REVIEW ARTICLE

Cardiovascular Regenerative Medicine at the Crossroads. Clinical Trials of Cellular Therapy Must Now Be Based on Reliable Experimental Data From Animals With Characteristics Similar to Human's

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It is now over 4 years since early reports of murine models raised high expectations that bone marrow cell transplantation to the postischemic myocardium could produce physiologically significant myocardial regeneration. In quick succession, a flurry of publications documented the capacity of a variety of other types of adult cell to produce similar results.

These publications were all controversial from the start because none addressed the mechanisms involved in the differentiation of transplanted cells. In addition, each report raised at least as many questions as it answered. Despite these obvious weaknesses, the first phase-I clinical trials were started immediately without any further animal experimentation. Today the results of more than a dozen trials are already in the public domain but we still do not have a single piece of solid data documenting whether any of the approaches used is capable of regenerating contractile cells in the human myocardium. This is one of the main reasons why the controversy over the effectiveness of this therapeutic approach is becoming increasingly heated. Moreover, skepticism about the efficacy, and even the feasibility, of inducing clinically relevant myocardial regeneration has increased to the point where it threatens the future of this nascent field. The present situation in myocardial generation contrasts sharply with that in neural regeneration. Although there is a solid and extensive body of knowledge on the origin, phenotype, and regulatory mechanisms of neural stem cells, the first clinical trials have only recently been started.

To move this field forward it is necessary to distinguish between the procedures needed to establish "proof-of-concept" and those that have the potential for widespread clinical application. In addition, the technique must be implemented in such a way that it continues to add to existing knowledge. It is our belief that, if the necessary information is to be acquired, we need: *a)* significantly more extensive experimental data from animals whose anatomical and physiological characteristics are similar to human's, including data on, for example, dose-effect relationships, the best form of administration, and the duration of therapeutic responses; and *b)* better understanding of the molecular mechanisms that determine whether cardiac stem cells and transplanted cells will either remain as stem cells or differentiate.

In summary, if we are to progress systematically in this area, we need better understanding of myocardial biology. Without it, we run the risk of holding back the field for decades, as happened with the first human heart transplants and with trials of gene therapy.

Key words: *Cellular therapy. Regenerative medicine. Stem cells. Cell transplantation. Myocardial regeneration.*

Medicina regenerativa cardiovascular en la encrucijada. Es urgente basar los ensayos clínicos sobre terapia celular en datos sólidos obtenidos en animales experimentales relevantes para los humanos

Hace ya 4 años que las primeras publicaciones de trabajos realizados en roedores crearon grandes expectativas sobre el potencial del trasplante de células de la medula ósea para producir una regeneración del miocardio con relevancia fisiológica. Rápidamente, en algunas publicaciones adicionales se documentó la capacidad de otras células del adulto para producir efectos semejantes.

Todas estas publicaciones fueron controvertidas desde el principio porque ninguna de ellas aclaraba los mecanismos de la diferenciación de las células trasplantadas. Es más, cada uno de estos trabajos dejaba al menos tantas preguntas abiertas como las que contestaba. A pesar de estas deficiencias, los primeros ensayos clínicos de

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ABBREVIATIONS

CSC: cardiac stem cells.
hESC: human embryonic stem cells.
MI: myocardial infarction.
CNS: central nervous system.

fase I se empezaron inmediatamente sin ninguna experimentación animal adicional. En la actualidad se han publicado los resultados de más de una docena de ensayos clínicos y todavía no hay una sola evidencia convincente que documente si los protocolos utilizados pueden regenerar células miocárdicas contráctiles en el miocardio humano. Ésta es una de las principales razones por las que la controversia sobre la efectividad de este tratamiento es cada día más vitriólica. El escepticismo acerca de la efectividad e incluso del potencial de la regeneración miocárdica clínica ha llegado a un nivel que amenaza el futuro de este campo en su infancia. La situación de la regeneración miocárdica contrasta claramente con la del campo de regeneración neuronal. A pesar de la extensa y sólida documentación sobre el origen, el fenotipo y los mecanismos reguladores de las células madre neurales, los primeros ensayos clínicos apenas se han iniciado recientemente.

Para progresar en este campo es necesario distinguir entre los procedimientos necesarios para establecer una «prueba de concepto» y los que tienen el potencial de una amplia aplicación clínica. Además, el método de implementación debe permitir una acumulación progresiva de conocimientos. Según nuestra opinión, para obtener la información requerida necesitamos: *a)* mucha más información procedente de animales cuya anatomía y fisiología sean relevantes para los humanos, incluidas la relación dosis/efecto, el modo óptimo de administración, la duración del efecto, etc., y *b)* un mejor conocimiento de los mecanismos moleculares que permiten mantener la «troncalidad frente a la diferenciación de las células madre cardiacas y/o de las células trasplantadas».

En conclusión, necesitamos entender mejor la biología del miocardio para avanzar en este campo de forma sistemática. De otra forma corremos el peligro de retrasar este campo durante décadas, como ocurrió con los primeros trasplantes de corazón y los ensayos de tratamiento genético.

Palabras clave: *Terapia celular. Medicina regenerativa. Células madre. Trasplante celular. Regeneración miocárdica*

INTRODUCTION

Despite the huge progress achieved during the last 50 years in treating many diseases, including cardiovascular ones, the fact is that in many cases the treatments available are only palliative. However, since these treatments are effective in resolving acute processes that were fatal in the past, they often extend the patient's life at the expense of leaving chronic disease as sequelae. These chronic sequelae, particularly those of the cardiovascular system, often lack effective treatment and the only viable choice to restore cardiac function compatible with an acceptable quality of life is cardiac transplantation. Unfortunately, this choice involves major drawbacks, from the logistic, economic, psychological and biological points of view, which reduce its usefulness and availability.

With mean age continuously increasing and the progressive aging of the population in developed countries, we are experiencing an increasingly large chronic disease epidemic whose treatment absorbs a growing fraction of human resources and health budgets. In many cases, this huge investment in health resources has yielded very discouraging results when its effect on the duration and quality of life is measured. Despite this, demands for advanced medical care continue to increase and threaten all national healthcare systems, whose choices range from imposing cutbacks on healthcare services by using different subterfuges to bankrupting public healthcare services. Currently, in the United States alone, there are more than 5 million post-myocardial infarction (MI) patients with chronic heart failure. Each year more than half a million new patients join this group, which has an annual mortality rate of approximately 18%, requiring around 30 000 million dollars for their treatment.[1] The general problem in this group of patients is a lack of functional contractile myocardial cells and an adequate coronary circulation to feed them. One of the major challenges of cardiovascular research during the last decade has been to find a way to make it possible to replace cells lost to infarction and thus prevent or reverse the process of pathological cardiac remodelling that causes heart failure in these patients.[2] Unfortunately, until recently, all attempts at cellular transplantation in the myocardium were condemned to failure a priori since the existence of cells with the suitable properties was unknown.

Given the situation summarized here, which is not significantly different from other areas of medicine, such as degenerative disease of the central nervous system (CNS), diabetes, etc., it is not surprising that the isolation of human embryonic stem cells (hESC) in 1998,[3,4] with their capacity for unlimited growth and potential to form all or most body cells, was received with enthusiasm by the medical-scientific community and society in general. Both the general and scientific press aroused widespread public interest by heralding in a new paradise offering an unending source of cellular, tissular and organ reimplantation to treat all manner of congenital and degenerative disease which would be available to everyone. Furthermore, they promised us that this

ongoing revolution would not produce the disasters described by A. Huxley in his book 'Brave New World', but would make it possible to leave behind the world of palliative medicine in which we live today and enter the new era of "regenerative medicine." This optimistic and global vision gave the impression that we were only a few steps away from reaching the "spring of eternal youth."

EMBRYONIC AND ADULT STEM CELLS: THE HARMFUL EFFECTS OF POLITICS WHEN INTERFERING WITH SCIENCE

Due to the fact that, up to the present, the development of hESC required the destruction of human embryos, a group totally opposed to their production and use rapidly arose, led by the Catholic church, conservative Protestant groups and several national governments headed by the United States. This controversy, still current in many countries, has created severe obstacles and limits regarding the production of and experimentation with hESC. As few things are more alluring than something that has earned the label "forbidden fruit," the barriers to using hESC have served to increase their attraction and the subjective medical potential of these cells. It is a generally accepted idea that the political-religious forces opposed to the generation and use of hESC are delaying the development of treatments for diseases as varied as Alzheimer's disease, diabetes, severed dorsal medulla, etc.

Although there is no doubt that, throughout history, the politicizing of science has had terrible consequences for science, and the case of hESC is no different, it is also clear that there is much excitement, exaggeration, frustration, and lack of balanced views and critical analysis in both camps, including in groups from the biomedical community. This deeply disturbing situation, one in which no quarter is given between the two camps, is out of proportion to the matters at stake. In order to restore balance and common sense to the discussion it would be useful for the supporters to recall that mouse embryonic stem cells (ESC) have been available since 1981[5,6] – more than a decade before the isolation of hESC– and at no time has their production or use been impeded. Despite this, and the fact that experiments on mice are much simpler than on humans and do not involve the immunological barriers affecting hESC, until now not a single chronic disease in mouse has been cured by ESC transplantation. On the other hand, those against using hESC should recall the disastrous predictions made about using DNA, contraception and in vitro fertilization, all of which turned out to be wrong. Furthermore, there is tangible proof that cellular therapy with adult stem cells has been one of the most brilliant cases of 20th-century medicine, as demonstrated by bone-marrow transplantation, among others.

The use of adult stem cells does not arouse the philosophical, political and religious fights triggered by hESC and so their use remains uncontroversial. Thus, the public, biomedical and political communities have mainly focused on hESC, practically ignoring the characteristics of and potential for adult stem cells. There is a great gap between the short- and medium-term potential of these two classes of stem cells and their level of visibility in the social, political and scientific arenas. This does not mean that both human and experimental animal ESC lack extraordinary value and potential. It is clear that these cells are irreplaceable for studying the regulatory genetic mechanisms of normal and pathological human development, as well as how the environment modifies these mechanisms. Embryonic stem cells provide a unique way to study the mechanism of action and side effects of certain drugs. However, their potential as therapeutic agents, if they have any, belongs to a far distant future. On the other hand, the therapeutic use of adult stem cells is a daily event in most medical centers and their clinical relevance will strongly increase in coming years.

The conflict of interest between many scientific, biomedical, political and religious pressure groups, who have very narrow agendas on both sides of the argument, stand out among the causes of the massive gap between the previously expressed point of view, on the one hand, and the consensus prevailing in the scientific literature and general press, on the other. This situation is made more critical due to the fierce competition for limited research support and the attention of the mass media. In addition, the discussion being conducted on the relative merits of embryonic and adult cells is based on outdated arguments that have remained static for the last 10 years and which have helped to increase the confusion and disinformation among the general public.[7]

The various pressure groups have co-opted this newly-formed discipline and turned it into a substitute for broader problems within the socio-ideological battles. The animistic stance of the extreme religious right, its opposition to abortion and even to in vitro fertilization has provided an excellent excuse to attack the use of human embryos in producing hESC lines, including abandoned embryos, frozen for years and destined to be destroyed. In order to avoid being labeled antiscientific and reactionary, these groups have become the most active proponents of adult stem cell use "instead of" hESC as a basis for advances in regenerative medicine and have organized strong pressure groups within the federal government of the United States and other countries. Due to the scorn and antagonism the scientific community has shown

toward the most strident antiabortionist groups, the latter's enthusiastic support for research into and use of adult stem cells has been the "kiss of the death" for this research field. A large proportion of the scientific community, part of the liberal section of society, and most top medical and scientific publications have responded with a knee-jerk reaction, but in the opposite direction, to the conservative pressure to totally prohibit hESC research and promote research with adult stem cells.

Due to specific political realities in the United States, the "progressive" scientific community is now worried about the possibility that advances in adult stem cell biology might be used by the conservative groups to further restrict hESC research, and eventually lead to it being completely banned. To avoid this possibility, many of the most prestigious and well-known researchers in this field, together with several of the most influential biomedical publications, have adopted a loose, but undeclared, policy of decreasing the visibility of adult stem cell research while giving maximum visibility to results with hESC.[8] This unfortunate and short-sighted attitude on the part of a large sector of the intelligentsia has further distorted research and public debate, created unrealistic expectations regarding the potential of hESC and affected the distribution of public funds for research. Whereas in the United States practically every state has responded to the blockage of federal funds for hESC research by creating a public research program with these cells, adult stem cell research languishes in its shadow.[9] Now, other nations throughout the world are blindly imitating the distorted scientific policy of the United States regarding the use of stem cells in the development of medical knowledge.[10]

The state of public discussion on stem cells contrasts profoundly with the biological and medical situation. As mentioned, in the heat of the discussion it is easy to forget that adult stem cell therapy has been one of the major medical successes in the second half of the 20th century. Since the first bone-marrow transplantation done by Thomas in 1956 to treat a terminal leukemia patient,[11] a huge number of patients have benefited from this therapy.[12] Bone-marrow transplantation, both autologous and heterologous, are now standard procedures in many hospitals.[12] In the 1980s, autologous skin grafts cultivated in vitro based on keratinocytes (skin stem cells) taken from the patient came into clinical use.[13] Regardless of their direct impact on the patients, these procedures were facilitated by the concurrent revolution that took place in the field of immunology (which in turn facilitated it) and that has had a strong impact on most medical specialties.

Until recently, the most serious factor holding back these advances in the use of adult stem cells, both in research and in the clinical field, was a lack of general knowledge regarding the mechanisms of cellular homeostasis in most adult tissues and organs. Up to the middle of the 1990s, the prevailing viewpoint in medicine and biology was that although tissues such as bone marrow, skin and intestinal epithelium demonstrated a strong capacity for self-renewal, this capacity was an exception restricted to a small group of tissues. The reigning paradigm was that most adult tissues and organs were either renewed very slowly (such as the endothelial wall of the vascular system) or did not have any self-renewal capacity (such as the heart and the CNS). It was practically an article of faith that from the postnatal period onward most tissues did not contain functional cells able to promote self-renewal in the parenchymal tissue (stem cells). An unavoidable outcome of this paradigm was acceptance of the idea that there was a continuous and progressive reduction in the number and function of parenchymal cells in most adult organs from infancy until death. The logical consequence of this viewpoint was that, with the exception of the three previously mentioned tissues with a clear capacity for self-renewal, any therapeutic intervention aimed at treating the loss of functional parenchymal cells in any other organ necessarily had to be palliative and aimed at preserving and/or improving the functioning of the organ's surviving cells. Returning the organ/tissue to the previous status quo had required the transplantation of identical cells donated by another individual or of cells able to differentiate into the cell type needed to make up the loss. Since the second option was unfeasible because it was thought that these types of cells did not exist for most tissues, thus, organ and heterologous cell transplantation was accepted as the only practical solution available. Despite the numerous problems and disadvantages of heterologous cell and organ transplantation, in fact its practice has become the intervention "par excellence" in several medical specialties. Despite the apparent success of this type of intervention, the lack of donors, its high cost and the side effects of immunosuppression have limited this therapy to a very small fraction of the patients requiring treatment. Given this scenario, there was great, and unsurprising, enthusiasm regarding the discovery of multipotent hESC[4,5,14-16] with the ability to differentiate into most, if not all, cellular types in the organism, because it opened up the obvious possibility of an inexhaustible source of cells and replacement organs.

ADULT STEM CELLS REASSESSED

In an almost unnoticed way, since it has not attracted the attention of the mass media and has not been an actor in the cultural battles of the last 15 years, the paradigm of cellular homeostasis in adult tissues has been under ongoing reassessment. Incontrovertible evidence has gradually accumulated that parenchymal cells in the vast majority of tissues, if not all, are in a

continuous process of self-renewal, with cells constantly dying at the same time as others are being born. Once this concept of ongoing cellular self-renewal in the adult was understood and accepted as a general feature of and central to homeostasis in organs and the body, it was a natural step to consider that there had to be a specific group of cells capable of regenerating each organ's parenchymal cells in order to preserve the cellular mass. Obviously, the adoption of this concept was rapidly followed by the discovery of stem cells in all adult organs/tissues.[17-29]

At the beginning of the 1990s, the only two organs remaining without winning a prize in the adult stem cell lottery were the heart and CNS, which became emblematic of tissues made up of postmitotic cells lacking any capacity for self-renewal.

In 1988, the formation of new neurons in adult bird brain was described,[30] then in mouse in 1992.[31,32] Neurogenesis in adult human hippocampus was reported in 1998.[33] Thus, during this decade, the heart remained isolated from the rest of the body as the only organ without the capacity for cellular self-renewal. Surprisingly, the heart's peculiar situation in relation to homeostasis in the rest of the body was accepted without argument by the cardiovascular research community, which continued to accept the current paradigm and support the unique status of cardiac cell biology. That is, it continued to treat to the heart as a postmitotic organ with no intrinsic capacity for renewal, where neither the death of myocytes nor their new formation played any role in cardiac cell homeostasis and, thus, both events could be completely ignored. The basis for this concept being so ingrained in the world of cardiology has mainly rested on two ideas: 1) that all cardiomyocytes are formed during fetal development or early postnatal life and are terminally differentiated in the adult, lacking any capacity to reenter the cell cycle; thus, all cardiomyocytes have the same chronological age as the body[34]; and 2) that adult heart does not have any intrinsic capacity to regenerate its parenchymal cells because it lacks a population of stem cells capable of producing new myocytes. Although it seems incredible, these two concepts, that had proved to be incorrect for the remaining organs, including the CNS, continued to be the basis for all therapies in use for treating ischemic heart disease and heart failure, and for all clinical trials of cellular therapy already completed or in progress. Fortunately, the role of the heart as the only organ untouched by stem cells in the evolutionary lottery did not persist for long and our first finding of stem cells with regenerative capacity in adult heart published in 2003[35] was rapidly confirmed by several independent groups.[36-41]

Given a neutral social and scientific setting, it would have been reasonable to hope that the identification of the capacity for self-renewal in all adult tissues/organs, together with the notion that cellular homeostasis requires an ongoing process of cellular renewal throughout the organism's lifetime, would have been sufficient to reassess the prospects of embryonic and adult stem cells. Such a reassessment, had it occurred, would have promoted parallel, but very closely connected, research with the two types of stem cells. In this way, discoveries concerning one cell type would have reciprocally advanced research into the other. Unfortunately, the discipline has not developed in this manner. In fact, research has generally mimicked the tone of public debate and, thus, a certain antagonism has arisen between some researchers from both fields. In addition, the discussion in the public arena and political circles continues to be based on the same arguments used 15 years ago, when it was thought that the regenerative potential for embryonic stem cells was not only unlimited, but would also be easy to implement clinically. On the other hand, the potential of adult stem cells did not exist for many tissues (since they did not have them) or was considered a biological curiosity and not an element essential to the organ's physiological homeostasis. It is clear that these arguments are not only outmoded, but do not reflect any biological or medical reality.[42]

The current situation has been and continues to be detrimental to the nascent field of regenerative medicine and it is vital that this should change as soon as possible. In order to emerge from this negative and self-destructive situation it is essential to eliminate politics from the scientific discussion and return to discussing science on its own merits and not because it fits or does not fit with certain philosophies. For this to occur, the level and quality of the discussion must be seriously raised and, at the same time, we need to increase our understanding of the biology of the two types of stem cells as rapidly as possible. Once we have more information available, this would be the time to assess the relative merit of the two cell types regarding specific medical targets, since it is likely that each cell type has a different optimal use.

A NEW MODEL OF CARDIAC CELLULAR HOMEOSTASIS

It is an incontrovertible fact that, in contrast to assertions based on poorly interpreted data[43] and clearly incorrect data,[44] adult cardiomyocytes are postmitotic terminally differentiated cells.[45,46] Despite this, it is also beyond doubt that the heart has a powerful capacity for regeneration, both in normal conditions and in reaction to various physiological and/or pathological stimuli.[47,48] The basis for this regenerative capacity lies in a small number of cells distributed through the atria and ventricles of the adult heart, including the human heart, that have the phenotype, behavior and regenerative potential of bona fide cardiac stem cells (CSC).[35] These cells are clonogenic, self-renewing and multipotent,

since they give rise to myocytes, endothelium and vascular smooth muscle, and have a practically unlimited capacity for expansion.[35,38] When injected into the borders of an infarction, the progeny of a single CSC is capable of regenerating the myocardium lost to massive infarction.[35] On average, the myocardium contains one CSC for every $1x10^3$ myocytes, a similar concentration to that of hematopoietic stem cells in bone marrow.[49] In normal adult myocardium, most CSC are inactive and outside the cell cycle, with only 2%-3% in the process of replication and differentiation to replace the myocytes and vascular cells lost during normal myocardial wear. However, in response to physiological or pathological stress (hypoxia, exercise, overload, cellular damage, etc.), most CSC (>95%) rapidly activate (Ellison et al., personal communication), multiply and differentiate into myocytes and vascular cells.[47,48,50] Such CSC activation can efficiently repair extensive diffuse myocardial damage and microlesions (Ellison et al., personal communication), but not severe segment loss as occurs in MI.

The identification of CSC has led to correctly interpreting the existence of a myocyte population in the process of cellular replication in adult myocardium[43,44] that until recently was misinterpreted.[43,44] At the same time, these cells made it possible to demonstrate that the death of myocytes and their new formation are the two sides of the coin of cardiac homeostasis where CSC play an essential and irreplaceable role.[50] The myocytes in the process of replication found in adult myocardium, and which are more abundant in a stressed heart,[47,48] are new myocytes created via CSC differentiation. It is now clear that the ongoing regeneration of myocytes and vascular cells throughout an individual's life is required for both cardiac homeostasis and to maintain its response capacity to different physiological and pathological stimuli.

A frequently raised objection by those skeptical of this new view of the myocardium concerns the fact that if the myocardium contains cells with regenerative capacity then it does not make sense that an MI evolves toward scarring instead of regenerating new contractile myocardium. What is forgotten is that blockage in a main artery of any organ, regardless of its quantity of stem cells (eg, skin, bone marrow, intestine, etc.), generally evolves toward scarring and not towards regeneration. It is very likely that this behavior is due to the fact that during the evolution of relatively long-lived organisms, adult stem cells were necessarily selected not to regenerate acute catastrophic segmentary lesions, but to repair minor lesions and regenerate the cells lost to normal wastage over time. If this reparative process had not existed then maintaining the function of most organs over the normal life-span would have proved impossible.

In addition to rejecting the unique biological status conferred on the heart and placing it in the same context as other organs with regenerative capacity, the viewpoint expressed here offers new options for treating processes secondary to the loss of contractile myocardial mass. This is the case because as long as the myocardium was regarded as a tissue without regenerative capacity, the only clinical choice for treating myocyte loss was to maintain or improve the function of the surviving myocytes or to replace the lost mass via cardiac transplantation. By identifying the regenerative capacity of the myocardium via CSC, which can be isolated and amplified in vitro[35] or stimulated in vivo,[51,52] it became reasonable to investigate methods that would enable us to exploit this potential to induce myocardial regeneration with autologous cells without the need for cellular transplantation.

MYOCARDIAL REGENERATION THROUGH CELLULAR TRANSPLANTION: A LONG-LASTING CONTROVERSY THAT COULD AND SHOULD HAVE BEEN AVOIDED

By the mid-1990s it was already clear that post-MI chronic heart failure was reaching epidemic proportions and becoming a serious public healthcare problem that could not be solved via cardiac transplantation, due to the shortage of donors, its prohibitive costs, and serious side-effects.[53] Since the myocardium was viewed as a tissue without any regenerative potential, the search for cells with the capacity to differentiate into or function as myocytes speeded up. Thus, different cell types, including skeletal myoblasts,[54-57] fetal cardiomyocytes[58,59] and ESC-derived myocytes[60] were transplanted into the myocardium of experimental animals. Shortly after, and despite the poor results obtained in animals, clinical trials began with skeletal myoblasts.[61] During this period of zeal in the search to replace myocytes lost to MI, several groups reported that bone marrow-derived cells, and more specifically, hematopoietic cells, were multipotent and thus capable of differentiating into a great variety of non-blood-cell types.[62-66] At the same time, and before some of these findings were challenged,[67-69] we had already demonstrated that there were human cells with myogenetic potential residing in the heart via the circulation and which differentiated into myocytes and vascular cells.[70] Based on these data, and together with the Anversa group, we decided to test whether bone-marrow cells enriched in hematopoietic cells were able to regenerate post-MI myocardium in mice. Surprisingly, the results were positive. The transplanted cells not only restored the number of myocytes lost to the infarction, but they improved ventricular function.[71,72] These results, although preliminary, aroused great interest and expectations regarding the potential of transplanting or mobilizing bone-marrow cells to the infarcted area to regenerate post-ischemic myocardium.[73-80]

Despite the interest aroused by these publications, the undeniable fact is that all these studies[71-80] were

both preliminary and incomplete and did not contain the information necessary to justify beginning clinical trials. To start with, none of these works specifically identified the cell type responsible for the myocardial regeneration. Thus, when other researchers were unable to replicate the results,[81-84] it was impossible to determine whether the cause of the discrepancy was technical or biological. Furthermore, none of these works established a dose-effect relationship, guidelines or methods for optimal administration or the long-term effect and fate of the transplanted cells. In addition, none of these publications investigated the mechanism responsible for the transplanted cells differentiating into myocardium.

The lack of solid information on the identity of the regenerative cells and on the biological process itself was compounded by a series of important practical questions that needed to be answered to be able to plan a stringent clinical trial that would not unnecessarily endanger the patients. Among the unknowns pending solution was determining whether the methods that seemed effective in regenerating mouse myocardium were directly applicable to organisms thousands of times larger, like humans, regardless of whether the biological process involved was similar or identical. Mouse ventricular myocardium weighs around 70 mg and is approximately 1 mm thick. Around 20 mg of myocardium forming a thin muscular layer is required to regenerate an infarction that has destroyed 30% of the ventricular mass. The left ventricle in most MI patients weighs around 500 g and is >1 cm thick. The formation of about 150 g of thick muscle wall with multiple arteries, arterioles and capillaries is required to regenerate 30% of the ventricle. This task is 7000 times bigger and qualitatively more complex than regeneration in mouse. Thus, the many unknowns which the mouse experiments had left unresolved foresaw a long period of experimental work before cellular therapy with bone-marrow cells, either through transplantation or mobilization with cytokines, was in a position where clinical trials could begin.

Contrary to this prediction, and despite the shortcomings regarding the animal data, the first clinical trial of myocardial transplantation of bone marrow-derived cells began immediately after the first data in mouse was published[85,86] without any additional animal experiments having been done. It is striking that the results of this trial were accepted for publication the day after their submission and were published less than 6 months after the results in mouse appeared.[85] There is no doubt that this was and continues to be the fastest transfer of an experimental protocol in mouse to human in the history of modern medicine. The results of this haste should serve as a warning concerning the danger inherent to beginning experimental protocols in humans before obtaining the preclinical information necessary to be able to plan a stringent clinical trial. But if this sequence of events had not already been sufficiently unfortunate, the report from the Strauer group[85] was interpreted by many cardiologists as the starting signal for a flurry of clinical trials where various protocols were used to transplant different types and mixtures of cells into human myocardium without any form of experimental validation in animals. Thus, the already highly questionable clinical trials of skeletal myoblast transplantation started previously by Menasché[61] without being validated by animal experimentation were followed by many groups which transplanted bone-marrow cells. These trials captured public attention and, due to the optimism of the researchers, created unrealistic expectations in potential candidates and the general public. This lack of caution on the road to clinical implementation brings to mind the wisdom of Clemenceau, the French Prime Minister during WWI, who said "war is too important to leave in the hands of generals." Perhaps it is time to raise the issue of whether the decision to begin clinical trials is too important to leave in the hands of the clinicians who want to conduct them.

Currently, the results from more than a dozen phase I clinical trials aimed at post-MI myocardial regeneration or treating heart failure via autologous bone-marrow cell transplantation have already been published,[85-106] and dozens more are in progress world-wide. As may have been expected, given the foregoing, the available results are inconsistent, confusing, controversial, and unconvincing, even when viewed in a positive light. Despite the great differences between the protocols used, the only common finding among the different groups is that bone-marrow cell transplantation in post-infarction and chronic heart failure is feasible and safe, at least in the medium-term, in the hands of interventionist cardiologists and experienced surgeons.[85-107] Unfortunately, since each group has used and continues to use different criteria for patient selection, cell preparation and selection, post-infarction method and transplantation time, criteria and parameters used to assess outcomes, etc, it is impossible to compare results between the different groups. A worrying fact is that, although most groups have detected improved ejection fraction in treated patients compared to the placebo group (when there is one), the differences in ejection fraction evolution in the different placebo groups is greater than the improvement detected in the treated groups. In this sense, it is worthwhile noting that the groups that detect greater improvement in ventricular function in treated patients are generally those which detect less positive evolution in the control groups, and vice versa. These results raise doubts, both concerning the validity of the reported improvements and the methods used to assess the results of the cellular transplantation. Thus, it is interesting to note that in the longest follow-up trial, the modest increase in ejection fraction detected at 6 months post-transplantation

disappeared at 18 months since the placebo group improved more than the treated one.[108] Still more disturbing is the fact that, although all the clinical trials done until now have been phase I trials and, as a result, designed to test the safety and replicability of the protocol, most publications emphasize the apparent effectiveness of the procedure.[85-106]

Despite the results of the clinical trials generally being presented with a positive bias, and the charged and poorly informed comments from some people on the periphery of this discipline,[109] it is increasingly clear that at the very least the transplantation methods used in humans up to the present do not produce the 'miraculous' results originally found in mice. Furthermore, several trials have not detected any effect attributable to transplanted cells,[98,105,106] although these trials suffer from flaws similar to those found in studies with positive results. Thus, it is surprising that, faced with this sobering and confusing situation, many clinical researchers working in this field try to ignore the fact that clinical myocardial regeneration is already in crisis even before moving beyond phase I trials.[107] Instead of reassessing the present situation and its causes, attempting to obtain the information needed to explore the reasons for the disparity between the expected results and those obtained, and modifying the clinical protocols based on relevant information, most of the "leaders" in this field are attempting to ignore the facts. Many researchers who, 4 years ago, boldly initiated premature clinical trials with no experimental data of their own, are now putting forward alternative interpretations to explain the marginal results they have obtained and, at the same time, to justify including patients in the same type of protocols,[95,104,110] sometimes endorsed by prestigious international societies.[107] The new fashionable target of clinical transplantation is not "regeneration", but the "paracrine" effect of cells transplanted into the surviving myocardium with or without "neovascularization" of the lesion,[95] or even the transplantation of new mitochondria into damaged myocytes.[111] As usual in this field, these alternative explanations have been published in quality international journals without any experimental data or clinical evidence supporting the proposed hypothesis. Similarly, clinical trials in patients on the waiting list for transplantation, which are poorly designed because they are unable to determine the fate of the transplanted cells, are presented as models of clinical research.[111]

The current situation is serious and threatens the future of this field. After having transplanted bone-marrow cells into more than 1000 patients there is still not a single solid piece of evidence demonstrating whether the protocols used are capable of leading to regeneration in the human heart. Furthermore, there are no data from animal studies that can help guide us through the confusion or clarify it, since, until now, all the experimental data in favor of regeneration have only been obtained in mouse[71,72,112] and even these have been challenged by some researchers.[81-84] Although unlikely, the possibility that the plasticity of bone-marrow cells to become myocardial cells may in fact be a property limited to rodents has not been explored.

The lack of solid experimental data on such a high-profile and clinically important topic is a mistake for which both basic researchers and clinicians are responsible. With few exceptions,[92,95] clinical researchers have not undertaken preclinical trials of the protocols and types of cells they have been transplanting into humans. In turn, basic researchers have squandered a lot of energy and resources on two totally irrelevant discussions both for basic research on regeneration and for its clinical application: 1) whether the "fusion" of the transplanted cells with the surviving myocytes could explain the "regeneration" detected in mouse,[113] forgetting that, by definition, cellular fusion cannot explain the formation of a single new cell; and 2) whether the immunohistopathological techniques with DNA labeling are suitable for identifying the formation of new myocytes.[83,84,114-117] While these discussions have paralyzed research on myocardial regeneration, hundreds of articles have been published using the same techniques to study the regeneration of other tissues, including the CNS.[33,118]

One of the outcomes of these internal conflicts is that this discipline has not generated any new information in the last 4 years regarding identifying cells with myocardial regenerative capacity or on the biological bases of the presumed beneficial effects observed in mouse and, possibly, in humans. Meanwhile, the number of clinical trials continue to proliferate as if the preclinical data that justify applying these procedures in humans were a completely resolved matter.

The cells used to produce myocardial regeneration in mouse were selected by c-kit expression, the membrane receptor for stem cell factor (SCF), a protein expressed in hematopoietic stem cells and in a small fraction of other bone-marrow cells and other tissues. However, in clinical trials, when the entire mononuclear fraction of the bone marrow is not used, the transplanted cells are selected on the basis of CD133 expression, an antigen of unknown function expressed in hematopoietic and endothelial stem cells, among others. However, not a single publication has demonstrated myocardial regeneration by using either the mononuclear fraction or CD133pos cells in animals or humans. Even though several publications have suggested that, at least in mouse, hematopoietic stem cells do not have cardiac regenerative capacity,[81-84] and that this capacity is probably limited to a cell type with the characteristics of mesenchymal stem cells from bone-marrow stroma,[76,80,119] clinical trials with bone marrow continue to be planned with the aim of transplanting the maximum

possible number of hematopoietic stem cells into the myocardium.

Leaving aside the fact that we still do not know the precise identity of the bone-marrow cells with cardiac regenerative capacity, not a single reputable publication has demonstrated the possibility of anatomically and functionally regenerating a myocardium with the mass and thickness of the human heart, a fact which cannot be extrapolated from the data obtained in mouse, as already discussed. Assuming that it is possible to repair human myocardium, there is no data on the type and number of cells necessary for this, which route and administration method are the most efficient, and so on. Given the state of this field, it is unsurprising that, despite the huge investment in material and human resources in myocardial regeneration clinical trials, it still remains impossible to show that a single human life has been saved or even extended. As a result, the argument put forward by some clinical researchers and defended by a consensus adopted by the European Society of Cardiology,[107] by which the severity of the clinical condition treated justifies the heterodox methods used until now, is highly unconvincing.

THE PUBLISHED AND ONGOING CLINICAL TRIALS ON CELLULAR TRANSPLANTATION ARE NOT EXPLORING MYOCARDIAL REGENERATION

Even putting aside the architectural challenge of mass, thickness, the complexity of the vascular system, and the type and organization of human myocardium fibers compared to those of mouse, the clinical trials involve serious flaws other than the identity of the regenerative cells. Accepting as a demonstrated fact that cells with regenerative capacity are bone-marrow cells with the characteristics of the stem cells which are included in the transplanted cells, and making the most conservative extrapolations of the data obtained in mouse and the most optimistic regarding the quantity of stem cells in bone marrow, the patients who have received the greatest number of cells[91] could have regenerated between 1 and 5 g of myocardium at most (in fact, far smaller quantities are involved). The problem is much more serious in the case of the skeletal myoblast transplantation where, at most, only milligrams of tissue[61] can be produced. Furthermore, it is undeniable that none of the methods available for measuring ventricular function, whether invasive or not, have the sensitivity needed to measure the functional contribution of 5 g of myocardium. Thus, no clinical protocol transplants enough cells for them or their descendants to have a detectable and direct effect on cardiac function, even if they survived and nested effectively, multiplied from 1 to 1000 and completely differentiated in cardiac tissue. This means that, even if the modest and transient positive functional results published up to now were real, they could not be the outcome of myocardial regeneration directly produced by the transplanted cells. The positive effects of cellular transplantation on ventricular function done until now, if real, must necessarily be due to a paracrine effect of the transplanted cells on the myocytes and stem cells in the surviving myocardium. Very recently experimental data have been obtained supporting this hypothesis.[120,121]

CALL FOR A MORATORIUM ON CLINICAL TRIALS OF CELLULAR TRANSPLANTATION IN THE MYOCARDIUM

Due to the currently chaotic situation in the field of myocardial regeneration via cellular transplantation, it is not surprising that the controversy concerning the effectiveness of this therapeutic modality is increasingly bitter and on the way to getting worse. As expected, skepticism concerning the potential and even the feasibility of producing cardiac regeneration with physiological relevance has gradually increased to the point of reaching a level that threatens to destroy this discipline's future at root. Sadly, both clinical and basic researchers in this field have contributed to the development of this situation and we have to accept our share of responsibility. There was no need whatsoever for myocardial regeneration as a scientific and/or clinical discipline to have developed in such an undisciplined and irresponsible way. Other specialties facing problems as difficult as those involving the myocardium, or even harder ones, have shown that is possible to follow a more sensible and productive course. For example, we only need to compare the state of confusion concerning cardiac regeneration via cellular transplantation with the field of neuronal regeneration in the CNS. Despite its earlier beginning and having generated more extensive and in-depth information on the origin, biology, and regenerative potential of fetal and adult neuronal stem cells obtained from many experiments with different animal models, including primates, the first phase I clinical trial with neuronal stem cells has just been approved by the Food and Drug Administration for the treatment of Batten disease (a neural ceroid lipofuscinosis).[122] If cardiovascular cellular therapy had evolved with similar caution, we would probably be in a better situation today.

Given that both clinical and basic researchers are equally responsible for the current chaos and confusion, it is vital to take decisions aimed at redirecting and focusing research on the use of stem cells for myocardial regeneration and its clinical application in the most productive way possible without putting patients at unnecessary risk. Fortunately, one of the most attractive and positive characteristics of scientific process is that,

given sufficient time, the mistakes made due to both commission and omission are always rectified. The challenge facing the medico-scientific community is to identify the corrections needed to avoid missing opportunities and, at the same time, to avoid affecting the patients adversely. As we have repeatedly pointed out, in the case of myocardial therapy with bone-marrow cells, we do not know the identity of the cells with regenerative potential. Thus, regardless of how detailed and careful the clinical protocols used are, it is impossible to know the number or condition of the effective transplanted cells. Therefore, it is impossible to assess the results of a given therapy when the identity of the therapeutic agent and the dose administered is unknown, especially if the results obtained are marginal or negative, as in the present case. Without this information, any changes made to the protocols to improve results are no more than shots in the dark. Thus, given the gaps in our knowledge and the tone of the current controversy, it is both of concern and strange that the regulatory bodies of both hospitals and public health-care services continue to approve new clinical trials whose probability of producing convincing results is minimal. As the protocols used, particularly in randomized trials, are invasive, not without risk to individuals and are of no possible benefit (bone-marrow extraction under anesthesia or general sedation, intracoronary injection a few days after infarction, both in the control group and treated patients, and so on), our opinion is that the time has come to thoroughly reassess both the basic data and the process most suited to implementing this information in clinical practice.To this end, we suggest placing a moratorium on new clinical trials on cellular transplantation in myocardium until the necessary information relevant to humans has been obtained from animal models. This will enable us to design clinical trials which, in addition to being safe for the patients, will generate interpretable results, whether positive or negative. The information obtained from trials designed in this way will in turn permit the rational modification of the protocols to gradually optimize the results.

In order to be productive, the suggested moratorium on clinical trials should be used to answer a core number of questions needed to design rational clinical protocols. Among these questions are the following:

1. Is the capacity of bone-marrow cells to differentiate into myocardial cells a property limited to rodents or is it shared with other species, including humans?

2. What is the identity of the bone-marrow cells capable of generating myocardial cells?

3. What is the short- and long-term fate of the cells transplanted into the myocardium of large animals?

4. Is it possible to obtain a sufficient number of autologous cells with therapeutic potential to directly produce quantifiable physiological results in hearts similar in size to those in human?

5. Is the mechanism of action of the transplanted cells a direct contribution to the contractile mass or a paracrine effect on the surviving myocardium?

6. What is the most effective route for administration?

7. What is the optimal time and approach for administration: during the acute, subacute or chronic postinfarction period?

8. What is the duration of the detectable beneficial effect on ventricular function in large animals?

9. What is the best predictor of a positive clinical effect?

Once we can answer these questions we will be in better position to reassess the potential of this new putative therapeutic modality. However, we should bear in mind that, even if the answers come out in favor of clinical development, this type of therapy still has to overcome three serious obstacles to its widespread application: *a*) it is based on an obsolete and flawed conception of cellular homeostasis of the myocardium and its regeneration; *b*) it will be difficult to determine the long-term fate of the transplanted cells in the context of human longevity; and *c*) the procedure is very expensive regarding human resources and materials and, thus, it will only be available to a very small proportion of the population candidate for myocardial regenerative therapy.

WHAT IS THE FUTURE OF MYOCARDIAL CELLULAR TRANSPLANTATION?

Slowly, but steadily, the research and clinical cardiovascular community is beginning to accept that adult myocardium has a significant and intrinsic regenerative capacity based on the presence of myocardial stem cells capable of regenerating myocytes and microvasculature. However, these concepts have still not been incorporated into the protocols designed to regenerate the myocytes lost to ischemic heart disease. All the clinical protocols without exception are based on the concept of the myocardium as tissue made up of differentiated postmitotic cells, lacking stem cells and, thus, having no intrinsic regenerative capacity. Consequently, regeneration is attempted by transplanting exogenous cells with contractile capacity (skeletal myocytes) into the myocardium or cells with the potential to convert into myocardial cells (bone marrow). This gap between basic knowledge and clinical protocols is mainly due to the short time this field has been under development, and it should be closed within a relatively short period. Given the information available on the biology of cardiac stem cells, and by extrapolating information regarding other organs, it is difficult not to be optimistic about the future of research on myocardial regeneration and its potential to revolutionize cardiovascular medicine.

Once the problems discussed here concerning cellular transplantation are solved, this kind of therapy might act as a bridge to a different therapeutic model. In the near future we should completely develop the methods, restricted to the experimental laboratory up to now, for achieving regeneration in the human heart by using its intrinsic regenerative capacity without having to use cellular transplantation in general and cells extrinsic to the heart in particular.[123-126] However, we should stress that, despite the promising future concerning these new possibilities, we should answer a series of questions like the ones previously listed regarding cellular therapy before beginning clinical trials with these new methods. Furthermore, once these questions are answered, the first clinical trials should be done in terminal heart failure patients on the waiting list for heart transplantation, to thus minimize the risks and, at the same time, ensure that we can document the effects of this therapy directly and in detail.

This is a suitable moment to recall that in clinical research, as in life, the more haste, the less speed. We cannot nor should not forget the negative impact on transplantation caused by Barnard[127] and the most recent problems in genetic therapy.[128-131]

In both cases, progress was delayed for years in the respective medical disciplines because clinical applications started before the necessary experimental information was obtained. If we absorb these historical lessons and act with responsibility and caution, this new discipline of myocardial regeneration, based on deep knowledge of heart stem cell biology, offers us the opportunity to prevent the onset of heart failure or radically alter its prognosis once it appears in a large number of patients with ischemic heart disease.

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