Advances in Experimental Medicine and Biology 741

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Stem Cell Transplantation

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STEM CELL TRANSPLANTATION
Carlos López-Larrea, Antonio López-Vázquez and Beatriz Suárez-Álvarez

Stem Cell Transplantation

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Springer Science+Business Media, LLC

Landes Bioscience

Springer Science+Business Media, LLC
Landes Bioscience

Printed in the USA.

Springer Science+Business Media, LLC, 233 Spring Street, New York, New York 10013, USA
http://www.springer.com

Please address all inquiries to the publishers:
Landes Bioscience, 1806 Rio Grande, Austin, Texas 78701, USA
Phone: 512/ 637 6050; FAX: 512/ 637 6079
http://www.landesbioscience.com

The chapters in this book are available in the Madame Curie Bioscience Database.
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Stem Cell Transplantation, edited by Carlos López-Larrea, Antonio López-Vázquez and Beatriz Suárez-Álvarez. Landes Bioscience / Springer Science+Business Media, LLC dual imprint / Springer series: Advances in Experimental Medicine and Biology.

ISBN: 978-1-4614-2097-2

Library of Congress Cataloging-in-Publication Data

Stem cell transplantation / edited by Carlos López-Larrea, Antonio López-Vázquez, Beatriz Suárez-Álvarez.
p. ; cm. -- (Advances in experimental medicine and biology ; v. 741)
Includes bibliographical references and index.
ISBN 978-1-4614-2097-2
I. López-Larrea, Carlos, 1953- II. López-Vázquez, Antonio. III. Suárez-Álvarez, Beatriz. IV. Series: Advances in experimental medicine and biology ; v. 741. 0065-2598
[DNLM: 1. Stem Cell Transplantation. 2. Gene Therapy. 3. Organ Transplantation. 4. Stem Cells. W1 AD559 v.741 2012 / QU 325]

616.02’774--dc23

2011040785

PREFACE

"All that lives must die, passing through nature to eternity"
—Hamlet (W. Shakespeare)

Organ transplantation has been the most important therapeutic advance in the last third of the 20th century. Its development has revolutionized medicine, as demonstrated by the fact that a large number of researchers in this field have been awarded Nobel Prizes.

In the beginning of this century, we are witnessing with great expectations the emergence of a new field of medicine related to the arrival of a new player on the scene: "**stem cells**" and their potential use in regenerative medicine. This volume aims to cover important aspects of the various facets of organ transplantation and regenerative medicine, with leading specialists in these fields setting out their vision. We try to rigorously explain current and novel scientific research in these fields—areas which arouse great interest from society in general, due to their potential use in modern medicine for the treatment of a great number of diseases.

The chapters of this volume are divided into four sections, **Section I** being devoted to the basic aspects of transplantation (immunological and pharmacological). A century has passed since the field of immunology was officially recognized with the award of the 1908 Nobel Prize to Elie Metchnikoff and Paul Ehrlich. They each developed concepts and points of view which led to the establishment of this new scientific discipline. Metchnikoff described the mechanism of phagocytosis and is considered the father of the field of cellular innate immunity. Ehrlich described the basis for acquired immunity through the production of antibodies. These two findings have become the cornerstones of this discipline, which has advanced very rapidly since the last century, providing an in depth understanding of the mechanisms related to organ transplant rejection. The first functioning transplants were of kidneys from living donors in the 1950s. During the '70s and '80s, the practice of organ transplantation stopped being regarded as an experimental therapy and became a reality in hospital medicine. Sir Peter Medawar pointed out that the rejection of transplant organs by the recipient body was mediated by an immunological reaction which could be modified. This was due mainly to the identification of the major histocompatibility complex (MHC) and the characterization

of the so-called human leukocyte antigen (HLA). On the other hand, the discovery of effective immunosuppressants (such as cyclosporin) meant a dramatic improvement in survival for patients and for grafts.

There are two main differences between organ transplantation and any other type of surgery. One concerns the need for organs to be donated by a living person or received from a deceased donor, and the other is the requirement for lifelong immunosuppressive medication in transplanted patients, with the associated risk of becoming vulnerable to infection and development of chronic diseases. Rejection is, as such, inherent to transplantation, and the fight against it is an important part of the history of this therapy. The medium and long-term challenges consist of overcoming so-called chronic rejection and finding a way to prevent it. The understanding of the mechanisms of immunogenicity and immunological tolerance is allowing the development of a new generation of immunossupressive agents which manipulate the immune system more precisely. Clinical trials are being undertaken to refine existing regimens.

The development of immunotolerance is, in a strict sense, the acceptance of the graft by the recipient without immunosuppressive medication. Several multicenter trials including Clinical Trials in Organ Transplantation (CTOT), a cooperative research program sponsored by the National Institute of Allergy and Infection Diseases, and Reprogramming the Immune System for the Establishment of Tolerance (RISET), an ongoing collaborative effort across the European Union are aimed at minimizing immunosuppression. Other future possibilities are open with the potential use of immunotherapeutic techniques that inhibit recognition by the immune system of the transplanted organ through the development of combined cell therapies (for example, generation of regulatory T-lymphocytes and mesenchymal stem cells).

However, despite this progress, the mismatch between supply and demand is, by far, the biggest problem in the world of transplants. Spain, the world leader in organ donation, has managed to avoid an excessive growth in waiting lists, but is still not capable of meeting all the demand for organs. One strategy proposed has been the use of animal organs. However, in order for xenotransplants to work, researchers must find the way to block immunological rejection. Moreover, at present, there is a moratorium on their use, based on the risk represented by the infection of humans with animal viruses.

During the last few years, we have seen the growth of a new area of knowledge: regenerative medicine. The scientific developments deriving from the discovery of so-called “stem cells” have raised great expectations given their potential use in tissue replacement. Tissue or cell transplants have become a potential therapeutic replacement for organ transplantation in the future. A “stem cell” is one that is undifferentiated and able to produce the specialized cells of various tissues and organs. Furthermore, these cells are capable of proliferating and growing in cultures indefinitely, which has been described as “cell immortality”. Many cell types fall within this definition of stem cell.

This volume deals mainly with the so-called embryonic and adult stem cells. The latter are the subject of **Section II**. The first effective cell therapy treatments were carried out using haematopoietic stem cells (HSCs) for bone marrow transplantation. HSCs are unique in their ability to migrate to various sites, ensuring the safety and integrity of their regenerative potential. Different chapters focus on the guidance cues and molecular pathways regulating HSC trafficking throughout the lifetime of the organism. Moreover, we know now that there are other places in the body where there are reservoirs of

adult non-haematopoietic stem cells. The presence of a small subpopulation of adult stem/progenitor cells in most tissues and organs provides the possibility of stimulating their in vivo differentiation potential, or of using their ex vivo expanded progenies for cell-replacement and gene therapies with multiple applications in humans without high-risk of graft rejection and major side effects.

An adult stem cell, therefore, has the possibility of self-renewal and of generating certain types of cells (multipotent stem cells) indefinitely, depending on their location, such as: cartilage, adipose, connective and muscle tissue. In relation to this, molecular signals of specific niches or microenvironments play a fundamental role in the maintenance of the differentiation potential. At present there is a drive to search for the factors that trigger the processes of regeneration from the stem cells in various locations in the body and how to control them. One of the emerging fields of research today is based on the existence of "tumour stem cells" and their implications in cancer development. Their similarity with adult stem cells will provide us with a new approach for the treatment of certain types of cancer.

Section III of this volume focuses on the description of embryonic stem cells. Pioneering work carried out by James Thomson (1998) demonstrated the possibility to derive embryonic stem cells from a human blastocyst for the first time. This was a milestone, opening a new field full of potential. The possibility of inducing these cells to differentiate into any type of tissue (pluripotency) and of having unlimited quantities of them provide new hope for treatment of many diseases. Also, the development of cloning techniques enables cells genetically identical to those of the patient to be derived, thus avoiding the risk of immunological rejection, a strategy commonly termed "therapeutic cloning". Several research groups have published studies on "cell reprogramming" techniques. For this purpose, reprogramming of somatic cells into pluripotent stem cells (iPSC) has been achieved by introducing four transcription factors in 2007 by Shinya Yamanaka's team. These results opened the possibility of using adult somatic cells and transforming them into stem cells similar to embryonic stem cells. Induced pluripotent stem cells overcome the problem of immune tolerance and the ethical issues faced by the use of allogenic embryonic and adult stem cells in patients. Applications of patient-specific induced pluripotent stem cells have focused on disease modeling, drug screening and therapy. Recently, iPS cells have been derived from patients with a variety of genetic diseases, such as Parkinson's, Huntington's, Down syndrome, muscular dystrophy, amyotrophic lateral sclerosis and others.

Section IV summarizes some ongoing approaches of stem cell regenerative medicine and also introduces recent findings from published studies and clinical trials. It is necessary to evaluate and demonstrate its effectiveness, biosecurity and real capacity to repair tissues that have been damaged by various conditions, such as neurodegenerative, cardiovascular and endocrine diseases, before their use in therapy. Some cell therapies had been established and approved for clinic use, such artificial skin, keratinocytes, chondrocytes, liver, cells of the corneal limbus and others. The present status of tissue bioengineered techniques in the regeneration of different organ systems is also reviewed. Synthetic biomaterials used to create scaffolds for tissue engineering applications have been limited in large part due to the lack of specific cell binding motifs that would allow cells to function properly. Creation of custom-made bioengineered organs by using scaffolds as decellularized native tissues seeded with autologous cells remains a major challenge.

Indeed, there are still many basic aspects to investigate and develop, such as: the specificity of molecular signals involved in the differentiation of stem cells into various cell lineages, the use of specific biomaterials supporting organogenesis and the development of biosecure gene therapies which can be transferred to clinical settings.

It is hoped that in the coming years better insight into the mechanisms underlying the beneficial effects of progenitor cells will help the design of more specific targeted therapies.

We would like to acknowledge the authors for their excellent contributions.

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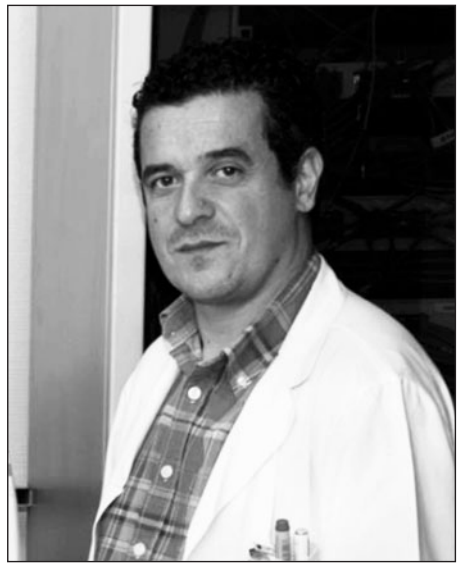

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ACKNOWLEDGMENTS

This work was supported in part by the “Red de Investigación Renal” (REDinREN), Spanish grant FIS PI080566 from Instituto “Carlos III” (European FEDER founds) and CAJASTUR.

CHAPTER 1

THE FRONTIERS OF ORGAN TRANSPLANTATION AND CELL THERAPY

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Abstract: The biggest problem in the area of organ transplantation is often the mismatch between supply and demand. Extrapolating the transplant waiting lists in Spain at the end of a year to the global population, more than one million people would be able to benefit from a transplant if there were enough available organs and adequate infrastructure. The first frontier and the most important is therefore the donation of organs. The aim of this chapter is to set out the most notable points concerning the various themes (donation, rejection, xenotrasnplants, tissue transplantation and stem cells therapy), and describe new avenues to be explored: The frontiers that it will be necessary to cross in order to continue the progress in saving lives and improving the health of hundreds of thousands of people across world.

In the last years, embryonic stem cells have become in the great hope of many millions of patients across the world. In theory, the possibility to have unlimited quantities of these cells, to culture them, and to make them differentiate into cells of the liver, nervous system or heart would in fact become the ideal solution for the treatment of millions of patients. It is quite plausible that what organ transplantation has represented in the 20th century, bringing down taboos and saving hundreds of thousands of lives, is going to be replaced by stem cell therapy in the 21st Century.

INTRODUCTION

In a book-like this, in which there are numerous references to the so-called "classical" transplantation protocols, as well as the new therapeutic approaches that are to become an alternative for a multitude of processes in the near or distant future, it seems reasonable to devote this first chapter to present an overall vision of the subject. The aim is to set out the most notable points concerning the various themes (Table 1), and above all, describe

Stem Cell Transplantation, edited by Carlos López-Larrea, Antonio López-Vázquez and Beatriz Suárez-Álvarez.

Table 1. Frontiers to define the donation and transplantation of organs, tissues and cells

1. Availability of organs for transplantation:
   a. Donation after brain death.
   b. Donation in cardiorespiratory arrest.
   c. Living donor transplant.
2. Prevention and management of rejection.
3. Xenotransplants.
4. Organ transplant.
5. Tissue transplant/tissue engineering.
6. Cellular transplant/cellular therapy.

new avenues to be explored: The frontiers that it will be necessary to cross in order to continue the progress in saving lives and improving the health of hundreds of thousands of people across world.

AVAILABILITY OF ORGANS FOR TRANSPLANTATION

The biggest problem in the area of organ transplantation is often the mismatch between supply and demand. In contrast to this limiting factor, the other problems, that we will go on to present as frontiers to cross, remain for the moment mere details. According to the data collected jointly by the Spanish National Transplant Organisation (*ONT*) and the World Health Organisation for the Global Observatory of Donation and Transplantation,[1] every year around 95.000 patients are recipients of solid organ transplants (excluding tissue and cell transplantation) (Fig. 1). It is not possible to know the exact demand for transplants due to the lack of an official waiting list and indeed of any transplant organisation covering a good part of the world. However, if we extrapolate the transplant waiting lists in Spain[2] at the end of a year to the global population, there would be more than one million people able to benefit from a transplant if there were enough available organs and adequate infrastructure. In other words, overall less than 10% of the candidates for this treatment obtain the organ needed and transplant policies are inevitably conditioned by the enormous mismatch that this represents.

The first successful transplants were those of kidneys coming from a living donor,[3] dating date back to the nineteen-fifties. However, it was the deceased donation which made possible the major development of all types of transplants, especially that of vital organs. The problem is that deceased donation has a fundamental limiting factor: The epidemiology of brain death, itself determined by a series of structural issues (availability of beds in intensive care units, number of respirators, general healthcare resources) and organisational factors.

The first frontier to target, and perhaps the most important one, is therefore the donation of organs. The remaining hurdles are of lesser importance since if there are no organs for transplantation, there is no point in anything else. Transplantation is the only medical speciality that in treating patients is dependent on another individual, dead or alive, who makes it possible by giving their organs. As regards donation of deceased-donor organs, since the establishment of the ONT[5-9] in 1989, Spain has been gradually pushing back the frontiers. Specifically, Spain has been progressively improving its organ donation

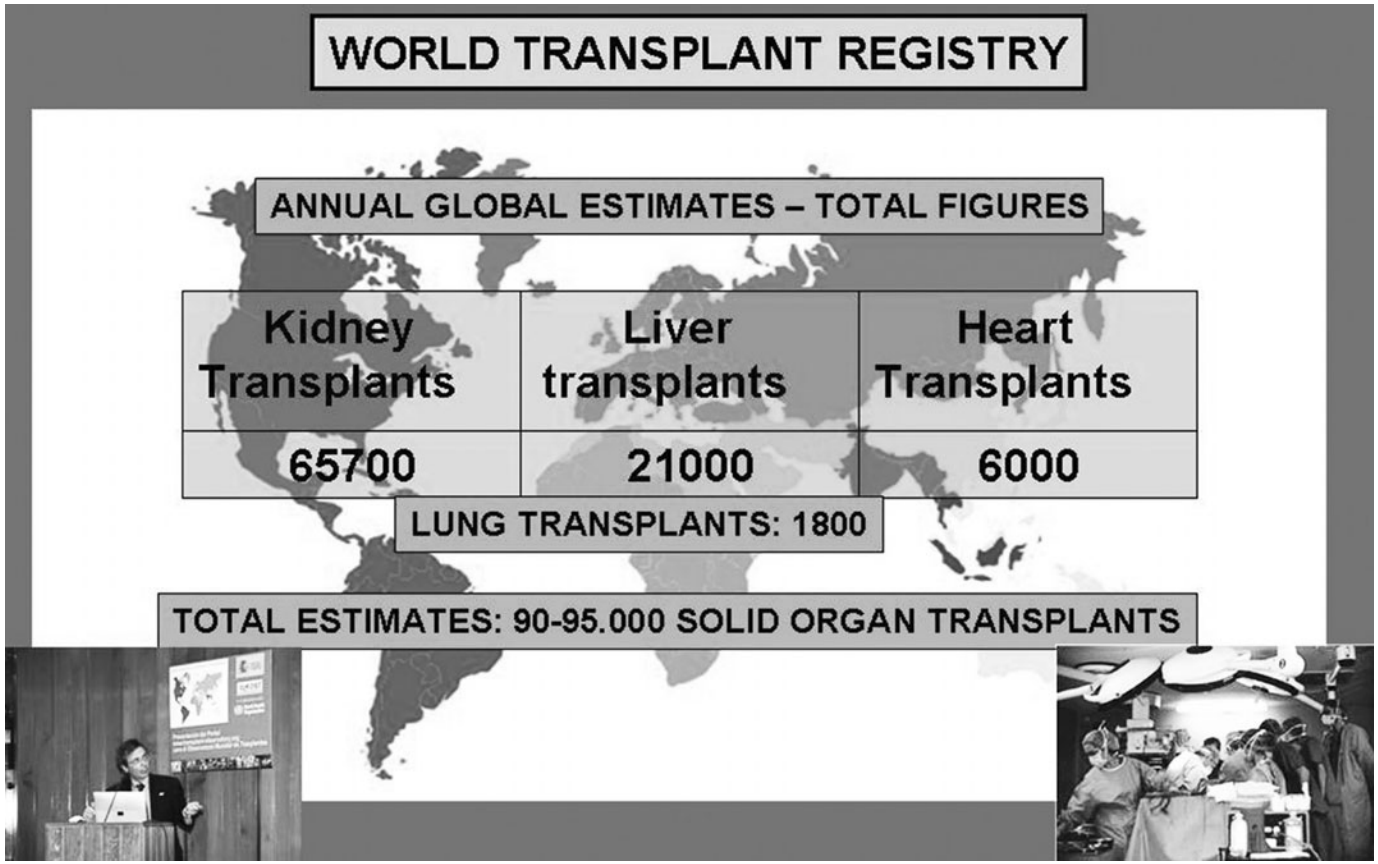

Figure 1. World Transplant Registry WHO/ONT: Overall figures for organ transplant worldwide. Reproduced with the permission of the Global Observatory on Donation & Transplantation (http://www.transplant-obervatory.org).

system and has reached and maintained 33-35 donors per million people (pmp), a level never achieved by any other country in the world. Since 1992, we have led the ranking in both generosity and organisation, but this should not lead to self-complacency and we should continue to strive for better results. In fact, although a national record of 1.606 donors was achieved in 2009, with a 34.4 donors pmp, 3-5 out the 17 Spanish Autonomous Regions usually reach levels above 40 donors pmp (2,9). Specifically, a small region as is La Rioja could boast of 74.2 donors pmp in 2007, and others such The Basque Country, The Canary Islands and Cantabria have exceeded 50 on several occasions. From this, we can state that with optimum organisational and structural conditions such as achieved in these Autonomous Regions, it should be possible to obtain similar figures elsewhere. Indeed, some Italian regions such as Toscana, that have adopted a model similar to ours, have also reached 40 donors pmp.[10]

Therefore, the ONT[2] has set as an objective achieving 40 donors pmp for the whole of Spain, an initiative called "Plan donación cuarenta". This would be a new achievement for Spain but also set the "Gold Standard" to be reached internationally, in which all the strategies with proven efficacy are put together to improve organ donation rates.

Up to now we have been talking about the typical brain-dead donors, who make all types of transplants possible, and who are obviously closely linked to intensive care units. These represent no more than 2-3% of those who die in a hospital, hence the interminable mismatch between supply and demand in this therapeutic procedure.

However, there is another minority group: Nonheart-beating donations or more descriptively donation after cardiac death (DCD). It involves those patients who suffer from cardiac arrest of any cause, and despite the usual procedures cannot be successfully resuscitated, leading to a shortage of oxygen in the central nervous system and the resulting irreversible damage. After all, from the beginning of time, this has always been and still is the most common way to die. Donation and subsequent organ transplant can only be achieved if at this moment, procedures for the cannulation and perfusion of organs are carried out.

This type of DCD, also referred as "uncontrolled" because it is not possible to know when they are going to happen, require an agile and efficient organisation. They represent just over 5% of Spanish donors, although in Barcelona and especially in Madrid, this percentage is much higher.[2,11] On the other hand, a new type of donor started to appear a few years ago for world transplant community. These are the so-called controlled DCD or Maastricht Catergory III donors: These are people with irreversible brain injury, who require mechanical ventilation but who are not brain dead. In this situation, and after informing relatives and receiving their consent, life-support is withdrawn until the heart stops, the individual dies and organs can be donated.[12,13] This form of donation, excluded in Spain in the Consensus Conference held in 1995 which opened the way to other DCD, although legally possible, is not being performed in Spain and most countries in Southern Europe and Latin America. On the other hand, it accounts for a third of the donations in The Netherlands and about 10% in the United States, where it represents the fastest growing-type of transplant, and there has been a similar pattern in other countries such as United Kingdom and Australia. Whether through the increasing uncontrolled DCD in large hospitals connected to good emergency services, or through the generalisation of controlled DCD, which despite all the ethical and legal issues for many countries and cultures, currently represents one of the greatest avenues of expansion worldwide in organ donations from deceased donors, the truth is that cardiac arrest deaths represent a

non insignificant way of increasing the number of donors, and therefore in the number of people who can benefit from these therapies.

A similar statement can be made with respect to live donation. In the nineteen-eighties there was a great development of donation from deceased donors, to the point that it was thought that live donations were going to become almost insignificant. Indeed, the Council of Europe recommended reducing its use as far as possible in a resolution of the Council of Ministers in 1987, as did the World Health Organisation in the early nineties.

However, things have not evolved as some expected. With the exception of Spain, where the donation of organs arising from deceased donors has not stop increasing since the late eighties, there is a growing mismatch between offer and demand for all types of transplant, but especially for renal transplants. That is why, given the improvement in the outcomes as a consequence of better drugs against rejection and the increase in medical recommendation for transplants that cannot be carried out in other ways,[14] the opinion of the transplant community and of the society in general has changed. These days, live transplantation has stopped being seen conflicting with deceased donor transplantation and has become a growing complementary alternative in countries. There is little doubt that for a given country, it is much easy to have a live kidney transplant programme than having a system for deceased donor kidney allocation that works. It requires only a relatively simple technique which does not need major facilities.

The consequence is that in several countries in Asia, Africa and, to a lesser extent, Latin America almost 100% of the renal transplants come from living donors. They represent 42% of the total in Latin America, 44% in Australia, and 36% in the United States, where there has been a real growth in this type of treatment. In the European Union they account for just 17%, with higher percentages, over 30-35%, found in northern countries and in Greece, and lower rates in Latin countries, accounting for 10% in Spain, although there has been an increase in recent years as a consequence of an active promotion policy.

While we are not going to analyse them here, the potential for expanding live donation, applicable not only to kidneys but also to the liver and, though to a much lesser extent, to other organs such as the lung, pancreas and intestine, are theoretically unlimited. However, in practical terms, there are two basic limiting factors: The potential damage to the donor and the commercialisation of organ donation, which according to the WHO affects 10% of all the live transplants carried out worldwide.

One hundred experts from 40 countries met in April 2004 in Amsterdam. The objective was to establish an international consensus on the standards that should be guaranteed to kidney donors.[15] The necessity to minimise the physical, psychological and social risks for the donor from the removal of a kidney, at the same time as optimising the benefit to the recipient and maintaining the public confidence in the donation process. The core of the document approved contains the recommendation of a thorough evaluation of potential donor's health to avoid any subsequent complications, as well as the need to provide extensive and detailed information on all the risks of the intervention in the short, medium and long-term, and the potential alternative treatments for the potential recipient. Another of the recommendations was the careful monitoring of the donor in order to detect any emerging complications, in to maximise the chances of successfully addressing the problem, as well as improving our understanding of the possible impact of the removal of a kidney. This is a very reasonable document, and its recommendations, should they be implemented in full, would go a long way towards eliminating the abuses currently occurring in many parts of the world. More recently, experts and organisations all over

the world have agreed the so-called "Declaration of Istambul"[16] which represents a clear consensus against the commercialisation of transplantation and transplant tourism.

The combination of the three forms of donation: Donation after brain death, donation after cardiac death and live donation currently enable 100.000 organ transplants every year.[1] There is no doubt that one of the frontiers to cross is improvement in these three modalities, to make these types of treatment available to anyone who needs them.

PREVENTION AND MANAGEMENT OF REJECTION—IMMUNOTOLERANCE

Rejection is inherent to the transplantation procedure, and the fight against it has been a key issue in the history of transplant therapeutics. Initial rudimentary immunosuppressive protocols have been replaced by current protocols in which drugs, discovered in recent decades, are increasingly combined in a personalised manner. It can be asserted that avoidance of short-term rejection is almost guaranteed with the use of current medications. This explains the excellent functional survival rates of the various grafts and indeed of the recipients for the majority organ transplants. Graft failure in the early months, although it may have an immunological basis, is mainly due to problems with respect to organ viability, the consequence of a greater use of donors with risk factors, as well as to surgical and infectious factors, and others associated with the clinical status of the recipient.

The challenge, the real frontier of immunosuppression, lies in the middle and long-term in two respects: To extend the good initial results to longer periods of time and to minimise the acute and chronic toxicity that goes with immunosuppressive treatment. The real problem of all types of organ transplant is the progressive functional deterioration, a consequence of so-called "chronic rejection". This is a complex and poorly understood phenomenon that causes the loss of a great number of grafts in the long-term and is associated with a notable morbimortality, as well as requirement for retransplantation. Another issue is the inherent toxicity associated with the long-term administration of the drugs, leading to a higher rate infections, tumours, metabolic disorders, etc. Evidently, all these problems are largely outweighed by the great clinical and social benefit of transplantation, but they do represent a serious problem that restricts long-term survival and quality of life of many patients. In fact, these have become the real sticking point in the clinical management of the transplanted patient.

The problems mentioned above could be largely solved if we understood the phenomenon of chimerism better.[17] This is a situation in which a transplanted organ or tissue, coming from the same or a different species, instead of being the object of a rejection immune response against the transplant, is accepted as if it is has been always part of the recipient. This event occurs spontaneously very rarely and its mechanism still remains largely unknown.

This type of "immunotolerance" has attracted the attention of many experts due to the future importance that its better understanding and control may have for the prognosis of transplanted patients. Another factor is the huge costs of these drugs. It is estimated that in the European Union, the annual expenditure is around 2.000 million Euro, with an average cost per patient between 6-9.000 Euro.

For a long time it has been known that in most cases transplanted patients who abandoned their medication whatever stage in the process loose the organ. Nevertheless, there is a proportion that progress well with no medication, thanks to the mechanism

of immunotolerance mentioned above. If the treatment is dropped on a patient's own initiative, little can be said, but removing the medication of a transplant recipient entails a risk of rejection, a responsibility that cannot be assumed by others. The development of a laboratory test that identifies these patients would be of great value in order to avoid running this risk, and this is subject of research in many groups both in Europe and in the United States.

However, research in this field is not only centred on the identification of potential immunotolerant patients, but also on inducing such a status.[17,18] The most promising lines of research indicate that the transplantation of haematopoyetic precursor cells of the donor themselves may be a way of inducing the desired immunotolerant status. This has been tried several times, for example, in the face transplantation carried out in France. However, currently, the unknowns by far exceed knowns. To date, anti-rejection drugs have been aimed at "blinding" the immune system. In this case, we would be intending to retrain it so that learns to co-exist "peacefully" with the new organ: A chimera in all the senses of the word.

XENOTRANSPLANTS

The concept of transplanting organs of other species' into human recipients is not new. In fact, we could state that there was an instinctive attempt in doctors to try to safe the lives of people suffering from renal, liver, and cardiac insufficiency long before the early foundations of modern surgical procedures were laid.[20] In the nineteen-nineties, there was the belief that xenotransplantation into humans might become the solution for the deficit of organs of deceased donors pig being the species with highest potential to become the ideal source of organs for humans.

Indeed, the production of transgenic pigs, that expressed in their cells human complement regulatory proteins, allowed the immunological barrier of hyperacute rejection to be overcome. Until then, this had been the prime limitation of pig organ transplantation into humans. However, in order to avoid these xenografts being rejected later, the use of an intense immunosuppressive treatment is necessary, and this is associated with severe toxic effects in the recipient. The next step is to determine if rejection could be avoided, with acceptable secondary effects for the recipient, using conventional treatments. If not, it might be necessary to resort to new genetic modification in the animals to be the source of the organs, or else to some other type of manipulation in the recipients. Only after we have solved these issues could long-term survival rates (more than 6 months) of pig organs in nonhuman primates be possible. It would then be possible to study the functioning of organs as well as the transmission of infectious agents.

At the end of last century, with the research at that stage of development, the process halted after the publication of results demonstrating infection of human cells by pig endogenous retrovirus and subsequent capacity for recombination. Although there have been no new indications of the danger of this retrovirus, there is a consensus that we should be cautious when considering clinical trials involving xenotransplants. This issue stopped quite a few lines of research; some others remained active, especially those based on cells rather than entire organs (pancreatic islets, hepatocytes, etc.).[20] There has been a lot of progress in the preparation of protocols for the prevention of diseases both in animal carers and potential recipients (especially the latter), and evidence suggests that, after a long pause, we may observe a new dawn in research on this fascinating subject.