INTRODUCTION

Myoblast Transfer Therapy in the New Millennium

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In 1996 at the 3rd Cell Transplantation meeting in Miami a major symposium on Myoblast Transfer Therapy (MTT) was organized by Jacques Tremblay (Montreal). This gathering marked a renaissance of interest in MTT, which had become unfashionable since the poor results of the clinical trials, and it served to put MTT firmly back on the "cell transplantation" agenda (1,26) along with pancreatic, hepatic, and neuronal cells (1). Eleven articles and an editorial from this symposium were published in a special issue of Basic & Applied Myology in 1997. Three years later at the Cell Transplantation meeting in Montreux (2), another workshop on MTT was held with a palpable difference in mood and feelings about the possibilities for this potential cell therapy. Six of these papers have been gathered together in this present issue of Cell Transplantation.

SURVIVAL OF CULTURED MYOBLASTS AFTER INJECTION IN VIVO

One of the most striking advances since the failed clinical trials has been recognition of the massive death of cultured donor myoblasts that occurs between 1 and 7 days after injection into host muscles, apparently due to a host immune response (5,6,8,16). An even more dramatic and massive death of donor myoblasts (80% of the injected cells have been destroyed within minutes) has now been demonstrated in the paper by Hodgetts et al. (15), although the mechanism for this earlier and more severe response is not clear. On the basis of these crucial observations it seems likely that most of the donor myoblasts in the clinical trials to date may have similarly perished within minutes of injection. Identification of these problems opens the way to develop strategies to overcome the immediate destruction of the injected myoblasts in addition to their further demise over the following week. Manipulation of the host immune response is one approach, and data from quantitative studies support the idea that host adaptive immune cells, CD4+, CD8+, and natural killer (NK) cells, contribute to both phases of the "death curve" (15). Depletion of such host cells by specific antibodies prior to myoblast injection results in a fourfold increase in initial survival of the donor myoblasts and these effects are sustained for up to a week in mice depleted of NK1.1+ cells. These and other modulations of the host immune response may hopefully be shown to enhance long-term survival of cultured donor myoblasts. There is now evidence that tissue culture procedures per se can modify the donor myoblasts and render them vulnerable when they are transferred in vivo. It was demonstrated that tissue culture can induce neoantigens on autologous cells and provoke a host immune response (17). Using whole-muscle grafts as the source of donor cells (rather than isolated myoblasts) it was shown that proteases have a highly deleterious effect on myogenic cells that are exposed to tissue culture conditions before transplantation in vivo (24). Because all isolated myoblasts are routinely exposed to proteases such as trypsin in order to remove cells from culture dishes prior to injection, this raises the unpleasant possibility that the proteases modify the surface of the myoblasts so that they provoke an immune response when exposed to the in vivo environment. This simple observation may also apply to other transplanted cells, and it focuses attention on approaches to improve the preparation of myoblasts for injection in MTT.

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SOURCE OF DONOR MYOBLASTS

A deeper appreciation of the source of the donor myoblasts is provided by three other papers. There is evidence that myoblasts extracted from skeletal muscle are not a homogeneous population. The observations of Petersen and Huurd (22) indicate that the type of innervation of the muscle (i.e., fast or slow twitch) results in different populations of donor myoblasts that preferentially fuse with each other and such "matching" is another factor to consider for effective MTT. In addition, the possibility of using (myogenic) stem cells derived from nonmyogenic tissues as a source of donor myoblasts is now attracting considerable attention for MTT (12,21), although one major problem with this approach would seem to be the relatively low (less than 1%) recruitment of such cells to the myogenic lineage. For example, it has recently been demonstrated that bone marrow-derived cells can give rise to myoblasts (9) and conversely that cells derived from muscle can give rise to bone marrow-derived cells (13,18), emphasizing an unexpected similarity and plasticity of such "stem cells." It has also been demonstrated that dermal fibroblasts can give rise to myoblasts, and the paper by Goldring and colleagues (10) shows that one inductive agent responsible for this conversion is β-galactosidase binding protein. The study of such inducers is crucial to try and improve the efficiency of conversion into the myogenic lineage. These studies raise the possibility of using nonmyogenic tissue from the patient as a source of autologous myoblasts for genetic correction prior to transplantation. It has long been recognized that cells within the thymus (myoid cells) have the capacity to give rise to cells that appear identical to conventional skeletal muscle. The potential for thymic cells to be used as another source of autologous myoblasts is supported by the in vivo studies of Pagel et al. (20), who show that donor cells of a clonal myoid cell line contribute to new muscle formation after injection into irradiated muscles of dystrophic mdx mice, although less success was obtained with a suspension of fresh thymic cells. It seems likely that many more studies exploring these options will emerge.

MIGRATION OF DONOR MYOBLASTS IN VIVO

Ideally, for successful MTT, the donor myoblasts should migrate away from the transplantation site and disperse throughout the host muscles. To date, few studies have investigated factors that enhance such migration of donor myoblasts in vivo. More effective dispersion from donor sliced muscle implants was demonstrated in host mice where the immune response had been modified (23), and the study by Torrente et al. (25) shows that enzymatic digestion of the extracellular matrix by metalloproteinases (MMPs) also improves the migration of injected donor myoblasts. The fact that the movement of myoblasts of the C2C12 cell lines was considerable in untreated muscles and was fourfold greater than for primary myoblasts in MMP-treated muscles confirms earlier observations of extensive movement of C2C12 cells in vivo (27) and emphasizes that results obtained using immortalized cell lines like C2C12 (that also form tumors in vivo) often differ markedly from those where primary donor myogenic cells are used (20,22). Because the latter are the likely source of donor myoblasts in human MTT, the probable behavior of "normal" cells in vivo must be carefully related to the (often superior) data obtained using immortalized cell lines.

CORTICOSTEROIDS AND MUSCULAR DYSTROPHY

Because of the tremendous interest in prescribing corticosteroids such as prednisolone or deflazacort to ameliorate DMD and the controversy that surrounds this, it is critical to define the precise effects of these drugs in vivo on all aspects of skeletal muscle necrosis and repair. It is known that they can reduce inflammation, increase myofiber hypertrophy [also demonstrated for clenbuterol (14)], and directly affect myogenesis in vivo. While deflazacort has been reported to decrease inflammation and increase myoblast proliferation, fusion, and myofiber size in regenerating mdx muscles (3), and studies of experimental injury concluded that deflazacort promoted muscle repair (19), prednisolone decreased the inflammatory cell response and had an adverse effect on new muscle formation (7). The recent study by Anderson and her colleagues (4) demonstrates a rapid increase in strength of mdx muscles treated with deflazacort and this was most effective in young mice, supporting the idea that (hypertrophied) myofibers may be "protected" from damage. Additional studies should clarify the effects of these drugs in relation to their long-term use in the clinical situation.

These six papers provide a strong indication of the diverse approaches and progress that is being made with research into MTT.

REFERENCES