, L-glutamine, biotin, dexamethasone, $CaCl_2$, trypsin, HEPES, and Giemsa stain. Vitamin C, fetuin, fetal bovine serum, penicillin-streptomycin, gentamicin, and all basal media were purchased from Gibco/Life Technologies, Inc. (Grand Island, NY). Selenium, linoleic acid, epidermal growth factor, insulin, and transferrin (human) were obtained from Collaborative Biomedical Products (Bedford, MA). Bovine fibroblast growth factor and bovine serum albumin were acquired from Intergen Co. (Purchase, NY). Bactopeptone was purchased from Difco (Detroit, MI). Plastic cultureware was purchased from Nunc Inc. (Naperville, IL).

Defined Media

Four defined media (Table 1) were tested with a sheep satellite cell strain [45] for their ability to maintain cell viability over a six day culture period or to induce variables of proliferation and differentiation. The ovine satellite cell defined medium (ODM) was developed originally to support the growth of primary rat- and ovine-derived satellite cells [14] and for this research was modified to include fifteen added components in Dulbecco's Modified Eagle Medium (DMEM). The defined medium (ITT) containing insulin, triiodothyronine, and transferrin was initially formulated for human diploid adipocyte precursor cells [12]. The defined medium (SFG) supplemented with epidermal growth factor originally supported the growth of 3T3 preadipocytes [50]. The defined medium (4F) containing transferrin, fibroblast growth factor and fibronectin was initially used in the study of the growth and differentiation of adipocyte precursor cells [53].

Animals and Cells

Non-transformed ovine satellite cell strains were obtained from clones isolated from the semimembranosus and semitendinosus muscles of a black-face wether lamb [45]. Muscle type was not a factor in deciding the outcome of characteristics measured in previous experiments [45].

Table 1. Defined media formulations. The concentrations of listed components are the amounts added to the basal media. The glucose levels in the three basal media are: DMEM- 4500 mg/L, DMEM/F12 (1:1)- 3651 mg/L, DMEM/F12 (3:1)- 3826 mg/L.

Components	ODM	ITT	SFG	4F
Basal medium	DMEM	DMEM/F12 (1:1)	DMEM/F12 (3:1)	DMEM/F12 (1:1)
FGF	20 ng/ml			25 ng/ml
EGF			0.8 nM	
Triiodothyronine		0.2 nM		
Insulin	1 nM	500 nM	1 nM	1800 nM
Dexamethasone	10^{-7} M			
Fibronectin	2 µg/ml			2.5 µg/ml
BSA	500 µg/ml			
L-Glutamine	2 mM			
Selenium	3.8 ng/ml			
Vitamin C	10 µg/ml			
Fetuin	500 µg/ml		300 µg/ml	
Biotin	200 ng/ml	8 µg/ml	244 ng/ml	
Linoleic acid	10 µg/ml			
$CaCl_2$	0.5 mM			
Transferrin	5 µg/ml	10 µg/ml	2 µg/ml	10 µg/ml
Bactopeptone	600 mg/l			
HEPES	10 mM	15 mM		
Pantothenate		17 µM	17 µM	

Culture plates (24-well) were coated with 0.5% pig skin gelatin [15]. Cells were plated at an initial density of 20,000 cells per well (100 nuclei per mm^2) for 24 hours in DMEM, supplemented with 10% fetal bovine serum (FBS). The wells were then washed thoroughly with serum-free DMEM, and one ml defined medium was added (day 0). Media were replaced at 24 hour intervals. Control cultures were maintained in DMEM-10% FBS or DMEM-1% FBS [14]. Penicillin-streptomycin (100 U per ml) and gentamicin (50 µg per ml) were added to all media. At 0, 24, 48, 72 and 120 h after treatment, cultures were washed with phosphate buffered saline (PBS), fixed with absolute methanol, stained with Giemsa, and total nuclei density was determined by enumerating the number of nuclei in 10 randomly chosen areas of each culture well at 400X magnification [13]. Nuclei were classified as being fused (myotube) if three or more nuclei were observed within a continuous cell membrane [15]. The experiments used ovine satellite cell strains of passage number three to five. All cultures were maintained at 37°C in a humidified atmosphere of 95% O_2 and 5% CO_2.

Insulin-like Growth Factor (IGF)/IGF Binding Protein Production and Identification

One-d or four-d old cultures were allowed to condition the ITT fusion medium for 24 or 48 h respectively. Conditioned media were analyzed for IGF-I levels after acid-ethanol extraction [11] using a double antibody radioimmunoassay [30]. The presence of IGF-I binding proteins was assessed in conditioned media which had been concentrated 4- to 5-fold using Centricon-10 concentrators (Amicon) (MW cutoff= 10,000). Seventy-five microliters of media were subjected to SDS polyacrylamide gel electrophoresis [36] in 12% gels in the absence of reducing agent. Separated proteins were transferred to nitrocellulose membranes using a semi-dry electrophoretic transfer apparatus (BioRad transblot, Richmond, CA), fixed overnight with glutaraldehyde [47], and hybridized to ^{125}[I]-IGF-I (5 x 10^5 cpm/ml) as described [32]. Nitrocellulose was exposed to Kodak X-OMAT AR5 X-ray film at -70°C for 5-8 days. Molecular weights of radioactive bands were determined by linear regression analysis of molecular weight standards (Kaleidoscope Prestained Standards, Sigma Chemical Co., St. Louis, MO).

Statistical Design and Data Analyses

All experiments used a completely random design. The first experiment tested the effects of four media on satellite cell fusion dynamics. Media was the treatment, and three to six replicates were used per treatment. The second experiment tested the effect of ITT medium on the fusion dynamics of various satellite cell strains. Cell strain was the treatment, and six replicates represented each treatment mean. Proliferation and fusion variables were analyzed using a one-way analysis of variance. When the treatment effect was significant, Tukey's least significant difference was applied to separate differences in individual treatment means at a significance level of $P < .05$. All analyses were

completed using GraphPad™ and InStat™ version 2.0 software [26].

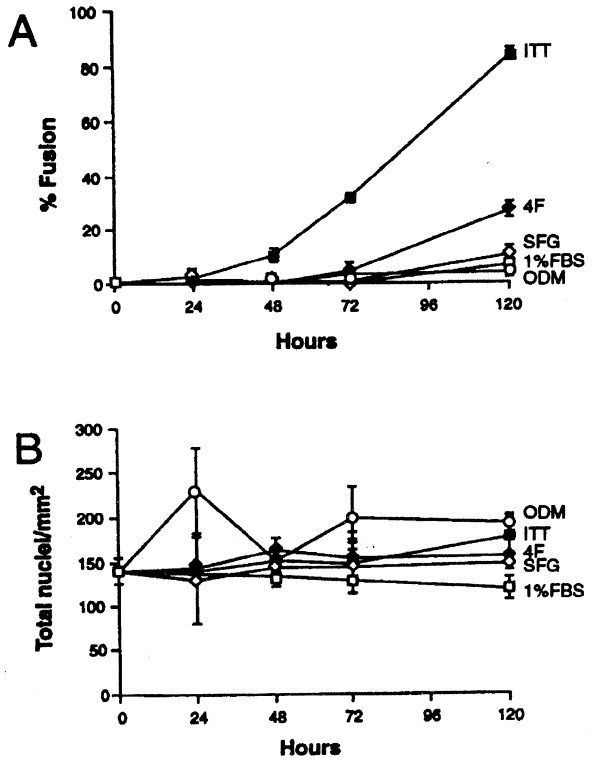
Results

Analysis of defined media

In an attempt to stimulate differentiation of sheep satellite cells, four defined media (Table 1) were assessed for the ability to induce fusion of the O_1 satellite cell strain into multinucleated myotubes. Differences were observed in the fusion of this strain in response to different media. ITT medium induced fusion in 84 ± 5% of the O_1 cells(Fig. 1A & 2A), while 4F medium induced 25 ± 7% fusion after 120 h exposure. ODM, SFG, and DMEM-1% FBS did not stimulate significant fusion of O_1 cells (P > .05). Over an eighteen month time frame, twelve experiments were performed using O_1 cells and ITT fusion medium. In all of these experiments, ITT induced higher fusion rates than those observed in other defined media or in 1% serum controls. The proliferation of O_1 cells in response to the four media was not different (Fig. 1B; P > .05), and no media stimulated significant increases in proliferation over initial plating density (Fig. 2B; P > .05). The morphological characteristics of the cultured O_1 cells before fusion were indistinguishable among the defined media. However, the myotubes induced by the ITT medium were very large and web-like compared to the smaller, more compact myotubes formed in the other defined media (Fig. 3).

Characterization of other ovine satellite cell strains

In earlier work in our laboratory, we noted differences among many ovine-derived nontransformed satellite cell



igure 1. Time course of the effects of four defined media and DMEM-1% FBS on the proliferation and differentiation of O_1 satellite cells. O_1 cells were plated at 100 nuclei per mm² in a DMEM-10% FBS medium. After a 24 h attachment period, cells were washed three times and defined media added. At 24 h intervals, the wells were fixed, stained, and evaluated for total and fused nuclei. Each point on the graph represents the mean ± standard error for 3-6 wells per treatment. (A) Percent fusion of O_1 satellite cells during 120 hour treatment period. (B) Total nuclei per mm² of O_1 satellite cells over 120 hour time period.

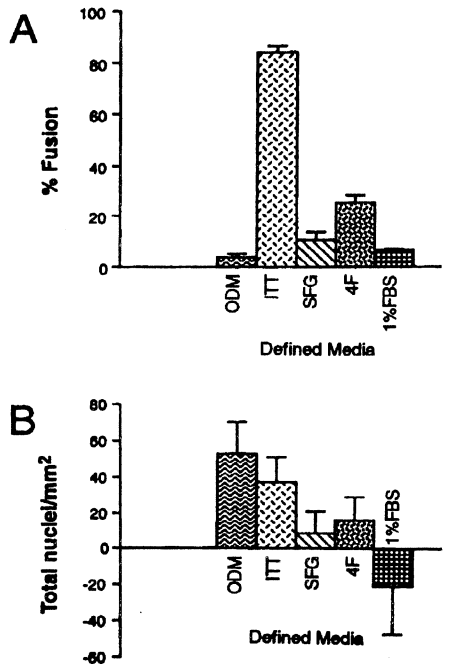


Figure 2. The effects of four defined media on the proliferation and differentiation of O_1 satellite cells. O_1 cells were plated at 100 nuclei per mm² in a DMEM-10% FBS medium for 24 h and then switched to the treatment medium. At 120 h after treatment all wells were stained and counted for total and fused nuclei. In both graphs the bars represent the mean ± standard error for 6 wells per treatment. (A) Data depict the percent fusion in four defined media and DMEM-1% FBS at 120 h. (B) Data represent the change in total nuclei per mm² (proliferation) at the end of the experiment (120 hours). Total nuclei per mm² at treatment application (140 ± 15) have been subtracted from the total nuclei per mm² at the end (ODM: 191 ± 10, ITT: 176 ± 23, SFG: 148 ± 9, 4F: 155 ± 4, 1% FBS: 118 ± 13).

strains in proliferation potential, differentiation, and myosin heavy chain (MHC) expression [45]. We therefore exposed nine ovine satellite cell strains [45] to the ITT fusion medium for 120 hours to examine differences in the responses of the cell strains to fusion induction. Eight of nine strains fused in response to ITT (Fig. 4A), and at 120 h, eight of the nine strains exhibited an increase in fusion over that produced by individual 1% serum controls ($P < .05$; fusion with 1% FBS: O_1= 6.3 ± 1.5%, O_2= 8 ± 4%, O_3=1.8 ± 3%, J_6=0.8 ± 1.6%, G_1=0%, I_1=22.5 ± 7.6%, I_2=1.8 ± 3.2%, 10A= 0%, N_3=0.5 ± 1.2%). In an earlier study using a medium containing 1% horse serum with six of these strains, fusion percentages were much lower than with the ITT medium (I_1, I_2, J_6, and 10A did not fuse; N_3 and O_1 fused 3% and 35% respectively)[45]. Differences were observed in the levels of proliferation of the nine ovine satellite cell strains with the ITT treatment ($P < .05$; Fig. 4B). Eight of the strains maintained or increased the nuclei number over that at treatment initiation. Cultures of the O_3 strain showed a decrease in nuclei number during the 120 hour period.

Effect of IGF-I treatment

In order to determine effects on mitosis and differentiation, 25 ng/ml IGF-I was added to ITT defined fusion medium at 24 hours post-plating. A five day time course design was used with three satellite cell strains (O_1, G_1, 10A). When added to ITT, IGF-I did not induce proliferation or fusion of the strains ($P > .05$, data not shown).

Detection of IGF-I and IGF-I binding proteins

Samples of conditioned fusion media from three ovine satellite cell strains (O_1, G_1, 10A) which differed in fusion potential were analyzed for IGF-I content by radioimmunoassay and for IGF-I binding proteins by ligand blot analyses. All IGF-I levels in conditioned media were at or below the detection limits of the RIA (1 ng/ml culture medium). Western ligand blot analysis of culture medium conditioned by the ovine satellite cell strains demonstrated unique patterns of IGF binding protein secretion (Fig. 5). After 24 h treatment with ITT medium, strains O_1 and G_1 displayed prominent IGFBPs which had estimated molecular weights of 36.0 kDa and 28.2 kDa. Both of these IGFBPs were not detectable in the nonfusing strain 10A at 24 h. At 120 h both IGFBPs persisted as faint bands in the O_1 strain, while the 28.2 kDa IGFBP was faintly present in the G_1 and 10A strains. Proteins migrating at a relative molecular weight consistent with other forms of IGFBP were not detected. When hybridized in the presence of 10^{-7} M IGF-I, ^{125}[I]-IGF-I did not bind to the culture medium, demonstrating that the observed binding was not due to nonspecific effects.

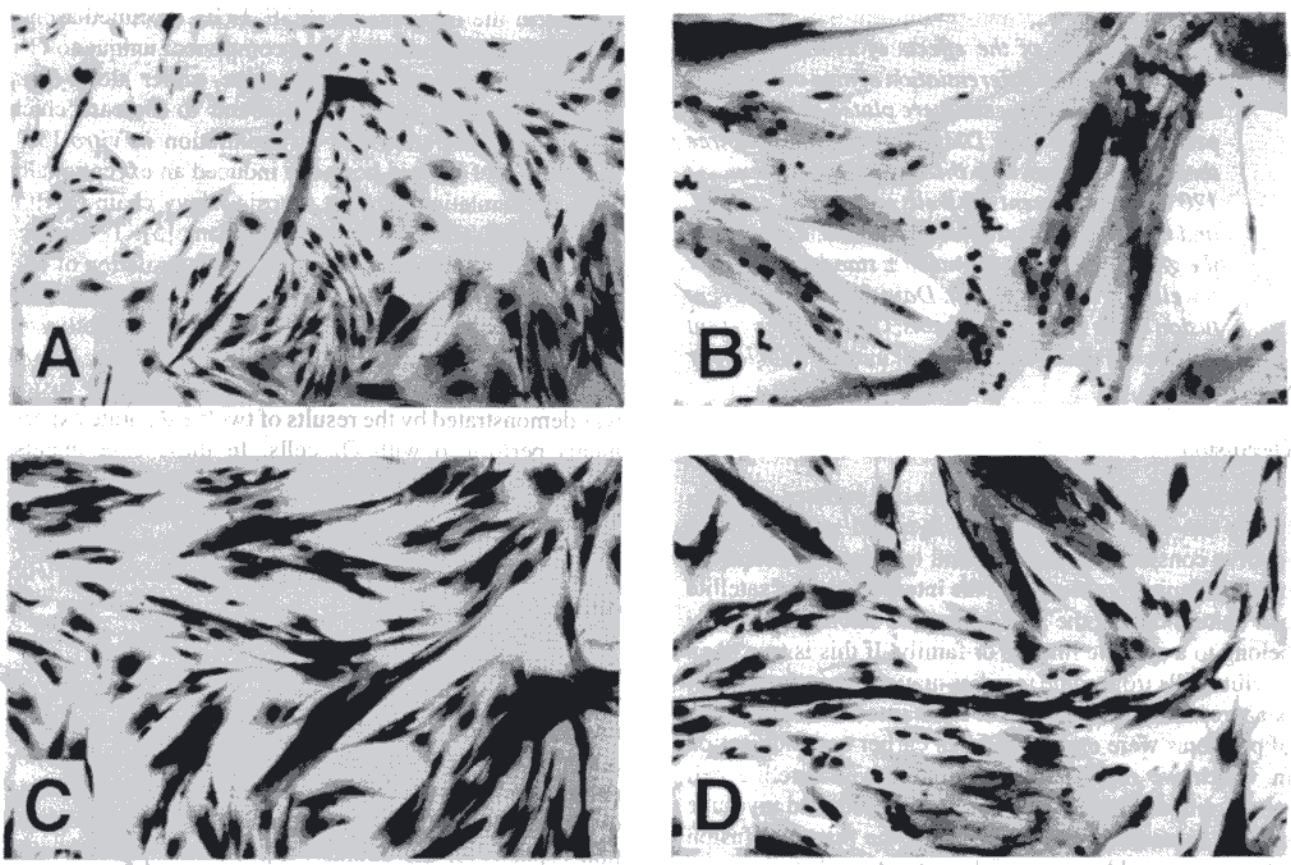


Figure 3. Photomicrographs (100 x) showing differences in myotube morphology at 120 h resulting from exposure of O_1 cells to four different media: (A) ODM (B) ITT (C) SFG (D) 4F. Cells have been fixed and stained with Giemsa.

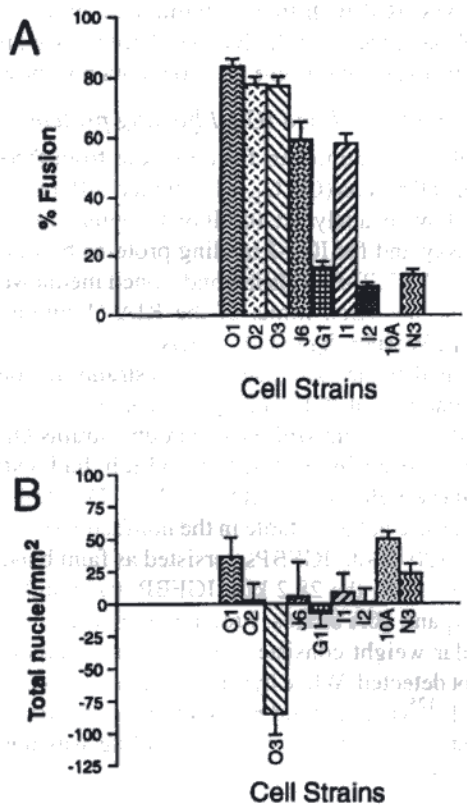


Figure 4. Comparison of the effects of ITT medium on proliferation and differentiation of nine ovine satellite cell strains. The cells were plated at 100 nuclei per mm^2 for 24 h in DMEM-10%, washed three times, and treated with the ITT fusion medium. At 120 h post treatment the wells were fixed, stained, and the total and fused nuclei counted. Each bar of the graph represents the mean ± standard error for 6 wells per treatment. (A). Data depict the percent fusion of the nine ovine strains. (B). Data represent the overall change in total cell number (nuclei per mm^2).

Discussion

Conducting research with satellite cell cultures and analyzing the subsequent data are dynamic processes that involve considerable patience and tolerance. Perhaps the most intriguing problem with the interpretation of satellite cell culture data is the possibility that satellite cells may belong to a specific lineage or family. If this is true, then satellite cells from some families may not be isolated at all or not repeatedly isolated in the same amounts. These types of problems were expressed in an earlier communication in which we reported that ovine-derived satellite cell strains differed in potential for both growth and myotube protein expression [45]. Because of the marginal fusion levels expressed by some of the satellite cell strains used in the previous study, it was difficult to make definitive comparisons and conclusions about lineages. We therefore initiated a series of studies to clarify differences among the satellite cell strains. Few defined media are available for delineating all aspects of myogenesis. Conditions that favor proliferation of cells usually are not optimal for differentiation or vice versa [28]. In our evaluation of defined media, the performance of one defined medium which had been formulated specifically for the maintenance of ovine satellite cells [14] was consistent with previously published results. In this medium both proliferation and fusion were low, allowing for observation of the effects of added growth factors. However, we needed a defined medium which maximized satellite cell fusion, which is an indicator of differentiation. With such a medium we could then reevaluate protein expression profiles or other variables of differentiation of the ovine satellite cell strains.

Of the four defined media that were examined, only the ITT medium induced significant fusion of the cells. The 84% fusion displayed by strain O_1 was well above the 35% fusion achieved in an earlier study with the same cells and a serum-containing fusion medium [45]. The reasons for the effectiveness of the ITT medium in promoting fusion are not known at this time. Both biotin and insulin are present in much greater amounts in ITT than in the ODM medium. Concordant with older protocols in which serum levels are reduced from 10% to 1% to induce myoblast fusion, the lack of several components in ITT which are present in the other media (FGF, EGF, dexamethasone) might lead to differention. One component unique to ITT is triiodothyronine (T_3). L-thyroxine, either alone or in combination with insulin, was found to increase chick myoblast proliferation and differentiation *in vitro* [35]. High levels of thyroid hormone induced an exceptionally early accumulation of adult myosin heavy chain (MHC) and early muscle maturation in humans [5]. T_3 also inhibited proliferation and stimulated differentiation of cultured quail myoblasts [40]. Future studies will address the mechanisms behind the fusion-stimulating capability of ITT.

The effectiveness of ITT medium at enhancing fusion was demonstrated by the results of twelve separate experiments performed with O_1 cells. In these experiments, ITT-treated O_1 cells demonstrated consistently higher fusion levels than those observed in other defined media or 1% serum. These studies also illustrated the stability of the O_1 satellite cell strain, as similar results were obtained with cells from passages 4-10.

The nine ovine satellite cell strains which we examined exhibited a broad range of fusion capacities. Previously, satellite cell clones derived from a single muscle of a muscular dystrophy patient also differed in amounts of fusion *in vitro* [58]. The current study corroborates earlier research in which the differences in fusion potential among ovine satellite cell strains were unrelated to the proliferation capacity of the strains [45]. Strain O_1 proliferated moderately during the 120 hours exposure to fusion medium and also had a high fusion percentage, while strain

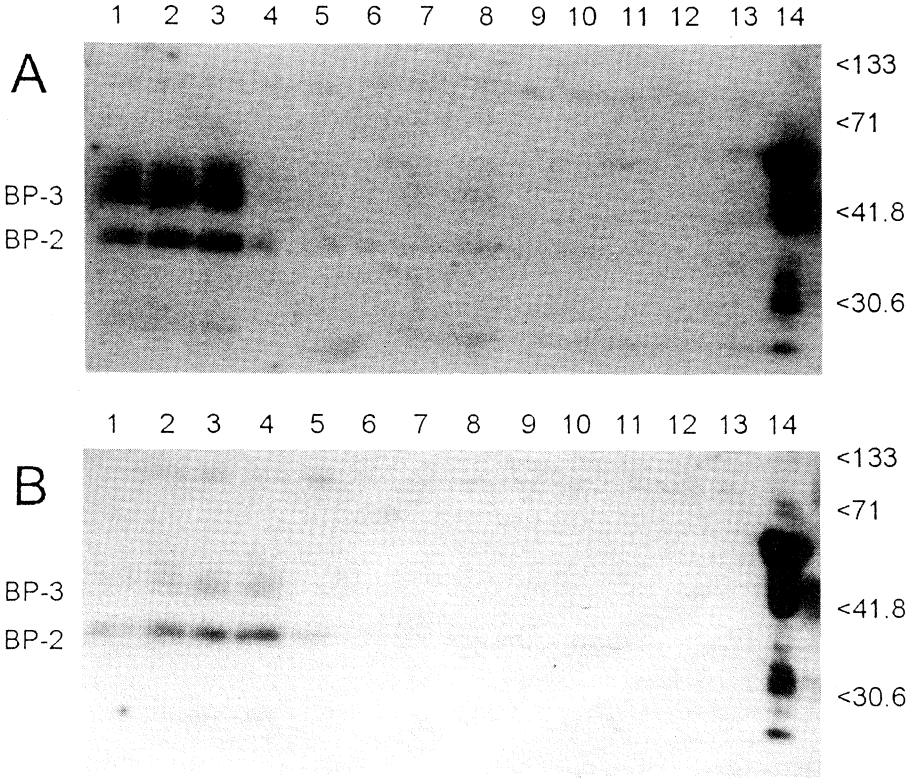


Figure 5. Autoradiographs of conditioned culture medium from three strains of ovine satellite cells. Medium conditioned by satellite cells for 24-48 h was subjected to SDS polyacrylamide gel electrophoresis and Western ligand blot analysis with ^{125}[I]-IGF-I. A. Lanes 1-3: cell line O_1, 24 h exposure to ITT medium; lanes 4-6: cell line O_1, 120 h; lanes 7-9: cell line 10A, 24 h; lanes 10-13: cell line 10A, 120 h; lane 14: 1l sheep serum. B. Lanes 1-4: cell line G_1, 24 h exposure to ITT medium; lanes 5-11: cell line G_1, 120 h; lanes 12-13: cell line 10A, 120 h; lane 14: 1 l sheep serum. Molecular weight standards (x 10^{-3} kDa) are shown to the right of each autoradiograph.

O_2 did not proliferate but displayed a high degree of fusion. Although the O_3 cells had a high proportion of cell death, the surviving nuclei were capable of high fusion rates. Regardless of the extent of proliferation, there was a high degree of fusion in the three O satellite cell strains. These results demonstrate that satellite cell strains obtained from the muscle of a single lamb differ in proliferative and differentiative responses to a defined fusion medium.

Insulin-like growth factor I has been shown to have mitogenic and differentiative effects on myogenic cells [2, 22, 34]. In studies with bovine satellite cells, IGF-I had no effect on proliferation but stimulated differentiation of the cells [27]. The fusion of turkey satellite cells was not affected by added IGF-I, although IGF-I stimulated the proliferation of the cells [39]. Florini et al [23] reported that IGFs stimulate proliferation and differentiation at low concentrations but inhibit differentiation at higher concentrations in L6 myoblasts. In our defined media studies, when three strains of ovine satellite cells were treated with 25 ng/ml of IGF-I, IGF-I did not induce proliferation or fusion.

There are several possible explanations for the lack of effect of the exogenous IGF-I on the satellite cell strains. It is conceivable that the high insulin level in the ITT medium could have masked the IGF-I effect by occupying IGF-I receptors. However, reduction of the pharmacologic level of insulin in the ITT medium (10^{-6} M) to a more physiological concentration (10^{-9} M) had no effect on the response of the cells to IGF-I. Satellite cells themselves can produce IGF-I [33], and high endogenous levels of IGF-I could block the effect of added IGF-I [39]. Assessment of IGF-I levels in conditioned media from three satellite cell strains, however, showed negligible levels of satellite-cell-derived IGF-I. In early studies by Dodson et al [13] it was determined that dexamethasone was required for IGF-I to have a pronounced effect on rat-derived satellite cells. The absence of dexamethasone in ITT as well as the observation that ITT itself is a strong inducer of differentiation may preclude the IGF-I effects on mitosis and differentiation. Other factors which could contribute to the lack of effect of IGF-I include scarcity of IGF receptors, cell density, species differences, or some other component

in the ITT medium. There is evidence that the autocrine secretion of IGF-II by muscle cells increases their rate of differentiation [24]. While we did not look at IGF-II, a next step would be to examine IGF-II levels in defined media conditioned by exposure to satellite cells.

Conditioned media samples from three strains were assessed for IGFBP and were found to have different IGFBP secretion patterns. Two strains of satellite cells which had a high fusion potential (O_1 and G_1) also secreted relatively high levels of IGFBP with molecular weights of 36.0 kD and 28.2 kDa. These correspond to the sizes of ovine IGFBP-3 and IGFBP-2, respectively [25]. The non-fusing strain of cells (10A) did not secrete significant amounts of any IGF binding protein. The tentative identification of IGFBP-2 and -3 in culture medium conditioned by satellite cells is apparently unique. An earlier report on studies using rodent myogenic cells identified IGFBP-4 and -5 secretion [38]. The secretion of IGFBP-2 and -3 by the sheep-derived satellite cells suggests that there may be species and developmental differences in the secretion of IGFBPs by myogenic cells. The significance of the presence of high levels of IGFBP-2 and -3 in media conditioned by the fusing cell lines O_1 and G_1 is not known. The binding proteins are present during the first 24 h of exposure to differentiation-inducing medium but decline to low levels after fusion is complete at 120 h. This suggests that the binding proteins may be involved in the early events accompanying differentiation of the satellite cells. The IGFBPs may sequester and inhibit mitosis-inducing IGF-I or may enhance the differentiating effects of IGF-I. The role of IGF-I, IGF-II, and other IGFBPs in modulating satellite cell differentiation is currently under investigation.

The concept that satellite cells may represent a heterologous population of cells distinctly different from other types of myogenic cells has received general support over the past decade. Data from the present study extend this idea [45] to suggest that individual satellite cell populations also differ in their proliferation and differentiation dynamics. Further, we have developed a defined medium which appears to maximize fusion of strains of ovine satellite cells over a treatment period of five days. Other ovine satellite cell strains respond to this medium by fusing to different degrees. Further experiments with this fusion medium found differences in the levels of IGF-I binding proteins produced by the ovine satellite cell strains. Thus, these ovine satellite cell strains and the ITT fusion medium could prove to be valuable tools for gaining additional insight into the cellular and molecular differences in satellite cell populations.

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