Myoblast Transplantation: a Brief Review of the Problems and of Some Solutions

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Abstract

The success of clinical trials of myoblast transplantation has been rather limited. This is due to three problems. The first one is the specific immune response against not only the major histocompatibility complex (MHC) but also against the minor antigens and against dystrophin produced following the fusion of the transplanted myoblasts. These specific immune responses can, however, be controlled by immunosuppressive treatments, the most effective so far being FK506. The second problem is the limited migration of the transplanted myoblasts inside the muscle. This would be due to their entrapment in the extracellular matrix. The migration of myoblasts was increased by pretreatments with bFGF or with Concanavalin A. These treatments may produce their beneficial effects by increasing the secretion of metalloproteinases (matrix degrading enzymes) by the myoblasts. The third problem, recently identified, is an inflammatory reaction which rapidly kills (3-5 days) up to 99% of the injected myoblasts. This reaction is effectively controlled in mice by a mAb against LFA-1, an adhesion molecule located on neutrophils, macrophages, natural killer cells and lymphocytes.

Key words: myoblast transplantation, Duchenne Muscular Dystrophy, immune response, inflammatory reaction, myeloperoxidase, myoblast migration.

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Clinical trials of myoblast transplantation in Duchenne Muscular Dystrophy (DMD) have been conducted by many research teams [29, 36, 45, 54, 56, 63, 65, 80, 81]. These trials have not produced clinically significant strength increases and most research teams have stopped their investigation. My research group has, nevertheless, observed the presence of dystrophin positive fibers and transient strength increases in a few patients following the transplantation of myoblasts obtained from donors perfectly compatible for the Major Histocompatibility Complex (MHC) [36, 80, 81]. The group of Miller reported dystrophin mRNA expression in a few patients [30, 65]. Law et al. [54, 55, 56, 58] have also reported the presence of dystrophin positive fibers and increases of strength in several patients. This group has not identified any problem with their clinical trial and is pursuing treatments in patients. Their clinical trial will therefore be discussed separately at the end of this review. The limited results in our trial were attributed to a specific immune response since antibodies against the donor myoblasts, myotubes and resulting muscle fibers were detected in some patient sera [75]. One of the antigens recognized by some patient sera was dystrophin, since the serum reacted against a 420 kDa protein present in normal muscle but absent from mdx muscle [75].

These limited results obtained in the clinical trials have led several basic research groups to reexamine in experimental models the problems associated with myoblast transplantation. These groups have identified three types of problems which can account for the limited results obtained in the clinical trials: 1) the specific immune response, 2) a limited diffusion of the transplanted myoblasts and finally 3) an inflammatory response responsible for the rapid death (within 3 days) of up to 99% of the transplanted myoblasts. These problems and their potential solutions will now be reviewed in more details.

The First Problem: the Specific Immune Response

The potential role of the immune system in myoblast transplantation has been largely underestimated by most of the research groups which have done myoblast transplantation in DMD patients. A strong immune response following myoblast transplantation in DMD patients was considered unlikely for two reasons [42]. First, it was observed that the MHC molecules which have a major role in the rejection of transplanted organs are not present on mature muscle fibers [2, 44, 72]. However, during inflammatory reaction, during regeneration and in DMD patients, muscle fibers do express MHCs [2, 14, 44] and even more important many research groups have shown even before

clinical trials that myoblasts do express MHC [4, 5, 12, 17, 32, 62, 64, 74]. The second experimental result which led to an underestimation of the potential immune response is the report by Karpati et al. [46] of successful transplantation of human myoblasts in mdx mice without any immunosuppression. Since the publication of that article, the discovery of the phenomenon of reverse mutation [31, 53, 67, 68, 88] has led to a new interpretation of these results. Indeed the dystrophin positive fibers observed in that experiment were most likely reverting fibers. Transplantation of human myoblasts in immunocompetent mdx mice has never been successful in my laboratory. In fact, this is a xenotransplantation (transplantation between different species) and this has so far never been successful with or without immunosuppression even for organ transplantation. Even before the clinical trials, there was evidence of a potential immune response. Indeed, there was evidence of poor survival of MHC-matched muscle transplantation in immuno competent animals [21, 66, 89]. As soon as 1981, Watt et al. [90] had shown that cyclosporine did improve the success of myoblast transplantation. It should also be noted that the very first article of Partridge et al. [70] showing the restoration of dystrophin by myoblast transplantation was done in nude mice which have a deficient immune system.

Because of the idea that MHC matching was not important for myoblast transplantation, most research groups used the father of the patient as the myoblast donor although he was rarely compatible for more than 3 out of 6 MHC class 1 genes. They have usually immunosuppressed their patients with cyclosporine A [29, 56, 63] or with cyclophosphamide [45]. My research group was the only one that screened more than 50 families with a DMD patient to find among the close relatives a donor perfectly compatible for MHC class 1 and class 2 molecules. This is probably why we were able to obtain the expression of dystrophin positive fibers in many of our transplanted patients and transient increases of strength in a few [36, 80, 81]. We also, however, observed in the serum of some of these patients the presence of antibodies reacting with dystrophin [36, 75]. This immune reaction against dystrophin is the best proof that some new dystrophin positive fibers were produced by our myoblast transplantations. Mendell et al [63] have also recently reported the presence of 10.3% dystrophin positive fibers in the myoblast transplanted muscle of one of their transplanted patients. It should be noted that the myoblast donor for this patient was histocompatible for 5 out of 6 MHC class 1 genes. In this case reported by Dr Mendell, it should be also noted that there is absolutely no doubt that the dystrophin observed was really in muscle fibers formed in part by the donor myoblasts rather than in revertant fibers. Indeed Mendell et al. [63] revealed the dystrophin with an antibody directed against a part of the dystrophin molecule coded by an exon which was totally missing from the patient gene. Gussoni et al. [30] also recently confirmed by in situ hybridization (using a probe specific for a dystrophin exon missing in the patient) that a few myoblasts from the donor survived in one of their transplanted patients and formed dystrophin positive fibers.

The importance of the specific immune response in the limited success of human myoblast transplantation was demonstrated by transplanting with great success human myoblasts in immunodeficient SCID mice which have no T and no B lymphocytes. Because they are immuno deficient, these mice are unable to reject transplanted tissues even from different species. When the SCID muscles were irradiated, destroyed with notexin and injected with a human myoblast clone, over 90% of their muscle fibers contained human dystrophin [37, 38, 39], indicating that clones of human myoblasts can form muscle fibers when there is no specific immune response.

Our research team obtained good evidence of cellular rejection following MHC incompatible allo- and xeno-transplantations of myoblasts in immunocompetent mice not immunosuppressed [23, 25]. The myoblast injected muscles were indeed infiltrated by CD4+ and CD8+ T lymphocytes. These infiltrating lymphocytes were activated since they expressed IL-2 receptors and a Th-1 cytokine (INF-γ) mRNA [28]. These lymphocytes also expressed granzyme B mRNA, an enzyme released by activated lymphocytes and natural killer cells to damage their target cells [24]. The rapid rejection of histo-incompatible myoblasts was confirmed by Irintchev et al. [40], Wernig [92] et al., Fan et al. [16] and Pavlath et al. [71]. Moreover, Hohlfeld et al. [33] have shown that *in vitro* myotubes are rapidly damaged by cytotoxic T cells and natural killer cells.

Myoblasts obtained from MHC compatible mice, but from a strain different from the host, were also rejected by immunocompetent hosts [9]. The poor survival of MHC compatible myoblast transplantation had also been noted before by the Partridge group [21, 66, 70, 89]. The rejection due to minor antigens was, however, slower than that observed following MHC incompatible myoblast transplantations. This rejection was attributed to minor antigen differences between the donor and the host. It should be noted that MHC compatible myoblast transplantations were also done by our team without any immunosuppression in DMD patients [36, 81] a situation very similar to these experiments in mice. Therefore, we now think that the incompatibilities for minor antigens were responsible for the rejection that we had observed in our patients.

Myoblasts from normal mice which were perfectly compatible for MHCs and for minor antigens were not rejected by immunocompetent mdx mice and resulted with time in the formation of an increasing percentage of dystrophin positive fibers, reaching up to 80% eight months after the transplantation even without any immunosuppression [87]. Some of these mice did, however, develop antibodies against dystrophin. These antibodies did not, however, trigger an effective rejection of the dystrophin positive muscle fibers. This experiment led us to think that the immune response against dystrophin was probably not

responsible for the rejection in our DMD patients of the muscle fibers of donor origin but that the rejection was rather due to the immune response against the minor antigens. In the mdx mice, the absence of rejection, despite the presence of antibodies against dystrophin, is possibly due to the intracellular location (under the plasma membrane) of dystrophin. Dystrophin would be exposed to the immune system by macrophages and dendritic cells only after damage to the muscle fibers. The absence of active rejection of the dystrophin positive fibers is good news not only for myoblast transplantation but also for gene therapy since the introduction of the dystrophin gene itself should not lead to an active rejection. However, for myoblast transplantation, the rejection can still be due to MHC or minor antigens unless an adequate immunosuppression is used. An alternative approach to avoid immune responses, is to introduce the dystrophin gene in the dystrophic myoblasts before their autologous transplantation. This has recently been done with success (for up to two weeks without any immunosuppression) in mdx mice using an adenovirus vector [18]. However, following the autotransplantation of myoblasts infected with an adenoviral vector as well as following direct gene therapy, there are possible immune reactions against the viral vector itself which remains to be investigated.

Following our demonstration of the immune response after myoblast transplantation, we focused our attention on identifying an adequate immunosuppressive treatment to eventually improve myoblast transplantation in DMD patients. In mice, one immunosuppressor (FK-506) produced very good results for MHC incompatible myoblast transplantation [51]. A lower number of dystrophin positive fibers was obtained following myoblast transplantation in mdx mice immunosuppressed with rapamycin [83, 84]. The lower number of dystrophin positive fibers with this immunosuppressor is possibly due to its interference with bFGF [10], an important factor in the proliferation of myoblasts. With FK506 up to 95% of the muscle fibers in a mouse muscle transplanted with transgenic myoblasts expressed β-galactosidase and dystrophin [51]. We have also recently obtained successful transplantations in monkeys using FK506 immunosuppression of myoblasts labeled with a retrovirus [48, 50]. We have also obtained successful myoblast transplantation using a combination of monoclonal antibodies (mAbs) directed against lymphocyte adhesion molecules (i.e., CD4, CD8 and LFA-1) and using CTLA4-Ig (a molecule which binds to the B7 adhesion molecule) combined with an anti-CD4 [26].

Vilquin et al [86] have shown that cyclophosphamide, one of the immunosuppressive drugs used in one clinical trial [45], killed all the transplanted myoblasts. This is because cyclophosphamide is an antitumoral agent which therefore kills rapidly proliferating cells such as transplanted myoblasts. Cyclophosphamide is used for immunosuppression following bone marrow transplantation because there is often following such transplantations a graft versus host reaction which leads to the proliferation of donor lymphocytes reacting with the host tissue. Our observation that cyclophosphamide kills the rapidly proliferating myoblasts certainly explains the failure of one of the clinical trials.

Our group has also investigated the immunosuppression with cyclosporine A following myoblast transplantation in mice and in monkeys (unpublished results). Although this immunosuppressive treatment has been shown as early as 1980 to improve myoblast transplantation [90], we have never obtained with this drug the level of success that we have observed with FK506 [51]. This perhaps explains the very limited success that was obtained in the clinical trials using that drug.

Myoblast transplantation in immunocompetent animals is definitively very immunogenic. In fact, there is cumulating evidence that myoblasts themselves are professional antigen presenting cells (APC). Indeed following stimulation with INF-γ, a cytokine present during the inflammatory reaction (see third problem), myoblasts not only express MHC classes I and II [32, 34, 74] but also cell adhesion molecules, i.e., ICAM-1 [20, 64], B7-1 and B7-2 [26] which are involved in the activation of lymphocytes. Moreover, Garlepp et al. [19] have recently shown that myoblasts can process and present the same antigens as APCs and do increase the secretion of IL-2 by lymphocytes. This is a definitive proof that myoblasts are APCs, are therefore part of the immune system and play an important role in triggering an immune reaction following the penetration of foreign substances in the muscle. It is therefore not surprising that following transplantation of myoblasts incompatible for major or minor antigens, they are rapidly rejected.

The Second Problem: Limited Diffusion of Transplanted Myoblasts

The low level of success of myoblast transplantation in DMD patients was also due to the limited migration of myoblasts injected in muscles [41, 43, 73]. This could be due to their entrapment within the extracellular matrix. This problem can possibly be overcome by growing the myoblasts in the presence of factors activating the secretion of metalloproteinases, i.e., the enzymes which degrade the extracellular matrix. Such enzymes are produced by macrophages, neutrophils and tumor cells and account for their rapid migration in different tissues. Our group has shown that incubation of the myoblast cultures with a high dose (100 ng/mL) of bFGF, a factor which can trigger the secretion of metalloproteinases [79], increased by 4 folds the success of myoblast transplantation. Indeed, following two series of injections with myoblasts grown with bFGF, up to 50% dystrophin positive fibers were obtained in mouse muscles not previously damaged with notexin and not altered by irradiation [49, 52]. More recent experiments by our group have also shown that addition of Concanavalin A to the culture medium increased by more than three folds the dispersion of myoblasts injected in a muscle [41]. This effect was again attributed to the secretion of

metalloproteinases by the fibroblasts present in the primary culture. More experiments are currently being done to confirm this mechanism.

Therefore, the limited migration of myoblasts injected in a muscle has contributed to the limited success of clinical trials of myoblast transplantation, but this problem may be resolved by increasing the secretion of metalloproteinases either by adding various stimulating components to the culture medium or by introducing a metalloproteinase gene in the myoblasts before their transplantation.

The Third Problem: Acute Death of the Transplanted Myoblasts

During the last two years, we have confirmed a phenomenon initially observed in Drs Karpati, Partridge and Grounds laboratories [7, 16, 35] that a high percentage (95 to 99%) of the myoblasts injected in a mouse muscle die during the first five days following their transplantation in immunocompetent mice. This mortality would be reduced when muscle slices rather than cells are transplanted [15]. Our results indicated that the rapid death of injected myoblasts was also present following transplantation in SCID mice (immunodeficient mice lacking T and B lymphocytes), in mice immunosuppressed with FK506 and following autologous transplantation [22, 28]. Therefore, these results ruled out a mechanism involving a specific immune reaction. However, myoblast death was reduced by roughly 50% when the host was lethally irradiated (over the whole body) three days before myoblast transplantation. This indicated that some host cells were contributing to the myoblast death. (It is important to note that in that experiment, the host was lethally irradiated only three days before myoblast transplantation, a longer delay might had further increased the myoblast survival by reducing even more the host cells participating in the inflammatory reaction). The possible involvement of host cells in the death of the injected myoblasts was indeed sustained by an infiltration of the myoblast injected muscle by macrophages and neutrophils within hours of the transplantation. This suggested us that myoblasts death was due to a non-specific inflammatory reaction. While Dr Karpati suggested that there could be an inflammatory reaction following myoblast transplantation, he did not anticipate that this would kill the injected myoblasts and thought that a low-dose of glucocorticoid would suppress it [42]. So far experiments in our laboratory using anti-inflammatory drugs (methylprednisone, ketoprofen, ibuprofen) have failed to reduce myoblast death. These drugs as well as other anti-inflammatory agents are still under investigation in our laboratory to control the myoblast death. However, myoblast death at three days after transplantation was reduced from 80% in controls to only 20% in animals treated with an anti-LFA-1 antibody [22, 28]. This antibody blocks the interaction between LFA-1 located on many types of leukocytes and ICAM-1 (and possibly other proteins) located on the myoblasts [64]. This prevents among other thing the respiratory burst of neutrophils thus possibly preventing myoblast death. Our laboratory has also recently demonstrated that myoblast death is reduced in hosts deficient in neutrophils or in macrophages [Merly et al, submitted for publication] Prevention of myoblast death in myoblast transplantation in DMD patients would probably greatly improve the success of this intervention.

Complement fixation may be involved in the initiation of the inflammatory reaction. It is interesting that Legoedec et al. [61] have found that the major proteins of the complement alternative pathway C3, factor B and factor H are produced constitutively by skeletal muscle cells. These syntheses *in vitro* were up-regulated by inflammatory cytokines (INF-γ and IL-1). This local complement expression by skeletal muscle *in vivo* may be implicated in the inflammatory process.

The inflammatory reaction following myoblast transplantation may be exacerbated by their production of IL-6. Indeed, Bartoccioni et al [6] found that myoblasts secrete IL-6 constitutively and that this secretion is greatly increased by TNF-a. Thus, the local production of cytokines that characterizes the inflammatory reaction, could stimulate myoblasts to secrete IL-6, which might add to the pro-inflammatory effects of IL-6 produced by activated macrophages and T cells.

The myoblast death produced by the inflammatory reaction can also be partially compensated by an increased proliferation of the surviving myoblasts. This proliferation can be increased by treating the myoblast cultures before their transplantation with various growth factors. Several groups have shown that bFGF increases the proliferation of myoblasts and reduces their fusion [11, 13, 77, 82]. Our group has shown that pre-treating the myoblast culture with bFGF greatly improved the number of hybrid muscle fibers observed after the myoblast transplantation [49] despite the inhibition of fusion produced by this factor [82]. This result may be due in part to the increase proliferating capacity of the surviving myoblasts. Our observation agrees with the observation of Anderson et al. [1] that bFGF is abundant near the regenerating muscle fibers of mdx mice. Austin et al. [3] have also reported that the leukemia inhibitory factor (LIF) increased the proliferation of myoblasts in culture. This factor may therefore have beneficial effects on the transplantation of myoblasts.

Clinical Trial Presently Conducted by Dr Law

Dr Law team was the first one to transplant myoblasts in the muscle of a DMD patient and to have claimed the restoration of dystrophin expression and some strength increases [54, 57, 58, 59, 60]. However, there have been many scientific criticisms for the trial that he is still currently conducting [69, 78].

First, the immune problem is not adequately controlled and investigated. Dr Law obtained myoblasts from biopsies of donors not related to the patients [54, 56, 57]. This means that the donors were most likely completely unmatched with the recipients for the MHCs, a situation which is avoided in organ transplantation. He immunosup-

pressed the recipients with only Cyclosporine A. Although Cyclosporine A is widely used for organ transplantation, it is usually used in combination with other drugs specially immediately after the transplantation to avoid the onset of rejection [8]. In organ transplantation, the patient is usually monitored for signs of rejection to adjust the medication. One possible way of doing so for DMD patients transplanted with myoblasts would be to verify whether the serum of the patient contains antibodies directed against the donor myoblasts [36, 81]. This is a simple non invasive procedure. Law has not reported any investigation of the potential immune responses in the patients that he transplanted. The immunosuppression of the patients with Cyclosporine A is stopped after three months [59]. Again this is not done for any organ transplantation. Law indicated that immunosuppression is not required after that period because the mature fibers formed by the fusion of the donor myoblasts do not express the MHCs [2, 44, 72]. However, unless the patient would be completely cured there will always be some degeneration and regeneration. During the regeneration, the myoblasts and the regenerating muscle fibers will express the MHCs [2] thus most likely triggering a rejection. Indeed, Irintchev et al. [40] have shown that in mice, immunosuppression with Cyclosporine A prevented invasion of T-lymphocytes and allowed differentiation of implanted myoblasts into myofibers as well as down-regulation of MHC expression. However, withdrawal of Cyclosporine A after 4 weeks triggered lymphocyte invasion and cytotoxic cell reactions with rejection of donor tissue. Although the vast majority of muscle fibers were MHC-negative 1-4 days after Cyclosporine A withdrawal, single small desmin-positive profiles were weakly positive for donor MHC. Parallel with the increase in the number of lymphocytes, larger numbers of small and large muscle fibers expressed high levels of either donor, host or both, class I - but not Class II - molecules. Surprisingly, immune reactions continued over several months, causing gradual loss of muscle tissue. Donor class I molecules persisted for more than 6 months after Cyclosporine A withdrawal, clearly indicating survival of donor muscle fibers despite ongoing rejection. It should, however, be pointed out that the Irintchev et al. [40] experiments were done with a cell clone that continued to proliferate and therefore to express the donor MHC. Pavlath et al. [71] reported the survival of hybrid muscle fibers formed by the transplantation of β-galactosidase labeled primary myoblasts even 4 months after the withdrawal of Cyclosporine A without any signs of an active rejection. This successful development of tolerance occurred only when the hosts were BALB/c or C3H mice, no tolerance was obtained with C57BL10 mice. This indicates that development of tolerance is a very complex process which differs from one mouse strain to another. It would therefore be surprising that development of tolerance could occur for all the DMD patients in Law trial whatever the donor and host MHC. Moreover, the experiments of Pavlath et al. [71] were done in non-dystrophic hosts and therefore the absence of muscle regeneration could account for the absence of continuous myoblast proliferation. The presence of proliferating myoblasts expressing the MHC may be required to trigger the rejection. In DMD patients, as in the experiments of Law, a complete absence of dystrophin negative fibers would be required to prevent any muscle regeneration. The possibility of a rejection following the interruption of the immunosuppressive treatment is an important point because Wernig et al. [91, 92] have shown that the rejection of the donor cells occurring after the interruption of a 4 or 8 week Cyclosporine A immunosuppressive treatment also damaged the hybrid muscle fibers formed by the fusion of donor myoblasts with the host fibers and resulted in a net reduction of the muscle strength. If such a rejection occurs in the DMD patients following the interruption of the Cyclosporine A treatment this could accelerate the development of their muscle weakness.

As evidence for the success of his myoblast transplantations, Dr Law has recently published pictures of cross-sections of one muscle of one patient containing dystrophin positive fibres six years after myoblast transplantation [60]. The number of dystrophin positive fibres in these pictures is certainly impressive. However, although this was considered at the beginning of the clinical trials of myoblast transplantation as a good evidence of a successful transplantation, this is no longer considered as a sufficient proof. Indeed, the revertant dystrophin positive fibers are more resistant than dystrophin negative fibers and their percentage may therefore progressively increase with the aging of the patient. Law could really demonstrate that dystrophin positive fibers are formed by donor myoblasts, by demonstrating that the donor dystrophin is present. This could be done by demonstrating the presence of an exon which is deleted in the patient dystrophin gene. This can be done by PCR amplification but an easier method is to use antibodies directed against segments of dystrophin coded by missing exons. This is the method that has been used with success by Mendell et al. [63] for one patient. Law has transplanted myoblasts so far to more than 130 DMD patients [59], several of these patients should have missing exons which if restored should produce a dystrophin segment detectable by a specific antibody. This is a very simple test which could demonstrate whether the myoblast transplantations done by that team are really successful. Law has not responded positively to an offer by 14 scientists to do these tests on a few of the patients that he has transplanted [69].

Other evidence presented by Law to claim some success is that the strength of the patient increased with time [56]. The most informative tables in that article are tables 2A, B and C. These tables show that an increase of strength of only 28% is obtained only in the ankle flexors (table 2A). There is a mean reduction of strength of 6% in the knee flexors 9 months after myoblast transfer (table 2C). One has to be careful that many of the other figures in that article are presenting the percentage of muscles which have an increase or a reduction of strength rather then the percent-

age of strength changes. In another article , Law et al. [57] calculated an average increase of strength of 41.3% but this was by including in the calculation of this average only the muscles that did show an increase of strength, i.e., those having a reduction of strength were included in another group to calculate an average reduction. Again, one has to be very careful, the strength may increase simply because of a placebo effect, i.e., only because the patients know that they have been transplanted. The patients are also followed by physiotherapists and make more exercise than before the transplantation, this could also contribute to a transient improvement of their strength. Both possibilities can not be neglected especially when the increases are only 30% [56]. Moreover, the presentation of the patient strength is also not normalized by taking into account the weight of the patient, a normalization which is necessary to clearly demonstrate the reduction of strength of DMD patients with age. This normalization is done in figure 3 of Law et al. [56] before myoblast transplantation. This normalization is not done for the tables of results after transplantation. Some of the transplanted patients are young and as they get older, their absolute strength increases simply because their muscles are growing. Another possible explanation for the strength increases observed by Law is that it is due to the immunosuppressive drug cyclosporine. Indeed the Dr Miller group has observed such an effect before [76] and has recently confirmed it in a new group of patients [65]. They reported that after 4 weeks of cyclosporine the strength increase in both the myoblast injected muscle and in the control muscle (ranging from 5 to 116%). A strength increase following the administration cyclosporine was, however, not observed by the group of Mendell [63].

Clearly the criticisms that I have made about the presence of dystrophin and increases of strength also apply to the clinical trial that has been conducted by my own team. This is why I now think that the best proof that myoblast transplantation had formed some dystrophin positive muscle fibers or at least some myotubes, is that some patients in our study made antibodies against dystrophin.

Dr Law has also not taken into account the second problem that I have mentioned, i.e., the limited diffusion of the injected myoblasts. He is indeed currently injecting myoblasts only at eight sites in very large muscles [59]. Most researchers agree that injected normal myoblasts do not move more than a few mm between the muscle fibers [43]. In 1996 during the Third International Congress of the Cell Transplant Society, Dr Law answered to this criticism that it is not the myoblasts which are moving but that it is the dystrophin mRNA which diffuses inside the muscle fibers. Our recent experimental results clearly indicated that the dystrophin mRNA and thus the dystrophin itself does not diffuse at more than 500 μm from the nucleus producing them [47]. Therefore, it is unlikely that only a few intramuscular injections will produce dystrophin throughout a muscle.

The third problem is the inflammatory reaction. Dr Law indicated that he is avoiding this problem by doing only a few intramuscular injections (i.e., 200 injections in 28 muscles) [59]. Although this indeed avoids a generalized inflammatory reaction throughout the muscle, this does not prevent an inflammatory reaction near the sites of myoblast injections. Our experiments indicate that the percentage of myoblast death is the same (around 76-80% at 3 days) whatever the number of injection sites. To try to improve the success of myoblast transplantation, Dr Law has progressively increased the number of transplanted myoblasts from 5 to 25 billions [57, 59, 62]. This massive effort of cell production is not helpful if 99% of the myoblasts are dead 5 days after their transplantation as indicated by Beauchamp et al. [7], Huard et al. [35] Fan et al. [16], and Guerette et al. [22, 28].

Conclusion

Although the initial trials of myoblast transplantation have produced only very limited results, continuous basic research on this procedure will improve the procedure. Current research in the development of tolerance, in the introduction of the dystrophin gene in the patient's own myoblasts and on transformation of fibroblasts into myoblasts may in the future reduce or avoid the immune problems. New growth factors added to the culture medium may further improve the proliferation and the mobility of the myoblasts in the host muscles. The inflammatory reaction will probably be eventually controlled by treatments with monoclonal antibodies against cell adhesion molecules and pro-inflammatory cytokines or by anti-inflammatory drugs. Myoblast transplantation has a great advantage over gene therapy, it remains the only treatment which could eventually not only introduce the dystrophin gene into the already existing muscle fibers but also increase their size and perhaps the number, thus increasing the strength of the DMD patients. The persistence of some of the donor myoblasts as satellite cells will also prevent the development of weakness.

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