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HUMAN SATELLITE CELL PROLIFERATION IN VITRO IS REGULATED BY AUTOCRINE SECRETION OF IL-6 STIMULATED BY A SOLUBLE FACTOR(S) RELEASED BY ACTIVATED MONOCYTES

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We previously showed that macrophages, besides their scavenger role, selectively induce rat myoblast proliferation in vitro by releasing soluble factors. In this paper we demonstrate a relationship between human-activated monocytes and increased human myoblast proliferation due to IL-6 autocrine secretion by satellite cells. Indeed in the supernatants of muscle cultures treated with activated monocyte-conditioned medium we show by means of an ELISA quantitation a higher autocrine secretion of IL-6 associated with increased myoblast proliferation. This suggests that a growth factor(s) secreted by activated monocytes stimulates IL-6 production by myoblasts and then regulates proliferation of satellite cells.

Skeletal muscle fibers result from the fusion of embryonic myoblasts into myotubes during the early stages of development. The myotubes then synthesize contractile proteins characteristic of mature muscle fibers. Some myoblasts, however, do not undergo fusion but remain quiescent (satellite cells), located between the sarcolemma and basal lamina of mature myofibers. After muscle injury, the muscle fibers degenerate and there is an inflammatory reaction which is accompanied by satellite cell activation and production of new myoblasts. In a few days, fusion of the newly generated myoblasts forms new fibers. One of the earliest visible cellular events in the skeletal muscle regeneration is the accumulation of polymorphonuclear leukocytes at the site of damage. Two days later, macrophages infiltrate and phagocytise the necrotic muscle. Afterward, myoblasts proliferate, fuse

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and regenerate the lost myofibers (1).

We have recently demonstrated that both in satellite cells and primary myoblast cultures of rat, the presence of macrophages is able to orchestrate the mitotic activity of muscle cell precursors by secreting soluble growth factors. Indeed macrophage and satellite cell co-cultures show a significantly increased number of differentiated myotubes when compared to control cultures of satellite cells alone. Additional experiments with macrophage-conditioned media revealed that the increase in muscle cell proliferation is mediated by acid-stable, heat-labile soluble factors released by macrophages. These results conclusively demonstrate that besides their scavenger role, macrophages can influence myoblast proliferation and muscle regeneration (2,3). It is well known that cytokines and growth factors can stimulate either myoblast proliferation or differentiation (4,5,6), and many of these are secreted by macrophages (7).

In this study we have extended our observations to man. We have demonstrated, by means of an ELISA quantitation, a higher autocrine secretion of IL-6 by myoblasts associated with increased proliferation of human satellite cells supplied with activated monocyte-conditioned medium. These results suggest that a growth factor(s) secreted by macrophages stimulates IL6 production by myoblasts which in turn causes the increased rate of myoblast proliferation during muscle regeneration.

MATERIALS AND METHODS

Activated monocyte cultures

Monocytes harvested from peripheral blood were activated in vitro by heterologous IgG or lipopolysaccharide (LPS) (8). Activated monocyte-conditioned medium harvested at day three of culture was added to myoblast cultures as described in (2,3).

Muscle cells cultures

Human satellite cells were obtained from biopsies of adult patients during toeletting of previous aesthetic surgery. Control cultures, seeded at 8,000 replated myoblasts per cm2, were grown in 10% FCS and 0.2% AB human serum (9). Three doses of 10% activated monocyte-conditioned medium were added to human myoblasts during the first week of culture.

SDS PAGE and immunoblot analyses of specific muscle markers

Quantitation of muscle growth was determined by SDS PAGE and immunoblotting of desmin content (2,3,10).

Cytokine determination

IL-1 and IL-6 were measured in culture supernatants by ELISA (Genzyme).

Immunocytochemistry

Cell cultures were stained with anti-desmin (Boeringher) and anti-embryonic myosin heavy chain (generous gift of Professor S. Schiaffino, Padova, Italy) monoclonal antibodies as previously described (3).

RESULTS AND DISCUSSION

In our experimental conditions early myotubes appear one week after myoblast seeding in human control cultures. A week later large and more numerous cross-striated multinucleated myotubes are present; they stain strongly with anti-embryonic myosin heavy chain antibodies. When human myoblasts are grown in the presence of either activated monocytes or of supernatant from activated monocyte cultures, myotubes are even more numerous and contain more nuclei (Fig 1). These observations are consistent with our results obtained with rat

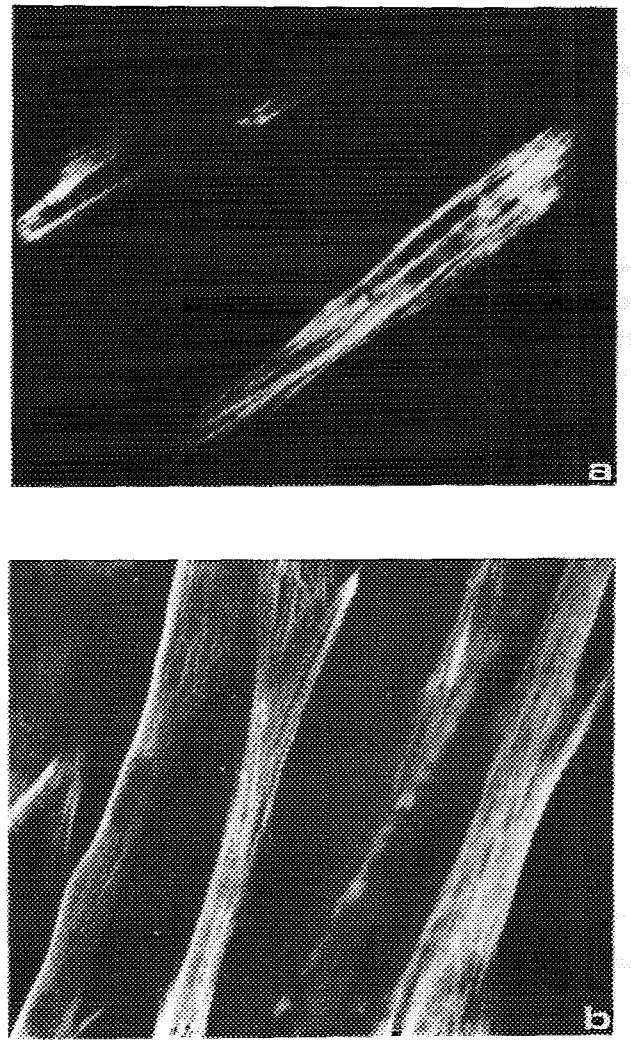

Figure 1. Effects of activated monocyte-conditioned medium on human muscle culture.
Key: a, control culture ; b treated muscle culture. Immunocytochemical staining with anti-MHCemb mAbs. Panel b,shows increased number of striated myotubes.
The Figure represents randomly chosen microscopic fields of a typical experiment.

macrophage-satellite cell co-cultures (2). The growth of human myoblast/myotubes, was quantitated by measuring the amount of desmin in the cultures by SDS PAGE separation and immunochemical analysis. We previously showed that the amount of desmin and myosin found in rat muscle cultures correlates well with the extent of cell proliferation and differentiation the cells have undergone (2,3). In human muscle cultures supplemented with activated monocyte medium the amount of desmin is 3 to 10 fold higher than in controls, thus demonstrating that myoblast proliferation is enhanced.

It is well known that cytokines and growth factors can stimulate either myoblast proliferation or differentiation (4,5,6), and many of these are secreted by macrophages (7). In particular, IL-6 and LIF have been shown to increase human myoblast proliferation in vitro (5,6). The production of IL-6, a pleiotropic cytokine not usually produced by normal cells, is induced in vitro in many cell types by a variety of stimuli. In human myoblasts in vitro IL-6 is constitutively secreted, and the adding of TNFα has been shown to have the potential to greatly increase its secretion (6).

To test the hypothesis that macrophages secrete specific factor(s) which may influence the autocrine IL-6 myoblast production, with consequent stimulation of myoblast proliferation, we measured by ELISA IL-6 concentration in the culture media. IL-1 was also measured as macrophage activity marker. Table 1 shows that the IL-6 content in the growth medium is low in comparison to that of the control muscle culture medium. The 10 fold higher value of IL-6 in muscle culture medium is in agreement with myoblast proliferation. After addition of activated monocyte-conditioned medium, IL-6 concentration in muscle culture medium increases more than five times in comparison to control cultures. Table 1 shows that also activated monocytes secrete high levels of IL-6, but the amounts of monocyte-conditioned medium added to muscle cultures cannot account for the very high levels of IL-6 in muscle treated cultures. Since IL-6 appears to be constitutively secreted by

Table 1. IL-1b, and IL-6 in human myoblast cultures supplemented with three-day Activated-Monocyte Conditioned Medium (AMCM)

	IL-1 β	IL-6
Growth medium	75 - 96 (2)	< 20 (2)
Muscle culture medium	31 - 75 (2)	242 - 1258 (3)
Activated-monocyte culture medium	187 - 500 (6)	156 - 700 (2)
Muscle culture medium supplemented with 3-day AMCM	196 - 520 (5)	1474 - 8000 (4)

Values are expressed as ng/l. In parentheses the number of determinations are given.

myoblasts, one can say that the higher the IL-6, the more enhanced the cell proliferation.

Our present results together with the one mentioned above (TNFα and LIF) suggest that cytokines released by macrophages could be essential for myoblast proliferation during muscle regeneration. On the other hand, since TNFα is known to be a mitogenic factor for fibroblasts (4), we cannot explain the selective proliferation exerted on myoblasts in primary cultures (2,3). It remains to be determined whether LIF or other unknown cytokines are responsible for the selective proliferation of myoblasts as a potential strategy in myoblast transfer therapy (11-13).

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