Instituts thématiques

Inserm

Institut national
de la santé et de la recherche médicale

Organ Transplantation

Research perspectives

Collective Expert Report
Summary and Recommendations

Inserm
Institut National de la Santé et de la Recherche Médicale
(National Institute for Health and Medical Research)

This document presents the summary and recommendations of the group of experts brought together by Inserm within the scope of the collective expertise procedure (Appendix 1) in response to the request put forward by the Agence de la biomédecine (Biomedicine Agency) regarding the transplantation of solid organs and the main direction of research in transplantation. This work is based on the scientific data available during the second half of 2008. The information contained in almost 3000 articles provides the basis for this expert report.

The Inserm Collective Expertise Centre co-ordinated this collective expert report.

Group of experts and authors

Monique BERNARD, Biological and Medical Magnetic Resonance Centre, CNRS UMR 6612, University of Aix-Marseille II, Marseille

Lucienne CHATENOUD, Department of Biological Immunology, GHU-Ouest Necker-Enfants Malades (Necker Hospital for Sick Children), Inserm U 580, University of Paris Descartes, Paris 5, Paris

Philippe COMPAGNON, Department of Hepato-Biliary and Gastro-Intestinal Surgery, Rennes University Medical Centre, Inserm U 522, University of Rennes 1, Rennes

Maria Cristina CUTURI, Institute for Transplantation and Transplantation Research, Inserm UMR-S 643, University of Nantes, Nantes

François DURAND, Department of Hepatology and Hepato-Gastro-Enterology Resuscitation Unit, GHU-Nord, Beaujon, Inserm U 773, University of Paris Diderot-Paris 7, Paris

Antoine DURRBACH, Department of Nephrology, GHU-Sud Bicêtre, Inserm U 542, University of Paris-South 11, Villejuif

Philippe GRIMBERT, Department of Nephrology and Transplantation, GHU-Sud Henri Mondor, Inserm U 955, University of Paris 12 Val de Marne, Créteil

Thierry HAUET, Department of Biochemistry, Poitiers University Medical Centre, Inserm U 927, University of Poitiers, Poitiers, Plateforme IBiSA, Surgères

Philippe LANG, Department of Nephrology and Transplantation, GHU-Sud Henri Mondor, Inserm U 955, University of Paris 12 Val de Marne, Créteil

Christophe LEGENDRE, Department of Adult Renal Transplantation, GHU-Ouest Necker Enfants Malades (Necker Hospital for Sick Children), Inserm U 580, University of Paris Descartes, Paris 5, Paris

Emmanuel MORELON, Department of Nephrology, Transplantation Medicine and Clinical Immunology, Lyon University Medical Centre, Inserm U 851, Claude Bernard Lyon 1 University, Lyon

Didier SAMUEL, Centre Hépato-Biliaire (Hepato-Biliary Centre), GHU-Sud Paul Brousse, Inserm UMR-S 785, University of Paris-South 11, Villejuif

Laurent SEBBAG, Medical-Surgical Cluster for Heart Transplantation, Lyon University Medical Centre, Inserm U 886, Claude Bernard Lyon 1 University, Lyon

Gabriel THABUT, Department of Chest Medicine B and Lung Transplantation, GHU-Nord Bichat-Claude Bernard, Inserm U 738, University of Paris Diderot, Paris 7, Paris

The following compiled a memorandum

Bernard CHARPENTIER, Department of Nephrology, Dialyses and Transplantation, GHU-Sud Bicêtre, Inserm UMS-S 542, University of Paris- South 11, Paris

Yvon LEBRANCHU, Department of Nephrology and Clinical Immunology, CHU de Tours (Tours University Medical Centre), EA 4245, François Rabelais University, Tours

Jean-Paul SOULILLOU, Institute for Transplantation and Transplantation Research, Inserm UMR-S 643, University of Nantes, Nantes

The following presented a communication

Dominique DEBRAY, Department of Paediatric Hepatology, GHU-Sud Bicêtre, Le Kremlin-Bicêtre

Patrick NIAUDET and Rémi SALOMON, Department of Paediatric Nephrology, GHU-Ouest Necker Enfants Malades (Necker Hospital for Sick Children), Paris

Scientific, editorial, bibliographical and logistic co-ordination

Fabienne BONNIN, Scientific associate, Inserm Collective Expertise Centre, Xavier-Bichat Faculty of Medicine, Paris

Catherine CHENU, Scientific associate, Inserm Collective Expertise Centre, Xavier-Bichat Faculty of Medicine, Paris

Véronique DUPREZ, Expert report manager, Inserm Collective Expertise Centre, Xavier-Bichat Faculty of Medicine, Paris

Jeanne ÉTIEMBLE, Director, Inserm Collective Expertise Centre, Xavier-Bichat Faculty of Medicine, Paris

Cécile GOMIS, Secretary, Inserm Collective Expertise Centre, Xavier-Bichat Faculty of Medicine, Paris

Anne-Laure PELLIER, Scientific associate, Inserm Collective Expertise Centre, Xavier-Bichat Faculty of Medicine, Paris

Chantal RONDET-GRELLIER, Documentalist, Inserm Collective Expertise Centre, Xavier-Bichat Faculty of Medicine, Paris

Iconography

Jean-Pierre LAIGNEAU, Inserm

Synopsis

Foreword

In 2007, over 275 000 Europeans were living with a transplant and thousands were awaiting organ transplantation. The increase in chronic diseases coupled with population ageing manifest in an increase in transplantation indications and, consequently, in requirements in terms of transplants. At the same time, the considerable drop in accidental deaths and deaths due to cerebrovascular accident has led to a decrease in the overall number of potential donors. Although organ recovery is currently on the increase, there is inevitably a shortage of organs for the foreseeable future.

In France, the number of transplantations has increased by 45% since 2000. In 2007, almost 12 800 people required organ transplantation and 232 patients died because of an organ shortage. The number of people on the waiting list increases by approximately 4% each year.

France has played a crucial role in organ transplantation, especially during the kidney transplantation pioneering phase, and subsequently for its successes in composite organ transplantation. Following surgical advances in kidney and heart transplantations at the beginning of the second half of the XXth century, the 1980s were marked by the development of the first medicinal products to control immune reactions triggered by transplant rejection. Despite greater knowledge of the cell and molecular mechanisms involved in rejection, the prevention of this phenomenon still depends on potentially toxic immunosuppressant molecules. Above all, the current situation heralds a change in practice with increasingly older patients undergoing transplantation using transplants retrieved from increasingly older subjects.

Transplantation is a good example of the integration in medicine of all the advances made in basic, biomedical, clinical, technological, epidemiological and ethical research in both human and social sciences as well as in public health. The work of the Agence de la Biomédecine (Biomedicine Agency) created in 2005 following the Établissement français des greffes (EFG) (French Transplant Agency) is based on these various disciplines.

In 2006, the Biomedicine Agency commissioned Inserm to carry out a collective expert evaluation to assess scientific, biomedical and clinical knowledge pertaining to various stages in the transplantation of solid organs and, based on this information, to define the main perspectives of research in transplantation.

In response to this request, Inserm created a multidisciplinary group comprising 15 experts – specialists in various fields of transplantation, physiology and basic and clinical immunology. The expert evaluation focused on the transplantation of vascularised organs (kidneys, liver, heart and lungs) excluding tissue and cell grafts. Human and social sciences, ethical and socio-economic issues, which constitute areas of investigation per se and/or assignments specific to the Biomedicine Agency have been excluded from this already extensive field.

The expert group has focused its attention on the following questions:

- What do we know at the present time about central and peripheral tolerance and applications to decrease (or suppress) immunosuppression?
- What do we know about the immunological and non-immunological mechanisms of rejection and the factors involved in short- and long-term rejection?

- What are the potential therapeutic targets to shift the tolerance-rejection balance in favour of tolerance?
- How can immunosuppressant therapy be optimised by focusing on the dose, bioavailability, combination, conversion or withdrawal of immunosuppressants? What are the perspectives regarding the use of à la carte (individual) protocols according to various individual (biological and pharmacogenetic) parameters?
- What do we know about new immunosuppression pathways in the quest for specificity?
- What are the scientific and technical advances geared to controlling graft quality?
- How does the ischaemia/reperfusion syndrome impact upon graft quality and a successful transplantation outcome?
- Can we identify markers of graft failure?
- What cell and molecular mechanisms are implicated in ischaemia/reperfusion?
- What are the options for expanding the number of donors without jeopardising successful transplantation: living donors, non-heart-beating donors, marginal donors?
- What is the best way of assessing potential donors and how does this evaluation impact upon the graft?
- What short- and long-term clinical results have been obtained in transplanted donors based on donor type? Can a recipient risk score be introduced?
- What types of donor "management" improve graft quality? What are the prognostic markers of organ quality?
- What do we currently know about surgery-, infection- and immunosuppression-related complications most frequently encountered after transplantation? How can these be limited?

The thrust of the analysis carried out by the expert group based on the international literature has focused on transplantation in adults. The expert group has enlisted the assistance of two specialists in paediatric liver and kidney transplantation in France in order to complete this evaluation.

Summary

Despite undeniable progress, organ transplantation faces recurring problems. From a medical standpoint, the recipient's immune system represents the main obstacle since it implements and co-ordinates a series of mechanisms aimed at destroying the allogeneic transplant, which it recognises as "foreign". Although immune response plays a key role in donor organ rejection or acceptance, numerous cell and molecular mechanisms actually condition the fate of the transplant (Appendix 2).

A large collection of immunosuppressants has been developed over the last 40 years in order to control the various forms of rejection. Based on optimised surgical techniques and preservation methods, immunosuppressant treatment has considerably increased the survival time of the transplant.

Immunosuppressants nevertheless have many disadvantages. The extent of the tissue distribution of their targets and their molecular state can trigger major iatrogenic effects. Generalised immunosuppression alters immunomonitoring mechanisms, increasing the frequency of infections and cancers as well as the morbidity with which they are associated. Immunosuppressant treatments, which are effective in combating acute rejection, have little effect on chronic rejection. In view of these limitations, the scientific community is attempting to develop strategies aimed at triggering tolerance vis-à-vis the transplant, i.e. a state of immunological hyporesponse specific to alloantigens. The majority of approaches are based on a common concept: to turn away self-tolerance mechanisms from their primary function.

Immunity tolerance in transplantation: Myth and realities

The progress achieved over the last twenty years in developing new immunosuppressant medication has substantially reduced the incidence of acute allograft rejection. The incidence of chronic rejection (the loss of the long-term function of the transplant) nevertheless remains very high, just like the morbidity and mortality rates associated with the chronic use of heavy immunosuppression. It seems that, in the future, the introduction of allograft tolerance will be the only way of overcoming these complications.

Strictly speaking, allograft tolerance is defined as the absence of any destructive reaction against alloantigens in the transplant by the host's immune system whilst the specific immune reactions of foreign or tumour antigens are preserved. This definition, which is entirely valid in the experimental context, must, however, be qualified when transferred to a clinical context in which it is difficult, if not impossible, to directly test the immune reactivity of the recipient to the donor's alloantigens. The term "operational tolerance" is coined to refer to a situation in which the long-term functional survival of the transplant is seen in the absence of chronic immunosuppression.

In animals, various therapeutic strategies have resulted in operational tolerance. Various problems of a practical and ethical nature have so far prevented strategies ultimately aimed at the total suspension of any immunosuppressant therapy from being transferred to the clinical situation. Based on evidence, the most effective way of acquiring transplantation tolerance is to use some of the immunological mechanisms that underpin physiological immunity tolerance. This is, in fact, an extremely effective approach. It is a case of

"reprogramming" the immune system to ensure that, whilst recognising the alloantigens, it does not generate an "aggressive" immune reaction towards them. Two concepts appear to be very promising for implementing strategies culminating in transplantation tolerance: central tolerance[1] through the depletion of alloreactive T-lymphocytes, and peripheral tolerance[2] involving T-cell regulation (which suppresses the effect of alloreactive T-lymphocytes).

During the 1950s, the Peter Medawar Group in Great Britain carried out experiments for which it received the Nobel Prize. The Group showed that newborn mice, the immune system of which was still immature, were particularly sensitive to the induction of tolerance following the injection of the donor bone marrow or allogeneic cells. This triggered immune mechanisms combining central and peripheral tolerance phenomena.

The reproduction of this type of phenomenon in an adult human being would involve drastic "conditioning" treatment of the recipient geared towards the total elimination of the entire hematopoietic system. So-called "intermediate" strategies have been tested in animals. These involve inoculating the donor's bone marrow cells into a recipient for whom "conditioning" does not imply complete myeloablation. This comprises partial myeloablation thanks to low-dose body irradiation combined with high-dose irradiation of the thymus compartment followed by short-term treatment with a polyclonal serum or monoclonal anti-lymphocyte antibody. After years of research conducted initially in mice and then in monkeys, a protocol has been devised to promote the very long-term, even indefinite survival of transplants without any immunosuppressant therapy. This type of approach was recently applied in a clinical setting in a small number of patients receiving renal allografts from living haplo-identical donors. These preliminary results are encouraging.

In mice, adequate "conditioning" of the recipient by administering antibodies targeting functionally important lymphocytic surface receptors has been seen to possibly trigger immune tolerance towards skin allografts. A key fact has been established: in rodents, immune tolerance can be induced with this type of strategy in thymectomised, adult hosts, thus proving that the underlying immune mechanisms arise from peripheral tolerance.

It would, therefore, appear that the immune system functions can be "reprogrammed" with biological products (monoclonal antibodies, etc.). Depending on their specific features, these products could eliminate the target cells or inhibit their function. They could also have an effect on the activation signals of certain specialist lymphocyte sub-populations or even effectively neutralise the action of cytokines or chemokines (which are subsequently involved in rejection mechanisms).

A number of these biological agents have demonstrated their ability to induce T-lymphocyte regulation. It must be emphasised that massive lymphocyte depletion does not appear to be a pre-requisite for inducing tolerance. In fact, many monoclonal anti-lymphocyte T antibodies possessing tolerogenic properties do not eliminate all of the T-lymphocytes.

All of the published data suggests that the mechanisms underlying the tolerogenic action of biological products are triggered to varying degree depending on cell depletion agents and immunoregulation involving both immune deviation and/or the induction of T-lymphocyte regulation.

It is important to note that the production of humanised or even human monoclonal antibodies, which are less immunogenic but better tolerated than the first generation of

[1] Central tolerance is established from the initial development sites of the lymphocytes (thymus, bone marrow).

[2] Peripheral tolerance is established in secondary lymphoid organs where the antigen is recognised (spleen, lymph glands, etc.)

antibodies introduced into the clinical setting, ensure that these therapeutic tools are used far more extensively.

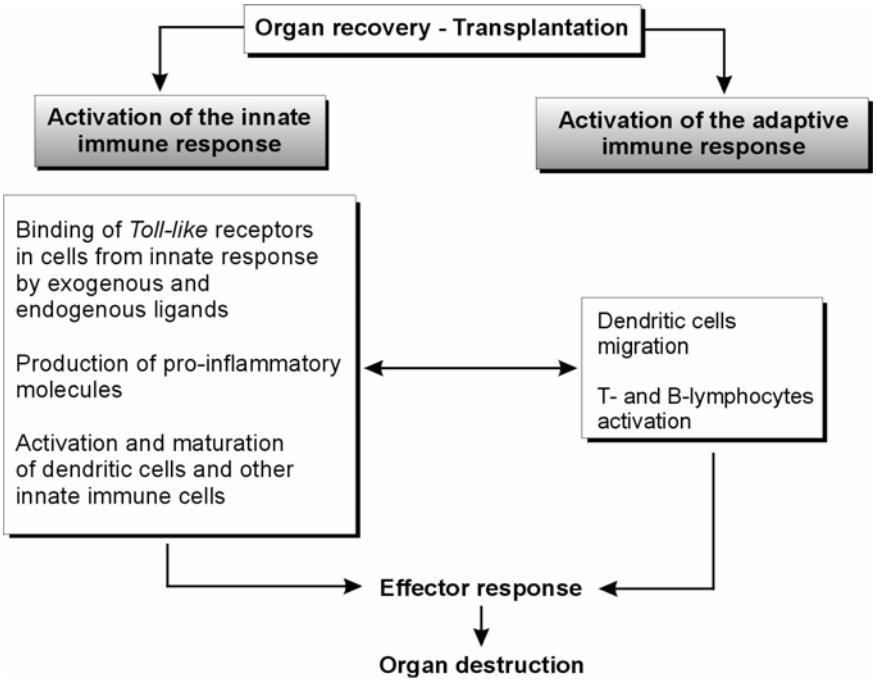
The *in-vitro* culture of specific sub-populations of immune cells that can be infused into transplanted patients in an attempt to "condition" them is an emerging treatment that has benefited greatly from the experience acquired in tumour immunotherapy. The two types of cell that generate the greatest interest are tolerogenic dendritic cells and regulatory T-cells. Although most of the studies focus on the use of donor dendritic cells, there is also evidence to show the marked immunoregulating capacity of recipient phenotype dendritic cells following adequate pre-treatment. The culture and expansion of regulating T-lymphocytes is another option. Recent data therefore shows that these cells, whether natural or adaptive, can be cultivated *in vitro* in an attempt to boost numbers whilst preserving their capacity for suppression.

Acute allograft rejection: Interaction between innate and adaptive response

Acute allograft rejection remains a problem in solid organ transplantation because it can trigger acute or chronic loss of graft function. It can occur from one week to several months after transplantation.

Two general immunological mechanisms are implemented during acute allograft rejection: the innate, non-specific immune response, which dominates the early phase of the immune response, and the donor-specific adaptive immune response resulting from the recognition of alloantigens by the recipient's T-lymphocytes.

Immediately after transplantation, injuries caused to the organ by retrieval procedure and ischaemia/reperfusion processes can trigger organ immunogenicity independent of the antigen. The innate response is initiated by danger signals, which activate the antigen-presenting cells, the dendritic cells of the transplant, leading to their differentiation and migration towards the recipient's lymphoid organs.



Interactions between innate and adaptive immune responses

In this way, naïf alloreactive T- and B-lymphocytes are thus stimulated and will become the effectors of the adaptive response. B-lymphocytes will produce alloantibodies and T-lymphocytes will migrate to the transplant. Other innate immune cells, such as polynuclear neutrophils, macrophages and NK (*Natural Killer*) lymphocytes also infiltrate the transplant in response to inflammatory stimuli, and can induce organ injuries via the production of pro-inflammatory molecules or by amplifying and supporting the adaptive T cell response. The attraction of mononucleated cells (monocytes, macrophages, etc.) to sites of inflammation results from the close interaction between inflammatory signals and chemokines. The inhibition of chemokines and their receptors has been shown to prolong allograft survival.

Different alloantigens can be recognised by the host's immune system during adaptive response: the major class I and II antigens from the donnor major histocompatibility complex (HLA in human), the minor alloantigens (allopeptides presented by class I or II molecules) and other antigens such as autoantigens or viral antigens recognised by cross-reaction with alloantigens.

Several cell types are involved in acute allograft rejection. The T CD4 lymphocytes (Th1, Th2 and Th17) and T CD8 lymphocytes contribute to the rejection. Cytotoxic T CD8 lymphocytes are involved in the effector response whilst T CD8 memory lymphocytes (Tm) are implicated in allo cross-reactivity responses. The presence of Tm cells in the recipient prior to transplantation increases the frequency and extent of acute rejection episodes. The mechanisms via which Tm cells recognise alloantigens could involve cross-reactivity between alloantigens and infectious agents or homeostatic proliferation (T-lymphocyte proliferation under conditions of lymphopenia).

An important role for B-lymphocytes and alloantibodies in acute rejection was recently described. The relevance of alloantibodies directed against the donor in the induction of acute rejection has been shown using very sensitive methods of detection of anti-donor antibody. For instance, this involves C4d immunofluorescence staining of peritubular capillaries in renal biopsies (suggesting the role of antibodies capable of activating the complement). This analysis could be important in the follow-up of patients and will allow to better adjust the treatment. The criteria for the diagnosis of acute humoral rejection in renal transplantation have recently been compiled by the *Banff Working Group.* These are morphological, immunohistological (C4d deposits, etc.) and serological criteria.

According to the "humoral theory in transplantation", there are the antibodies produced by cells that destroy the transplant. If the antibodies act as effectors of the acute rejection, their elimination should result in a reduction in immunosuppressant treatment. Alloantibodies found in patients mostly recognise antigens of the major histocompatibility complex HLA, HLA-related minor alloantigens and non-HLA antigens such as those of the ABO blood group. The presence of anti-donor alloantibodies is associated with a poor prognosis for graft survival.

Chronic rejection: An imbalance between aggression and adaptation

If the transplant survives acute rejection, its functions may seem normal for a certain period of time, varying in length from one person to the next. Chronic rejection occurs in approximately 50% of transplanted patients. Since the early 1980s, one-year survival of renal organ transplants has increased significantly, nowadays exceeding 90%. Nevertheless, the long-term results have scarcely altered and, in particular, the percentage of transplants lost each year after the first year of transplantation has not changed.

Chronic rejection is characterised by the slow, constant occlusion of the arteries, veins and other tubular structures in the transplanted tissue. Vascular occlusion triggers ischaemia leading to necrosis and tissue fibrosis. Our understanding of the mechanisms involved in the onset of chronic rejection has improved considerably thanks to animal models (rodents), which have been used to recreate arteritic cell injuries in various heart transplant or allogenic vessel models. An increase in the intima causing a reduction in the size of the vessel followed by destruction of the internal elastic lamina (one of the three layers of the intima) have been highlighted during chronic rejection. Thickening is associated with accumulation of the extracellular matrix and the proliferation of myofibroblast cells.

The involvement of lymphocytes in the origin of chronic rejection injuries has been investigated in genetically debilitated mice. These models show that activated T-lymphocytes are needed to initiate the chronic rejection phenomenon. The existence of C4d deposits in immunofluorescence suggests the role of anti-HLA antibodies capable of activating the complement. The onset of chronic rejection is not, however, simply the change-over from acceptance to chronic rejection but a continuum between these two states, based on a subtle balance between aggression factors (cytotoxic T-lymphocytes, antibodies and complement, etc.) and the mechanisms of survival and adaptation of target cells.

In addition to activating the complement, the antibodies can bind to the surface molecules of target cells or even recruit other cells. An endothelial cell activation stage is related to the expression of various receptors on the surface of these cells, and the synthesis of numerous growth and endothelin 1 factors. Endothelin 1 promotes the stimulation of smooth muscle cells and, indirectly, the local synthesis of angiotensin II. It also triggers the local recruitment of inflammatory cells and activates coagulation by promoting platelet adhesion and releasing thromboxane A2. Finally, it stimulates the differentiation and proliferation of cells that synthesise the extracellular matrix involved in chronic rejection injuries, namely the myofibroblasts.

The presence of class I and, more particularly, class II anti-HLA antibodies is an independent risk factor in chronic rejection. In the case of renal transplants, over 80% of patients with allograft glomerulopathy have anti-HLA antibodies, 85% of which are directed against a class I or class II antigen. However, other antibodies (*MHC class I-related molecules A and B,* anti-endothelial cell antibodies and anti-vimentin antibodies, etc.) are also linked to structural changes observed during chronic rejection. The minor antigens can thus stimulate the extracellular expression of a certain number of components in the cytoskeleton resulting in lymphocyte stimulation.

Fibroblast cells are important components in chronic rejection injuries. They have several different origins: differentiation of stem cells circulating in endothelial cells or in myocardiocytes, the transdifferentiation of differentiated endothelial cells into myofibroblast cells and the transdifferentiation of epithelial cells (renal tubular cells) into myofibroblasts.

This differentiation of cells into myofibroblasts and their expansion involves numerous growth factors implicated to varying degree in the initiation of epithelio-mesenchymatous transdifferentiation and the expansion and migration of these cells. The factor most widely investigated nowadays is TGF-β (*Transforming Growth Factor*-β). Other growth factors (*Hepatocyte Growth Factor, Bone Morphogenic Protein*) antagonise TGF-β action, either by blocking the TGF-β activation pathways or by allowing myofibroblast cells to reacquire an epithelial cell phenotype. The perfusion of one of these factors (*Bone Morphogenic Protein*) in a chronic rejection model can inhibit the onset of chronic rejection. Molecules involved in the activation of endothelial cells could also play an initiating role or intervene to perpetuate chronic rejection injuries. This applies to endothelin I and angiotensin II. It has recently come

to light that the presence of anti-angiotensin II receptor antibodies is linked to the onset of chronic rejection.

During various episodes of immunological and non-immunological aggression, the endothelial cells will be responsible for the formation of an extracellular matrix by synthesizing growth factors or cytokines. The accumulated extracellular matrix comprises a fibrotic lesion. This lesion can be prevented or broken down using blocking molecules or by activating various tissue proteases including metallo-proteases. These approaches have yet to be validated in clinical models.

A certain number of clinical risk factors have been associated with transplant dysfunction. The transplantation of an organ from an elderly donor is associated with an increase in the incidence of acute and chronic rejection. This is correlated with the onset of organ ageing resulting in the release of pro-inflammatory cytokines with molecular expression inducing neo-antigens either directly or indirectly. Moreover, different stress situations triggered by ischaemia or infection (viral, bacterial and fungal, etc.) can contribute to this rejection. Local regulation of the innate immune system should limit the impact of these events on the onset of chronic rejection.

Prevention and treatment of rejection: Immunosuppression

Regardless of the organ in question, the treatment of rejection is primarily based on prevention and then on curative treatment if preventive measures fail.

The prevention of rejection is based on immunosuppressant treatment, which is essentially adapted in line with the recipient's "immunological" risk (history of immunisation by transfusion, grafts, etc.). This immunosuppressant treatment involves combining several drugs with different mechanisms of action but it is currently based on anticalcineurin. Induction treatment[3] with a biological immunosuppressant (anti-lymphocyte or anti-interleukin 2 receptor antibody) is often initially prescribed for several weeks in order to boost the overall level of immunosuppression. It allows nephrotoxic anticalcineurins to be introduced at a later stage.

Immunosuppressants used in solid organ transplantation

Class of immunosuppressant	Immunosuppressant
Corticosteroid	Prednisolone Prednisone Methyl prednisolone
Anti-proliferative	Azathioprine Mycophenolate mofetil Sodium mycophenolate
Calcineurin inhibitor	Cyclosporine Tacrolimus
TOR inhibitor	Sirolimus Everolimus
Anti-lymphocyte polyclonal antibodies	ALG ATG ALS
Monoclonal antibodies	Muromonab-CD3 Basiliximab Daclizumab

[3] This is a treatment that allegedly reduces the incidence of acute rejection in the 3 months following transplantation.

Prevention is currently effective since the incidence of acute rejection is below 15%. The role of mTOR inhibitors (*mammalian target of rapamycin*) in the treatment of rejection is still unclear except perhaps in heart transplantation where these new immunosuppressants can prevent the clinical advance of a transplanted myocardium to vasculopathy.

The curative treatment of acute rejection is relatively homogeneous at the present time. Acute cell rejection is treated with high doses of steroids in the case of less severe forms of rejection and anti-lymphocyte antibodies in severe forms. Acute humoral rejection is treated with non-standardised therapy combining steroids, plasmapheresis, immunoglobulins (intravenous administration) and anti-CD20 antibodies. Progress is needed in order to make new, more specific and better tolerated treatments available for the management of acute cell rejection, to standardise humoral rejection treatment (studies underway) and to provide molecules that have an actual effect on plasmocytes and memory B-lymphocytes and on the complement-dependent effector response.

The treatment of chronic rejection is still in its early stages and is based on the possibility of clearly defining chronic rejection and separating immunological and non-immunological mechanisms in an attempt to establish the need for increased or even modified immunosuppression or, conversely, a decrease in immunosuppression. Histological analysis clarifying transplant biopsies steered by a clinical sign or laboratory result or targeted towards screening should promote a better understanding of the physiopathology of this type of rejection and thus facilitate more appropriate treatment. This histological classification should be refined by developing and validating all types of biomarkers, and by defining biomarkers for fibrosis or fibrogenesis of chronic rejection or even nephrotoxicity or viral infection. The relevant tools are already in place and tests are underway.

Treatment optimisation: adaptation and individualisation of immunosuppression

The quest to optimise immunosuppression is currently based on several strategies: the removal of drugs that are less well tolerated (anticalcineurin and steroids), the optimal use of available drugs (pharmacological adaptation), treatment individualisation thanks to pharmacogenetics in particular and induction or screening strategies for tolerance.

Some studies carried out in an attempt to minimise anticalcineurin dose levels have shown that this approach does not increase the incidence of acute rejection. Conversely, the results obtained in terms of improving renal function remain mixed.

Conversion strategies comprise a gradual reduction in anticalcineurin up to withdrawal by replacing this class of immunosuppressants with a non-nephrotoxic immunosuppressant. In patients with a stable kidney function, this strategy was initially carried out with mycophenolate mofetil away from transplantation (delayed conversion). The improvement observed in terms of kidney function was not off-set by the consequences of over-risk of rejection due to the withdrawal of anticalcineurins. The results suggest that the main advantage of withdrawing anticalcineurins and the delayed conversion with non-nephrotoxic sirolimus (mTOR inhibitor) is a significant, early reduction in the incidence of cancer. Furthermore, the early withdrawal of cyclosporine (anticalcineurin) and its replacement by sirolimus certainly boosts the function of the transplant but at the cost of an increase in the incidence of acute rejection. Finally, within the scope of conversion studies carried out in patients resenting with chronic transplant dysfunction, the early replacement of cyclosporine by mycophenolate mofetil stabilises or improves kidney function.

As regards strategies geared to the initial, non-introduction of anticalcineurins, it seems that the role of mTOR inhibitors used as soon as possible after transplantation is currently very restricted due to a significant increase in the incidence of acute rejection. Belatacept, a molecule that blocks the costimulation signal between the dendritic cell and the T-lymphocyte looks promising for the future and is currently being tested in patients with a low immunological risk, in subjects with a high risk of anticalcineurin-mediated nephrotoxicity and in delayed conversion in stable patients.

Most of the steroid reduction studies published definitely show an advantage in terms of a reduction in the incidence of complications such as dyslipidaemia and arterial hypertension but, at the same time, highlight an increase in the incidence of acute rejection episodes, which is not always accompanied by a harmful effect on mid-term transplant survival.

Tolerance-inducing strategies are aimed at achieving withdrawal of post-transplantation immunosuppression due to specific recipient-donor tolerance. A reduction in the toxicity of the various drugs used obviously constitutes a considerable advantage in addition to the disappearance of clinical signs of over-immunosuppression such as infection and cancer. Several studies highlighting the genuine induction of tolerance in transplant recipients were reported in 2008. These look extremely promising for the future.

Pharmacology (pharmacokinetic, pharmacodynamic and pharmacogenetic) resources have also been used to optimise the adaptation of immunosuppressant therapies with the two-fold objective of limiting their toxicity and individualising dosage. Anticalcineurins are medicinal products with a narrow therapeutic spectrum, which means that the margin between efficacy and toxicity is narrow. In addition, there is also considerable intra-individual and inter-individual variability. Evaluation of the area-under-the-curve as an indicator of exposure to immunosuppressants (pharmacokinetics) has been used in the case of mycophenolate mofetil in particular with even more controversial results vis-à-vis a reduction in the incidence of acute rejection. Pharmacogenetics, which investigates the effect of genetic variability in certain metabolism genes on immunosuppressant activity, is probably more innovative even if its application in transplantation is still in its infancy. The dose of tacrolimus required in order to obtain a value within the therapeutic window varies in the presence of certain alleles (CYP3A). The exact role of these interactions in clinical practice, in terms of how quickly function is restored and the early prevention of acute rejection, has yet to be defined.

These new technologies should allow immunosuppressant therapies to be individualised more effectively not only to prevent toxicity and boost efficacy, but also to ensure overall suitability.

New immunosuppressants: Other efficacy criteria

The current limitations of immunosuppressant therapies are the lack of efficacy in chronic rejection and reduced efficacy in humoral and cell rejection coupled with their overall, essentially renal, toxicity. Moreover, an increase in the level of non-specific immunosuppression and the intrinsic properties of certain drugs promote the onset of post-transplantation cancers. This risk is increased as the recipient population ages and is more exposed to the risk of cancer. Furthermore, the number of immunised patients at high immunological risk awaiting organ transplants is increasing and the use of so-called "marginal" transplants, which are particularly susceptible to immunosuppressant nephrotoxicity, is becoming increasingly common practice during a period characterised by organ shortage.

Consequently, new immunosuppressants are required to have a novel mode of action to complement that of existing immunosuppressants, a good benefit/risk ratio and no nephrotoxicity. They should also be effective in the treatment of acute and chronic rejection, possess anti-tumour properties and be devoid of any pro-tumour effect. More specifically, immunosuppressants that control memory cells, block alloantibody synthesis and inhibit processes that extend chronic rejection are required. Finally, we need drugs that can trigger tolerance and, at the very least, that do not block the induction of tolerance.

The clinical development of immunosuppressants is governed by the complex regulations of clinical research and national health agencies. Many of the molecules that look promising during *in-vitro* studies and laboratory animal experiments are not granted marketing authorisation for humans because of their side effects, which are often detected in latter stages, or their low benefit/risk ratio. Drugs currently under evaluation are also subject to the same fate if the results of phase III studies are unknown.

Immunosuppressants under development

Molecules	Type	Development stage
ISA 247	Signal 1 inhibitor Cyclosporine analogue	Phase III in renal transplantation
AEB 071	Signal 1, signal 2 inhibitor Protein kinase C inhibitor	Phase II
Belatacept	Signal 2 inhibitor CTLA4 and IgG1 fusion molecule	Phase III in renal transplantation
CP 690, 550 and NC 1153	Signal 3 inhibitor JAK3 inhibitor	Phase II
Humanised anti-CD3	Signal 1 inhibitor	Phase II in renal transplantation
Alemtuzumab	Humanised anti-CD 52 monoclonal antibody	
Rituximab	Anti-CD 20 antibody	

ISA 247 is a cyclosporine analogue, a lymphocyte activation signal 1 inhibitor. It seems to be more effective than cyclosporine in preventing acute rejection and is less nephrotoxic. It is being developed in the following indications: psoriasis and renal transplantation. It remains to be seen whether the advantages described are evident in a large patient population.

AEB 071 is a potent protein kinase C inhibitor. It inhibits signal transduction via the T-lymphocyte receptor (signal 1) and co-receptor CD28 (signal 2). Administered via the oral route, it possesses good clinical safety. Phase II clinical trials are underway, combining AEB 071 with everolimus, tacrolimus and mycophenolate mofetil. Clinical tolerance appears to be good and AEB 071 does not seem nephrotoxic. It therefore ranks well to replace calcineurin inhibitors.

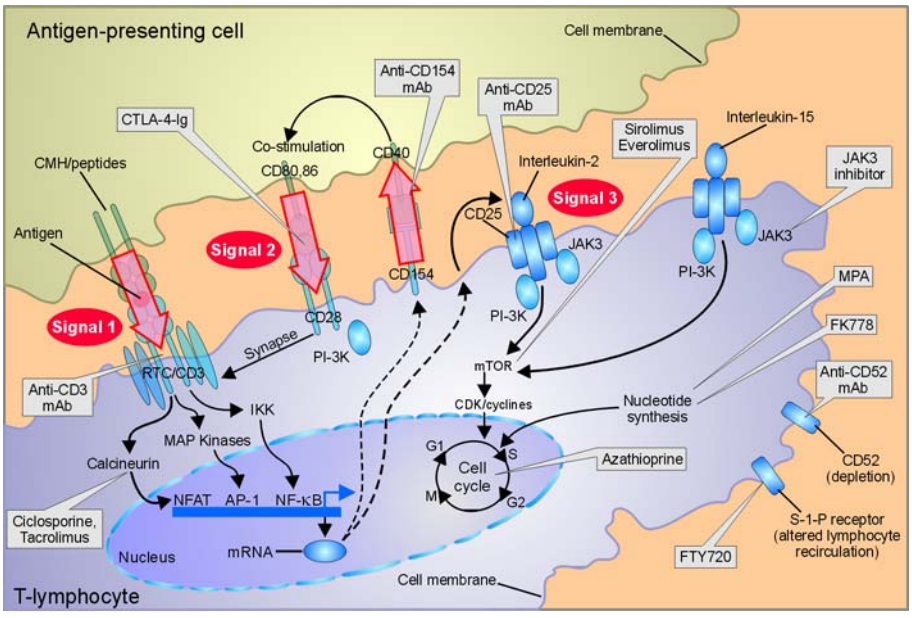
Belatacept is a fusion molecule comprising CTLA4 and an IgG1, modified in order to boost affinity for CD80/CD86. It specifically blocks signal 2 and is currently under investigation in phase III clinical trials. A phase II renal transplantation clinical trial confirmed efficacy similar to that of cyclosporine and without nephrotoxicity in the management of acute rejection. The strong points of this fusion molecule are its original mode of action, efficacy and excellent clinical tolerance coupled with its potential to trigger tolerance. Its weak point is that it has to be administered via parenteral injection, but this could be useful for promoting therapeutic compliance. It is not specifically effective in chronic rejection and has no anti-tumour efficacy.

Tyrosine kinase inhibitors JAK 3, CP 690,550 and NC 1153 inhibit the transduction of the signal mediated by 5 cytokine receptors, namely IL-2, IL-7, IL-9, IL-15 and IL-22. The mode of action is original and specific to immune system cells. Preclinical studies reveal similar efficacy to that of cyclosporine in preventing acute rejection. Phase II clinical trials are currently being carried out. The main limitation is anaemia linked to crossed blockage of tyrosine kinase JAK 2 associated with the erythropoietin receptor.

Humanised, non-mitogenic anti-CD3 molecules display considerable potential for the prevention and treatment of acute rejection in organ transplantation and the treatment of cell-mediated auto-immune diseases such as auto-immune diabetes. Well tolerated, these molecules are currently at the phase II/III stage for auto-immune diabetes, and at the phase II stage in the treatment of renal allograft rejection. Humanised anti-CD3 molecules trigger tolerance in murine models by promoting the emergence of regulating T-lymphocytes, which are, in fact, highly promising, future immunosuppressants.

Alemtuzumab is a humanised anti-CD52 monoclonal antibody that depletes T-lymphocytes, B-lymphocytes and monocytes. It is used in induction therapy and to treat acute, corticosteroid-resistant rejection. It can reduce the immunosuppressant maintenance, anticalcineurin and corticosteroid doses. Controlled, prospective, comparative studies with thymoglobulin or IL-2 anti-receptor antibodies are needed to define its role in induction therapy in transplantation.

Rituximab is an anti-CD20 antibody that depletes B-lymphocytes by apoptosis. It triggers deep-seated, lasting, peripheral lymphopenia. Widely used over the last 4 years in the treatment of antibody-mediate auto-immune diseases, it could reduce the synthesis of anti-HLA antibodies. Its efficacy must be confirmed in prospective, randomised studies that are not currently available.



Sites of immunosuppressant action during immune response (according to Halloran, 2004)

AP-1: activating protein-1; CDK: cyclin-dependent kinase; MHC: major histocompatibility complex; IKK: IkB kinase; JAK 3: Janus kinase 3; mTOR: mammalian target of rapamycin; NFAT: nuclear factor of activated T-cells; NF-kβ: nuclear factor-kβ; PI-3K: phosphoinositide-3-kinase; TCR: T-cell receptor; S-1-P: sphingosine-1-phosphate

The efficacy evaluation criteria used to develop new immunosuppressants over the last 15 years must be modified due to an improvement in transplantation outcomes and the reduction in the incidence of acute rejection – the principal efficacy criterion in most studies. Composite efficacy criteria must allow the short-term evaluation of long-term changes in the

transplant and must also take into account the function of the transplant, histology through routine biopsies and genomic biomarkers. Current prospective studies are in the process of validating these biomarkers.

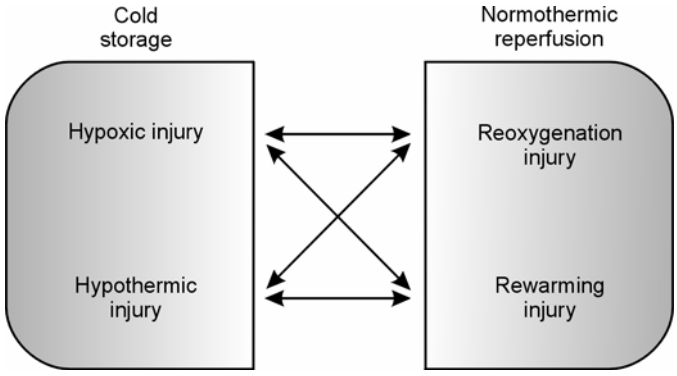
Studies must be carried out for at least three years in order to evaluate chronic rejection. The toxicity of immunosuppressants and nephrotoxicity in particular, must be one of the principal criteria. Lastly, future immunosuppressants could be evaluated in targeted populations such as recipients with a high immunological risk, elderly recipients and patients receiving a marginal transplant.

Ischaemia / reperfusion syndrome: What are the mechanisms involved?

The stages in transplantation, which range from retrieving the organ from the donor, preserving the transplant (ischaemia phase) through to its implantation in the recipient (reperfusion), are accompanied by molecular, cell and tissue changes in the transplant. The physiopathological processes responsible for transplant injuries are defined as ischaemia/reperfusion (I/R) syndrome in organ transplantation. I/R syndrome manifests as an alteration in organ function, partly due to persistent vasoconstriction disrupting the blood flow.

The damage caused by ischaemia/reperfusion promotes acute rejection and, in particular, the formation of chronic injuries on the transplant. The most tangible effect of I/R is the delayed graft function. I/R also has a significant impact on primary graft failure, which is incompatible with recipient survival, and for which retransplantation is the only treatment available.

Ischaemia/reperfusion syndrome is associated with hypothermia and organ hypoxia during preservation, and with reoxygenation during reperfusion. It should be noted that this syndrome also incorporates lesions, which can appear in the donor during brain death. This causes pro-inflammatory lesions to appear and activates endothelial cells.



Origins of ischaemia/reperfusion in the transplant

Maintaining the viability of the transplant during its ischaemic transfer from donor to recipient is mainly based on hypothermia, which is deliberately applied to reduce metabolic activity. Tolerated periods of cold ischaemia vary depending on the organ: 24 hours for the kidney, 10 to 12 hours for the liver, a maximum of 8 hours for the lung and 6 hours for the heart. Prolonged cold ischaemia is an independent risk factor for the non-functioning or dysfunction of the transplant. Data confirm, for instance, 57% survival at 5 years for transplanted livers when cold ischaemia exceeds 15 hours compared to 67% with cold ischaemia of less than 12 hours' duration.

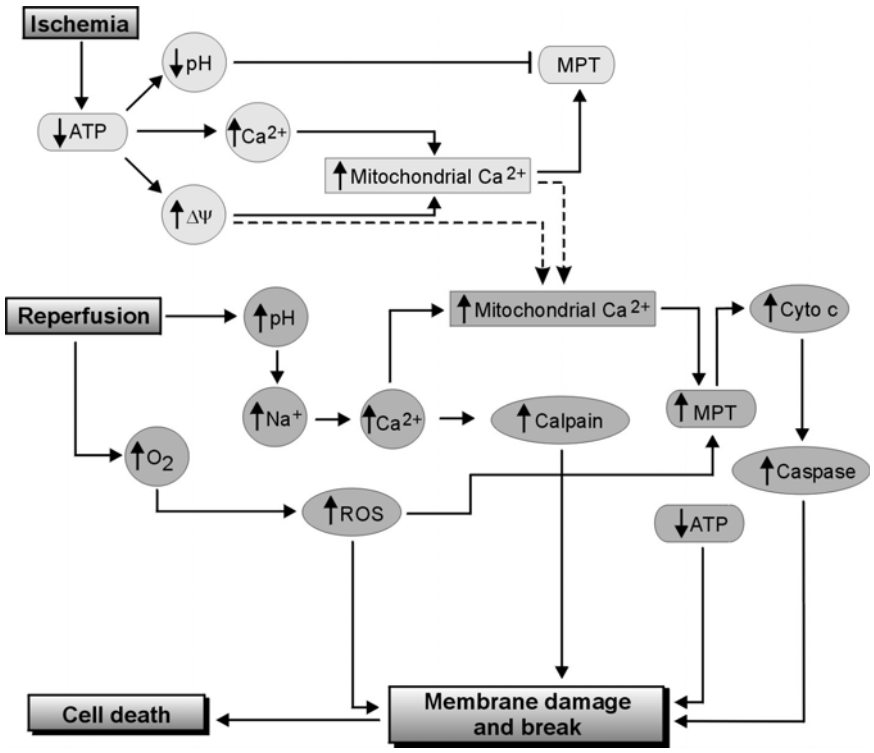
The potential use of organs from older donors or donors who suffered cardiac arrest to increase the number of organs available for transplantation requires suitable, high-performance preservation methods based on a better understanding of the cell and molecular mechanisms associated with I/R.

Although a fundamental requirement, the chilling of organs has harmful repercussions on the tissues due to oxidative stress (production of reactive oxygen species) and inflammation (cytokine production), which are probably responsible for the exacerbation and, above all, persistence of this condition. Significant structural changes in the cytoskeleton result in dislocation of the endothelial cells.

As regards the cells, several metabolic pathways are affected: inhibition of the Na^+/K^+ ATPase pump causing cell oedema, rapid depletion in ATP reserves, and problems associated with calcium and anaerobic glycolysis homeostasis responsible for intracellular acidosis. An increase in Ca^{2+} concentration triggers mitochondrial dysfunction by disrupting its membrane permeability.

Depending on the residual ATP level (depending on the duration of the ischaemia), this dysfunction will manifest as apoptosis or necrosis. The ATP concentration therefore acts as a "switch" between these two types of cell death. When the transition of mitochondrial membrane permeability is accompanied by ATP depletion (prolonged ischaemia), the apoptotic signal is blocked and necrosis develops. *On the other hand,* if glycolytic substrates are available, deep ATP depletion is prevented and the process leads to apoptosis.

The few molecular studies of gene expression during I/R carried out to date focus on the reperfusion phase. They have highlighted the role of certain signalling pathways such as pro- or anti-apoptotic pathways, the HIF (*Hypoxia Inducible Factor*) pathway or heme-oxygenase 1.



Principal pathways leading to cell death during ischaemia / reperfusion (according to Murphy and Steenbergen, 2008)

Cyto c: cytochrome C, ROS: Reactive oxygen species, MPT: mitochondrial membrane permeability transition pore, $\Delta\psi$: mitochondrial membrane potential

I/R is also implicated in the link between the injuries that it generates and innate immunity via maturation of dendritic cells and the *Toll-like* receptor pathway.

The identification of the physiopathology of I/R should improve its clinical and therapeutic management. An understanding of the mechanisms of physiological adaptation to ischaemia-induced stress is undoubtedly one of the most promising avenues of research in terms of medical applications and the development of suitable preservation techniques. This understanding must be acquired globally and should involve the use of integrated methods currently available such as genomics, proteonomics and metabolomics.

Ischaemia/reperfusion syndrome: Therapeutic strategies

The therapeutic resources currently available to prevent I/R syndrome are mainly hypothermia at 4°C and the composition of preservation solutions. Several limiting factors must, however, be considered: the undesirable effects of hypothermia itself, the lack of fast, simple methods to evaluate organ viability, the inevitable appearance of I/R, the presence of hypoxia and rewarming. Preservation performance in cold ischaemia is based essentially on hypothermia-induced inhibition of metabolism, a short preservation period, the suppression of cell oedema thanks to impermeant agents (polyethylene glycol, etc.) and, during reperfusion, the stimulation of energy metabolism by growth factors.

Preservation solutions vary considerably from one transplantation centre to the next and are always subject to modifications concerning optimal ionic composition and the use of new pharmacological agents in particular. The general consensus tends towards solutions containing a minimal quantity of potassium due to the harmful effect of the latter on endothelial function. The pharmacological agents recommended in recent publications for limiting organ changes include numerous nitric oxide (NO) donors, MAPK (*Mitogen Activated Protein Kinase)* inhibitors, new anti-oxidising agents and pleiotropic compounds such as erythropoietin (EPO) or statins. The conditioning (temperature, oxygenation, short or continuous perfusion) of transplants during the period of arrest (especially cardiac and pulmonary), is still subject to controversy.

Continuous, hypothermic (4°C) or normothermic (37°C) perfusion is a protection strategy that maintains a supply of oxygen to the organ. Normothermic perfusion also allows the problems associated with cold ischaemia to be avoided. This perfusion technique also enables toxic products that have accumulated in the tissue to be eliminated, the cell pH to be controlled, transplant visibility markers to be measured, cytoprotective agents to be released and sub-optimal organ viability to be improved by "post-conditioning". In warm ischaemic models, preservation by normothermic perfusion could significantly improve graft survival.

Various donor pre-treatment strategies have been investigated in order to improve organ tolerance to ischaemia/reperfusion. Beneficial effects on graft survival have been obtained by directly protecting the donor using pharmacological agents likely to inhibit harmful molecules or to strengthen protective metabolic pathways, but treatment specificity is still reduced whilst costs are high. Although (anti-apoptotic or anti-oxidative) gene therapy is both appealing and effective, numerous problems still beset this therapeutic strategy, at least in small animals.

Surgical strategies such as ischaemic pre-conditioning[4] can be envisaged in routine clinical applications. Brief reperfusion periods alternating with reocclusion applied at the start of

[4] Ischaemic pre-conditioning comprises a brief period of ischaemia followed by reperfusion, thus protecting against severe, posterior ischaemia/reperfusion.

reperfusion (post-conditioning) are also capable of triggering a protective effect by preventing the mitochondrial membrane permeability transition pore from being opened.

Recent publications highlight the option of acting on transplants during the I/R period not only to limit the alteration in organ function, but also to reduce its immunogenicity and boost protection against the immune host response. At the present time, innate immunity activation clearly plays a major role in ischaemia/reperfusion-related damage and contributes to organ failure as well as acute and chronic rejection. The oxidation of non-native proteins (by reactive oxygen species produced during I/R), activates TLR (*Toll Like Receptors*) in innate immune system cells and particularly in dendritic cells capable of triggering the adaptive, allo-immune response. On a more general note, various molecules are likely to be exposed during ischaemia/reperfusion. These act as antigens, thus altering acceptance of the transplant. This implies new developments in terms of protection against I/R aimed at reducing the immunogenicity of the transplant.

Renal transplantation: Extending the donor pool

Despite a significant increase in the number of organ procurement intended for renal transplantation in France in recent years, the shortage of organs for transplantation due to the epidemiology of chronic renal disease poses a major problem.

In France, 2911 renal transplantations were carried out in 2007, 3510 new patients were added to the waiting list during this period and 6491 patients were awaiting renal transplantation as at 1 January 2008 (data provided by the Biomedicine Agency[5]).

There are consequently 3.3 candidates for every transplant that can be used during the year. This epidemiological situation calls for an increase in potential sources of transplants. Several strategies are being developed along these lines to extend the source of transplants (to include organs from so-called "marginal" donors in particular), to develop grafts from living donors, to encourage the use of organs recovered after cardiac arrest and to carry out transplantation with incompatible ABO grafts.

The shortage of transplants and the demographic changes affecting donors have led to the definition of the so-called "marginal" donor concept. This refers to organs retrieved from elderly donors who have died from cardiovascular causes or who present with cardiovascular risk factors or a reduction in glomerular filtration. Criteria have been established, mainly from studies of American registers, highlighting scores that accurately define organs retrieved from these donors. The use of such organs is, by definition, associated with a shorter organ survival time compared to that obtained with so-called "optimal" transplants. The long-term survival of marginal transplants is 5 to 15% less than that obtained with so-called optimal transplants. The survival of patients transplanted with a marginal kidney is nevertheless better than that recorded in dialysis patients on the waiting list, with an average increase in life expectancy of the order of 5 years.

These transplants nowadays constitute a precious source of organs and future stakes are, therefore, based on implementing strategies to optimise their use. Based on recently identified factors that condition the transplantation success rate, these strategies include:

- Transplant selection methods based on clinical or even histological criteria;
- Criteria for allocating and defining the donor/recipient match;

[5] The 2007 evaluation of organ procurement for renal transplantation in France can be viewed at the following address://www-agence-biomedicine.fr/fr/rapport_2007.

- Immunosuppressive strategies;
- Evaluation of preservation techniques.

Transplantation involving living donors, which was a marginal practice in France up to the revision of the bioethical laws (law No. 2004-800 of 6 August 2004), has witnessed significant developments since this period. International publications reporting on experience acquired with the transplantation of organs from living donors all show that the survival of a renal transplant obtained from a living donor is significantly better than that recorded with organs recovered from deceased donors. The data contained in the 2007 North-American UNOS (*United Network for Organ Sharing*) register confirmed an 80% survival rate for the transplant after five years compared to 67% for organs retrieved from deceased donors. Patient survival was also significantly better than that recorded with organs transplanted from deceased donors. HLA compatibility does not significantly affect the outcome of transplantation using living donor organs. Although the age of the donor conditions recipient survival, the survival rate recorded with an organ transplanted from an elderly living donor is always higher than that obtained with a kidney transplanted from a deceased donor of the same age. In the case of ABO-incompatible living donors, long-term survival results obtained in Japan and the United States are comparable to those obtained in the case of an ABO-compatible donor/recipient match. The use of a novel immunosuppressant agent specifically targeting B-lymphocyte populations, namely the anti-CD20 antibody, has led to ABO-incompatible transplantations being carried out without splenectomy. The success rate with this approach is entirely comparable to that obtained in transplantations involving routine splenectomy.

Donor and recipient evaluation strategies are particularly important in order to obtain a better definition of indications for transplantation with living donor organs.

Since the early 1990s, many European countries and the United States have been developing transplantation programmes using organs recovered from non-heart-beating donors (NHBD). These organs are retrieved from donors selected in accordance with the criteria compiled by the so-called International Maastricht Classification.

Classification of non-heart-beating donors (according to Van Raemdonck *et al.*, 2004)

Categories	Criteria
I	Dead on arrival
II	Unsuccessful resuscitation
III	Awaiting cardiac arrest
IV	Cardiac arrest in a brain-dead donor
V	Eqivalent to group II, but occurring in hospital

In France, organ recovery is feasible only from donors in categories I, II and IV.

The incidence of primary graft non-function is of the order of 4 to 6% for non-heart-beating donors and is henceforth comparable to that observed for heart-beating donors. All of the publications show identical transplant survival rates at one, five and ten years with comparable organ functions regardless of whether the deceased donor is brain death or has suffered cardiac arrest. The excellent results obtained can be attributed to improved donor and recipient selection, compliance with warm and cold ischaemia timescales and the emergence of significant therapeutic innovations in donor and organ management. Donor selection is of paramount importance for successful transplantation from a non-heart-beating donor and the risk factors for the recipient have now been clearly identified, such as the presence of glomerulosclerotic and fibrotic lesions, or a history of cardiovascular disease.

Therapeutic innovations include extracorporeal circulation for donor management and perfusion machines for transplant preservation, which significantly reduce the incidence of delayed graft function. A pilot programme focusing on organ recovery from "non-heart-beating donors" has recently been implemented in France.

The recovery of organs from non-heart-beating donors represents a considerable resource. Overall, results are satisfactory in cases where optimal technical infrastructure and logistics are employed.

Liver transplantation: Marginal donors and alternative approaches

The main limitation for liver transplantation in France as in other western countries is the difference observed between the number of organ donors and the number of patients who could benefit from transplantation. Given this deficit, the general consensus is that transplantation must only be offered to patients who are most likely to benefit from this approach. In practice, the transplantation indication is open to discussion if life expectancy after transplantation is less than 50% at one year. Despite this selection process, the number of potential recipients continues to outweigh the number of donors.

Approximately 1000 liver transplants are carried out annually in France (1061 in 2007). 1200 to 1300 new candidates are added to the waiting list each year (1348 new entries in 2007 with 575 still on the waiting list in early 2008). The death rate in waiting list is around 10% per year.

The shortage of donors coupled with excellent transplantation results have led to the gradual expansion of donor selection criteria to include organs transplanted from so-called "marginal" donors. Liver transplantation from non-heart-beating donors[6] or living donors is also increasing. Finally, regardless of the actual donor, alternative transplantation techniques lead to the use of marginal transplants.

An ideal donor would be a 40 year-old, brain dead, trauma victim with stable haemodynamics and devoid of steatosis or any other underlying, chronic parenchymatous lesion at the time of organ recovery and with no transmissible disease.

In the case of an "ideal" donor, the risk of graft non-function or graft dysfunction culminating in death or retransplantation is less than 5%. By definition, a marginal donor is a donor with one or more different characteristics compared to an ideal donor. Some factors that do not affect the risk of graft dysfunction, such as transmissible diseases, must be taken into account in the marginal donor definition.

Brain death has numerous circulatory and metabolic repercussions, which may impact upon subsequent liver graft function. However, provided that the liver parenchyma is normal, the liver is one of the organs most resistant to these types of complication.

Marginal transplants retrieved from elderly donors or donors with steatosis are more sensitive to cold ischaemia. Macrovesicular steatosis, which is an entirely benign disease that is reversible in the non-transplanted population, is a major risk factor in early transplant dysfunction. The capacity to regenerate and tolerate ischaemia/reperfusion injuries differs considerably from that observed with non-steatotic transplants. Steatosis in excess of 60% is usually contra-indicated in transplantation. Regardless of the degree of steatosis, the presence of related lesions (marked inflammatory infiltrates and fibrosis, however subtle) is

[6] Although authorised by decree, organ recovery from a non-heart-beating donor is not yet carried out within the scope of liver transplantation in France.

another contra-indication. The steatosis regresses when the transplant starts to function satisfactorily. There is no conclusive evidence to suggest that this has a significant impact on long-term graft survival, regardless of other risk factors. It can be assumed that specific, combined measures (reduction of cold ischaemia, appropriate preservation solution, decrease in liver volume) could extend the use of steatotic lesions. The potential increase in the number of donors is significant.

In recent years, the average age of donors has increased in France as in most other European countries and the United States of America. There is no formal donor age limit for liver transplantation. However, the ability of the liver to regenerate decreases with age. Advanced age is an independent risk factor in graft failure, and the corresponding risk is on-going. The age of the donor also has a particularly marked impact on recipients presenting with the hepatitis C virus (HCV). The recurrence of hepatitis C is more severe and the clinical course of fibrosis more rapid when the donor is elderly. The risk is exacerbated with donors over 40 years of age. Apart from the specific case of hepatitis C, no other high-risk group has been identified in relation to older donors.

Bacterial infection documented in the donor (including a central nervous system infection) is not a contra-indication to liver transplantation. On the other hand, HIV infection is a current contra-indication for transplantation. The use of donors with "cured" hepatitis B, positive anti-HBc antibodies (Ab) $\pm$ positive anti-HBs antibodies is also feasible within the scope of an exceptional protocol in France. Moreover, as a general rule, the presence of cancer or a history of cancer in the donor represents a contra-indication in transplantation.

The split liver technique is widely advocated since two recipients can share an organ retrieved from just one donor. Thus a liver transplant recovered from a brain-dead patient can be split into 2 autonomous semi-transplants (right and left). However, only organs of optimal quality can be split. Transplants which are prone to a risk factor (steatosis, advanced age, etc.) cannot be split because the cumulative risk is too high. Children are the main recipients of a left transplant. Although the paediatric waiting list is short compared to that of adults, an adult can rarely be transplanted with a left transplant. This type of procedure calls for excellent co-ordination and close geographical proximity between a paediatric transplantation team and an adult transplantation team. These conditions are met in only a few regions of France. Similarly, two transplantations from a single split transplant are seldom carried out in one centre at the same time.

The transplantation of a split transplant from a living donor is technically feasible. In children, the left transplant is usually taken from one of the parents. The results of living donor to child transplantation are equivalent to or slightly better than those obtained with organs retrieved from brain-dead donors. In adults, a right transplant weighing at least 0.08 to 0.1% of the body weight is generally used in order to obtain a sufficient mass of liver parenchyma. The donor retains the left liver, which must represent at least 35% of the volume of the native liver, so as to prevent post-surgical liver failure. Although the living donor to adult technique looks promising, it is limited by several factors including a stringent legal framework. Furthermore, the donor must have a normal liver devoid of any underlying disease. In view of this, only a minority of transplantation candidates can benefit from a living donor organ. Even if the donor is not at any obvious risk from the operation, the surgical risk associated with right hepatectomy is far from negligible. It is a major procedure associated with a morbidity rate of 20 – 25% and a mortality rate of the order of 0.2%. This risk must be assessed bearing in mind that such invasive surgery is not personally required by the donor who is, by definition, healthy.

Transplantation from non-heart-beating donors is an attractive alternative for increasing the number of donors. It involves the retrieval of a transplant from a subject who has suffered

and not recovered from a cardiac arrest. Both the "warm" and "cold" ischaemia periods must be as brief as possible. A routine biopsy to rule out any underlying hepatic lesion is strongly recommended.

Carried out under routine conditions with excellent results in terms of both recipient survival and donor safety in industrialised Asian countries, adult transplants involving living donor organs is stagnating or regressing in both Europe and the United States. The expansion of this technique chiefly depends on logistic factors with the appropriate awareness and training of emergency outpatient departments, the creation of management networks and the training of the organ recovery teams. The expansion of transplantation from non-heart-beating donors could increase the number of available transplants by 10% to 20%.

Finally, the question of optimal allocation of marginal donors to certain recipient categories could be addressed using a donor risk score, sufficiently large databases and statistical modelling techniques.

Heart transplantation: New perspectives for optimising the donor pool

Over 2000 heart transplants have been carried out in France over the last eight years with an annual waiting list of approximately 700 patients. 366 transplantations were performed in 2007. Median recipient survival is 10.8 years following transplantation and 13 years after the first year. 70% of transplanted patients are currently estimated to survive for 10 years. Progress in cardiology ensures the survival of an increasingly large population and allows patients to claim transplantation either directly or during mechanical circulatory assistance. Despite these successes, the annual number of patients awaiting heart transplantation is twice that of transplant recipients, and 10% of them die without receiving an organ transplant. The optimisation of access to heart transplantation depends on optimisation of the donor pool, recipient selection and the protection of the organ to be transplanted.

Experimental data have shown that the suddenness of brain death affects the physiology of the myocardium essentially due to catecholinergic disruption. Unlike other organs, the very process of brain death is, therefore, likely to alter the myocardium and disrupt donor evaluation and selection. The principal alteration is a decrease in left ventricular contractility, and an evaluation of its reversibility is the key selection criterion. These potential changes must, therefore, be taken into account before deciding whether or not the donor heart is compatible with the scheduled transplantation project. Acceptable haemodynamic parameters have been defined and a donor resuscitation strategy has been standardised. Recommendations were clearly outlined at the 2001 Crystal City Consensus Conference with recourse to thyroid hormones, vasopressin, methyl prednisolone and insulin. More recently, beta-adrenergic donor blocking has been proposed by some authors on the basis of experimental and clinical studies.

The initial criteria defining ideal transplants have been reviewed and extended to increase the availability of cardiac transplants. These criteria focus on the donor's age and weight, tolerance to moderate, left ventricular hypertrophy, the acceptance of moderate coronary diseases and tolerance to transplant injuries accessible to *ex-vivo* repair prior to transplantation.

The donors' age has substantially increased over the last 15 years. The average age is currently over 30, and 8% of donors are over 50. The greatest proportion of older donors can be found in Europe where 19.6% of donors are over 50 years of age compared to just 10% in the United States. A young donor age is a well-established success criterion in heart

transplantation. However, heart transplantation with an organ retrieved from an older donor is preferable to no graft at all, and related factors must be taken into account in order to correctly assess the prognosis. Similarly, with current therapeutic protocols, age does not appear to influence the vascular disease of the transplant. However, results regarding the long-term survival of patients transplanted with the hearts of donors over the age of 50 remain controversial.

Recent data taken from the International Register of the Heart and Lung Transplantation Society (2008) confirm the prognostic value of the duration of ischaemia over the 5- to 10-year survival period. The weight of the recipient, that of the donor and the donor/recipient weight ratio are also key factors that condition cardiac graft prognosis. It is important to integrate data relating to the duration of the ischaemia and the weight of the donor and recipient in all of the factors influencing graft quality, including the donor's age.

It is still difficult to evaluate donor myocardial dysfunction, based on initial ultrasound scans and evaluation of the left ventricular ejection fraction. It would appear that between 25 and 50% of rejected hearts are unsuitable because of unsatisfactory ultrasound scans. Left ventricular ejection fraction is a parameter that varies over time and which can be modified by donor resuscitation conditions.

Various avenues are being explored to optimise the cardiac donor pool but they are proving difficult to apply. Post-conditioning (intervention before the graft is finally restored) improves the function of ischaemic rat hearts undergoing hypothermic cardioplegia for 4 hours. Its application to patients has only focused on protection against infarction to date and transplantation results are pending. Perfusion machines are currently the subject of active research in heart transplantation. They should reduce the duration of graft ischaemia, ensure their evaluation and make accessible a certain number of organs that have been rejected to date.

Lung transplantation: How to overcome the shortage of transplants

Approximately 200 lung transplants are carried out every year in France. Despite recent improvements, survival following lung transplantation is still deceiving, and is of the order of 50% at 5 years. These factors must be taken into account when considering how to manage the shortage of lung transplants. As in all sectors of solid organ transplantation, the number of patients on the waiting list largely exceeds the number of transplants available, resulting in long waiting times and a high waiting list mortality rate. Although 223 lung transplants were carried out in 2007 (154 involving both lungs, 49 involving one lung and 20 cardiopulmonary procedures), the number of transplants required each year is estimated to be in the region of 300 or 400.

Several avenues have either been investigated or are in the process of being investigated in an attempt to overcome this shortage of lung transplants. In lung transplantation, potential transplant sources include brain-dead donors, living donors and non-heart-beating donors.

Brain-dead donors represent the largest source of lung transplants worldwide and the only source in France. An important study was carried out in France concerning the registration of the number of brain-dead patients, which doubled between 1996 and 2007 to reach 24.7 organ donors per million inhabitants, ranking France in second place amongst the European countries. Lungs (even one lung) are seldom retrieved from brain-dead donors. The cause of death (mainly trauma) and the repercussions of resuscitation (nosocomial infections) frequently trigger a profound change in lung function, which is incompatible with lung transplantation. The acceptance of a transplant for transplantation is a difficult decision

based on factors that are hardly objective. Selection criteria were defined in a perfectly empirical manner right from the outset of transplantation. The expansion of these selection criteria in 2003 substantially increased the number of patients transplanted with at least one lung from a brain-dead subject (from 9.8% in 2000 to 15.8% in 2006). Although post lung transplantation survival does not appear to suffer as a result of this policy, studies analysing the impact of expanded selection criteria on patient survival are, for the most part, small-scale and use hazardous methodology. A specific effort should be made to determine the impact of transplant characteristics on the fate of recipients in order to guide lung transplant surgeons when accepting a lung transplant for transplantation. The introduction of a scoring system should objectively quantify graft "quality".

The retrieval of transplants from living donors is a marginal activity in lung transplantation (3 transplantations performed in the United States and none in France in 2006). The ethical problems posed by this intervention (considerable donor morbidity) and the fact that, contrary to reports with other organs, lung transplants from living donors are not superior in terms of recipient survival rate or the frequency of acute and chronic rejection, explains why this procedure is gradually being phased out.

Transplantation using organs from non-heart-beating donors is soaring for both lung and other organ transplants. The feasibility of this technique in lung transplantation was initially demonstrated in various animal models, highlighting the excellent tolerance of the lung to warm ischaemia. This method was transposed to humans in early 2000. The results of lung transplantations carried out using organs from non-heart-beating donors have been published in Spain. In the 17 patients transplanted between 2002 and 2007, survival at 1 and 3 years did not differ significantly from that observed following transplantation with organs retrieved from brain-dead donors. These initial promising results have yet to be confirmed. This technique is not currently authorised in lung transplantation in France.

For the time being, no thought has been given to the surgical technique employed. In France, over 70% of lung transplants involve the transplantation of two lungs, with practices varying considerably from one transplantation centre to the next. The superiority of double-lung transplantation has not, however, been confirmed in all of the patients (patients over 60 years of age) or in all of the indications (pulmonary fibrosis). Routine, single-lung transplantation performed in patients over the age of 60 or suffering from pulmonary fibrosis, for instance, perceptibly increases the number of transplantations carried out with a constant number of donors. Similarly, consideration of the sub-groups of patients who would gain maximum benefit from transplantation should facilitate the more effective use of the limited number of lung transplants available.

Post-transplantation complications: Infections, heart and metabolic diseases

Organ transplantation remains a procedure fraught by numerous complications. Although considerable progress has been made, immunosuppression used to prevent the rejection phenomenon increases both the risk and the severity of complications associated with the surgical procedure.

Early complications include surgical (graft non-function or dysfunction), vascular and infectious complications.

Graft non-function and dysfunction are observed in all forms of transplantation, manifesting in the early stages post-surgery. This may be due to the quality of the transplant, the retrieval techniques used, the duration of cold ischaemia or the presence of haemodynamic shock in the donor. In renal transplantation, these complications are associated with a poorer graft

survival rate and, in some cases, are irreversible. In liver transplantation, dysfunction as opposed to non-function generally occurs, and the graft will function again after a few days. In cases of prolonged graft dysfunction, however, the risk of recipient infection is high and the post-surgical morbidity rate is increased. In some cases, liver retransplantation must be decided as a matter of urgency. This is always a difficult decision since the mortality rate following emergency liver retransplantation is 50%. Graft non-function is the main obstacle with heart and lung transplants, and explains the need for a very short cold ischaemia period for both these organs.

Vascular complications occurring after renal transplantation include stenosis, thrombosis and haemorrhagic complications. The principal complication following liver transplantation is hepatic artery thrombosis, which is more common after a split-liver transplant, paediatric graft or in cases where a family donor is used. If diagnosed early, repeat surgery can be carried out, otherwise liver retransplantation is generally the short-term solution. Other complications frequently encountered include biliary and haemorrhagic complications and intra-abdominal collections, which are still quite common.

Infectious complications are mainly observed after transplantation. They generally occur in the first three months post-transplantation. Various trigger factors include the patient's condition at the time of transplantation, the duration of the procedure, surgical complications, failure of the graft to regain function and the intensity of immunosuppression.

Bacterial infections are extremely common in the first few days following surgery, regardless of whether these are collected infections, septicaemia, urinary infections or pulmonary infections. Fungal infections are less common and are often associated with the duration of resuscitation and graft function. The main fungal infections encountered are candidiasis and aspergillosis. The prognosis relating to diffuse aspergillosis, which was once catastrophic, remains serious but has improved following the launch of new anti-fungal agents.

Viral infections are also commonplace and some of them are highly typical of organ transplantation. In the case of cytomegalovirus-induced infection, the problem is either due to a primary infection, superinfection or post-transplantation reaction. Primary infections are more severe and occur in a context where the donor is seropositive and the recipient seronegative. They can be serious, causing graft dysfunction, pneumopathy or even a systemic infection requiring urgent treatment. These complications are seldom life-threatening but can nevertheless trigger the onset of acute or chronic rejection.

Infections due to the Epstein Barr Virus (EBV), frequently reported in children, are especially severe in the case of primary infection, and can trigger the onset of post-transplantation lymphoma. The prevention of primary infection is, therefore, extremely important and the monitoring of EBV by PCR (*Polymerase Chain Reaction*) is of paramount importance. The treatment of lymphoproliferations can range from a simple reduction in the dose of immunosuppressants combined with anti-viral treatment or chemotherapy in the case of confirmed lymphoma.

Herpes simplex 1 and 2 viral infections are rare and exceptionally severe. Herpes 6 infection, which is little known, can be associated with post-transplantation hepatitis and trigger rejection. Herpes 8 primary infections or Herpes 8 reactions can be linked with the onset of Kaposi syndrome, and are rare.

Hepatitis B (HB) and hepatitis C (HC) viruses have the greatest impact on transplantation. In patients who have undergone transplantation due to chronic, HBV-induced liver disease, the infection is essentially due to a recurrence of the virus. In recent years, a combination of nucleoside or nucleotide analogues with anti-HB immunoglobulins has reduced the

recurrence of hepatitis B to less than 10%. This condition can, however, still be acquired following liver transplantation when the transplant has been retrieved from a hepatitis B donor. Prophylaxis may be required in this situation. Reactivation of the hepatitis B virus may also be triggered in the recipient following kidney, heart or lung transplantation. Post-transplantation immunosuppression may trigger reactivation of the virus. Unlike viral recurrence, contraction of the hepatitis B virus during transplantation is fortunately extremely rare. Following liver transplantation, the recurrence of hepatitis C is constant when the patient undergoes transplantation due to HCV-induced cirrhosis with the virus being present in the blood, which applies to 90% of cases. Current methods to prevent recurrence are not very effective. Viral recurrence will trigger the rapid onset of chronic hepatitis followed by cirrhosis (20 – 25% of cases of cirrhoses at 5 years) and jeopardise the long-term survival of transplanted patients. Hepatitis C acquired post-transplantation is extremely rare. In renal transplantation, patients already infected with HCV, may experience a chronic disease advance in chronic hepatitis C towards cirrhosis.

The donor may transmit numerous infectious diseases. Viruses, bacteria or parasites may, in fact, be localised in the graft cells or in the lymphocyte cells accompanying the graft. The most commonly transmitted viral infections are cytomegalovirus, the EBV virus and Herpes 8. The rabies virus and hepatitis are transmitted less often. Among the bacterial infections, the syphilis bacillus can be transmitted whilst the most frequently transmitted parasitic infection is toxoplasmosis.

One of the main strategies adopted in an attempt to reduce post-transplantation morbidity is the prevention of infectious bacterial complications by preparing the recipient for transplantation, reducing surgical complications, the correct use of antibiotics and adequate modulation of immunosuppressants. Viral infections must be prevented by donor/recipient matching, especially for cytomegalovirus, the use of anti-viral agents and, once again, the modulation of immunosuppressants.

Cardiovascular and metabolic complications are also linked to immunosuppression and are delayed. The overall cardiovascular risk is increased in transplanted patients compared to the general population. These cardiovascular complications are the primary causes of death in the long term following kidney and liver transplantation. Every effort must be made to assess the risk prior to transplantation and to prevent these complications: prevention of dyslipidaemia and diabetes, smoking cessation, dietetic measures and physical exercise.

The frequency of arterial hypertension varies from 20 to 50% in liver transplantation and is promoted by the use of corticosteroids and calcineurin inhibitors. In transplantation, arterial hypertension impacts upon the long-term overall cardiovascular risk. This complication must be prevented and treated by modulating or modifying immunosuppressants and by administering anti-hypertensive drugs.

The onset of diabetes following liver transplantation is the most common metabolic complication. Cases of pre-existing diabetes are also exacerbated. Risk factors include the use of corticosteroids, calcineurin inhibitors (especially tacrolimus), the presence of HCV, obesity and all dysmetabolic diseases. These complications may affect graft survival (especially in the case of kidney transplants through arterial involvement) and patient survival. Prevention is based on immunosuppression with or without low doses of corticosteroids, a decrease in or premature withdrawal of corticosteroids and modification of immunosuppression. The treatment of diabetes must always be aimed at balancing blood glucose levels and obtaining suitable long-term results.

Dyslipidaemia is a frequent problem. Risk factors include transplantation, obesity, dietary habits, genetic factors, the use of high doses of corticosteroids or mTOR inhibitors. Prophylaxis and treatment are mainly based on dietary changes.

Obesity is becoming a common problem. Weight gain and an increase in the body mass index are observed following liver transplantation with 14% of transplanted patients presenting with a BMI of over 30.

The onset of diabetes following liver transplantation is the most common metabolic complication. Cases of pre-existing diabetes are also exacerbated. Risk factors include the use of corticosteroids, calcineurin inhibitors (especially tacrolimus), the presence of HCV, obesity and all dysmetabolic diseases. These complications may affect graft survival (especially in the case of kidney transplants through arterial involvement) and patient survival. Prevention is based on immunosuppression with or without low doses of corticosteroids, a decrease in or premature withdrawal of corticosteroids and modification of immunosuppression. The treatment of diabetes must always be aimed at balancing blood glucose levels and obtaining suitable long-term results.

Dyslipidaemia is a frequent problem. Risk factors include transplantation, obesity, dietary habits, genetic factors, use of high doses of corticosteroids or mTOR inhibitors. Prophylaxis and treatment are mainly based on dietary changes.

Obesity has become a common problem. Weight gain and an increase in the body mass index are observed following liver transplantation with 14% of transplant patients presenting with a BMI of over 30.

Post-transplantation complications: Calcineurin inhibitor nephrotoxicity

Apart from their immunosuppressive potential, calcineurin inhibitor immunosuppressants have in common a nephrotoxic effect, which has long-term harmful consequences, regardless of the type of organ transplanted.

Nephrotoxicity can be acute, functional and regressive with a reduction in calcineurin inhibitors, or chronic and irreversible. In clinical terms, this manifests as acute or chronic kidney failure. The diagnosis can be corroborated by blood assays of calcineurin inhibitors (although the correlation between toxicity and overdose is relatively low, especially on an individual scale) on the one hand, and on the other hand, by examining the histological lesions induced, some of which are highly indicative (tubular, isometric vacuolisation or arteriolar hyalinisation) whereas others are sequelar (interstitial fibrosis, tubular atrophy). The natural history of nephrotoxicity lesions can be accurately determined from renal biopsies, which are part of the screening process.

The physiopathology of calcineurin inhibitor nephrotoxicity has not been clearly elucidated. It is essentially due to vasoconstriction and its short- and long-term consequences. Vasoconstriction *per se* is linked to an increase in endothelin I production, an increase in sympathetic tone, modification of the prostaglandin/thromboxane ratio in favour of vasoconstriction, activation of the renin-angiotensin system and oxidising stress. This leads to the synthesis by tubular cells of pro-fibrosing molecules such as TGF-β and interstitial fibrosis. Tubular cells are ultimately the site of induced apoptosis. The endothelial cells are also targeted by calcineurin inhibitors.

These mechanisms, which have long since been established, are supplemented by emerging mechanisms, which could be involved in calcineurin inhibitor nephrotoxicity. The first of these concerns epithelio-mesenchymatous transition during which the tubular cells acquire a myofibroblast phenotype. After transplantation, these phenotypical modifications are detected in the tubular cells following screening biopsies carried out 3 months post-

transplantation. This phenomenon, which is triggered by calcineurin inhibitors, can also be generated by ischaemia/reperfusion.

Another potential mechanism is based on the appearance (*in vitro* and *in vivo*) of the endoplasmic reticulum stress phenomenon induced by calcineurin inhibitors. This effect has been highlighted in the renal tubular cells of animals and humans. Endoplasmic reticulum stress could be involved in the epithelio-mesenchymatous transition mechanism of tubular cells.

Finally, there is experimental evidence pointing to calcineurin inhibitor-induced transition lesions that are no longer epithelio-mesenchymatous but endothelio-mesenchymatous.

Only greater understanding of the nephrotoxicity mechanisms triggered by calcineurin inhibitors will improve the management of this side effect in transplant patients.

Post-transplantation complications: Increased risk of cancer

Cancer is one of the main, delayed post-transplantation complications impacting upon the quality of life and survival of transplant patients. This is confirmed by all of the information listed in national and international registers. The French database Cristal, which lists 47 000 transplant patients (taking all organs into account), identifies 7% of this population as having had at least one solid organ cancer (excluding the skin). The register of the International Society for Heart and Lung Transplantation lists 26% of cancers (including the skin) in heart-lung patients 8 years after transplantation.

The distribution of cancers for the various organ transplantations is relatively similar even if nuances linked to the age of the transplantation population (lymphoma in children) or exposure to carcinogenetic risk factors (e.g. smoking in heart-lung patients). One of the main predisposing factors in neoplasm formation in transplant patients is the pre-existing disease that led to the transplant: renal cancer in renal transplantation and hepatocellular carcinoma associated with hepatitis B and C viruses in liver transplantation. These risk factors for the development of cancer in post-transplant subjects also include exposure to UV rays, genetic predispositions and viral infections.

For most cancers in transplant patients, the onset is also triggered by immunosuppressant therapy, which is essential for transplantation. The relationship between cancer and immunosuppression has been the subject of numerous publications and models. Current immunosuppression is based on the association between anticalcineurins, puric base inhibitors and corticosteroids. Immunosuppressant induction, which accompanies most organ transplantations performed nowadays in Europe and especially procedures involving polyclonal antibodies, has also been suspected of potentiating the risk of haematological complications in the long term. Some immunosuppressants (e.g. azathioprine) are more likely to cause cell mutagenesis followed by cancer than others.

Skin cancers and post-transplant lymphomas (PTLD – *Post-Transplant Lymphoproliferative Disorder*) are the forms of cancer most frequently encountered in transplant patients. Skin cancers, especially spinocellular carcinomas, are very common in solid organ grafts with increased incidence away from transplantation and ultimately affecting more than half of transplant patients. Spinocellular carcinomas are more aggressive in transplant patients than in non-immunodepressed subjects and are compounded by local recurrences (12% of cases) and metastases (8% of cases). The length of time to onset depends on the patient's age when transplantation is carried out, skin type, sun exposure and immunosuppression, but averages 7-8 years in patients who undergo transplantation at the age of 40. Taking all skin

tumours into account, 40% of patients present with new tumours within one year of diagnosis and 67% within two years. Multiple or recurrent lesions are associated with profound immunodepression. A reduction in immunosuppressant therapy may limit these lesions.

Several factors are associated with the onset of lymphoma in transplant patients: young age, the induction of immunosuppression by anti-lymphocyte antibodies, serological status for the EBV virus and HLA status. Lymphoma prognosis is dominated by a major risk of death. 80% survival at 5 years in kidney transplant patients amounts to just 65% in kidney transplant patients suffering from lymphoma.

Comparison of age-adjusted main cancers rates in kidney transplant patients vs. non-transplanted American population (according to Kasiske *et al.*, 2004)

	Cancer rates in men[1]				Cancer rates in women[1]			
		Years post-transplant				Years post-transplant		
Site	NT[2] Pop	1	2	3	NT[2] Pop	1	2	3
Skin								
Skin (non-melanoma)	24.0	2017.1	2333.3	2160.2	14.3	851.6	1306.8	1320.5
Melanoma	19.0	60.4	77.5	131.3	12.1	99.9	58.4	63.5
Lymphomas								
Non-Hodgkin's	22.0	882.0	345.1	150.7	15.7	667.5	337.5	456.7
Hodgkin's	3.2	37.9	12.4	98.6	2.5	11.5	0.0	93.5

[1] Rates per 100 000 subject-years in the American population and per transplant patients. All of the values are standardised according to age bracket in the American population listed in 2000; [2] Non-transplanted American population.

Transplantation teams have recommended various strategies to minimise immunosuppressant therapies and recourse to new therapeutic classes in an attempt to reduce the risk of cancer. Obtained from the mTOR protein antagonist group, the new molecules everolimus and sirolimus possess anti-tumour activity both *in vitro* and *in vivo*. This effect is exerted directly (anti-proliferative) and indirectly (anti-angiogenic). These molecules are currently at the clinical development stage, which has already touched on the specific indications for use in oncology.

Other immunosuppressant therapeutic categories specific to the host-graft relationship and therefore less likely to cause cell disruption are currently under development.

Apart from these pharmacological perspectives, other avenues must be developed in order to improve the survival and quality of life of transplant patients. These concern the high-performance identification of risk factors or genetic predisposition factors, the compilation of exhaustive registers specifically dedicated to this pathological sector, namely cancer, with pre-transplantation screening and targeted post-transplantation follow-up. Lastly, patient education and the training of transplantation teams are key objectives, the impact of which is of paramount importance.

Paediatric transplantation: Mainly liver and kidney

Seventy-one paediatric liver transplants were carried out in France in 2007. Eighty new patients were entered on the waiting list during this period and 39 young patients were

waiting for a liver transplant as at 1 January 2008 (information obtained from the Biomedicine Agency[7]). The global context of paediatric liver transplantation in 2007 was characterised by 2 years' stability in terms of waiting list inclusion and transplantation coupled with an organ shortage. The demand for transplants still exceeds actual feasibility despite an improvement in organ recovery methods over the last three years.

The indications for paediatric liver transplantation include chronic cholestatic diseases, generally of neonatal onset and progressing towards biliary cirrhosis. These conditions rapidly trigger delayed growth in terms of both height and weight. Bile duct atresia alone represents more than 50% of the indications. The diseases indicated in paediatric transplantation (usually carried out before 5 years of age) pose little threat of graft-related recurrence.

The results of liver transplantation carried out in a situation of donor-recipient blood group incompatibility appear to be satisfactory in recipients under 12 months old. However, the use of transplants recovered from donors under 12 months old poses the increased risk of arterial thrombosis and graft failure. A reduction in the survival rate has also been reported with transplants obtained from donors over 50 years of age. Whole liver transplantation is the simplest technique but this requires an appropriate match in terms of size between the transplant and the recipient, and represents only 15 to 20% of the total number of paediatric liver transplants carried out since 2000. Liver reduction and split transplant methods allow any discrepancies between donor/recipient weight to be reduced. Finally, retrieval of the left hepatic lobe from a living donor can be carried out in one of the parents, which allows the procedure to be programmed under good general conditions using a high-quality graft.

Depending on teams, immunosuppressant treatment combines cyclosporine, corticosteroids and mycophenolate mofetil or tacrolimus and corticosteroids. The current trend is the early withdrawal of corticosteroids during the post-surgical phase and even their elimination from primary immunosuppression regimens. The global rate of graft chronic rejection leading to liver retransplantation is less than 5% in most cases.

Most of the teams report a patient survival rate of over 70% at 10 years. Primary or secondary graft dysfunction (following hepatic artery thrombosis) and infectious complications represent the primary cause of premature death. The three main situations leading to retransplantation are: secondary graft dysfunction following vascular thrombosis (> 40% of cases), primary graft dysfunction, post-ischaemic biliary complications and chronic graft rejection.

Following liver transplantation, an excellent increase in height is generally observed in children during the first 3 years following transplantation. Full-term pregnancies resulting in the birth of normal infants have been recorded in women who underwent transplantation during childhood. The post-transplant intellectual development of children is normal in most cases. Quality of life following paediatric transplantation, which is assessed on the basis of self-evaluation, has hardly been investigated but appears to be relatively satisfactory.

A kidney transplant is the optimal treatment for kidney failure in children. Terminal kidney failure affects boys more often than girls (60% versus 40%). Obstructive uropathies together with renal hypoplasia and dysplasia account for 30% of the causes of terminal kidney failure. Corticosteroid-resistant idiopathic nephritic syndrome is the third cause in order of frequency, representing 12% of the causes of terminal kidney failure. The survival rate for kidney transplant children is far greater than that recorded for children on dialysis, regardless of the age at which transplantation was carried out. As the median half-life of a

[7] http://www.agence-biomedicine.fr/annexes/bilan2007/organes9_greffe_ped/9_1/9_synthese.htm

kidney graft in children is approximately 20 years, a second transplantation will be required in the majority of cases.

Seventy-six paediatric kidney transplants were carried out in France in 2007. During this period, 88 new patients were added to the waiting list and 61 children were waiting for a kidney transplant as at 1 January 2008 (data obtained from the Biomedicine Agency[8]). Eleven of these 76 young patients received an organ from a living donor. The proportion of children transplanted with a kidney from a related living donor (usually one of their parents) has ranged from 7 to 19% over the past 5 years compared to 52% in North America. The survival of transplants from living donors is significantly higher than that recorded with cadaveric organs, with a difference of 10% at 5 years post-transplantation. "Pre-emptive" transplantation using an organ from a living donor either dispenses with dialysis or shortens the period of dialysis, thus improving the growth and quality of life of the child in question.

Immunosuppressant treatment protocols (including antibody therapies) are currently being assessed in an attempt to restrict the use of corticosteroids and reduce the dosage of anticalcineurins possessing nephrotoxicity and playing a key role in chronic allograft nephropathy. Combination with tacrolimus, mycophenolate mofetil and corticosteroids is the most common strategy at the present time. The side effects of mTOR inhibitors (hypercholesterolaemia, pneumonia, anaemia, lymphoceles and delayed healing process) limit their use.

An increase in the incidence of malignant tumours and lymphoproliferative syndromes in particular, has been observed following paediatric kidney transplantation. The North American Register lists the incidence of malignant tumours for the first three years following transplantation in 0.96% of recipients in a cohort of children who underwent transplantation between 1987 and 1991, and in 3.6% after 1996.

Although infections due to cytomegalovirus (CMV) and pneumocystis are prevented by effective, prophylactic therapy, the incidence of infections due to the BK virus has significantly increased. The onset of BK virus-induced nephropathy is diagnosed on average 10 months after transplantation in 4.6% of transplanted children.

The major problem for patients following kidney or liver transplant is therapeutic non-compliance in adolescence, which causes delayed graft dysfunction. In the case of liver transplantation, this is the primary cause of graft failure 10 years after transplantation. This problem of poor compliance associated with immunosuppressant therapy is responsible for at least one-quarter of kidney graft failures in adolescents.

[8] http://www.agence-biomedicine.fr/annexes/bilan2007/organes9_greffe_ped/9_1/9_synthese.htm

Recommendations

Organ transplantation currently remains the only therapeutic strategy for most diseases resulting in the irreversible loss of vital organ (kidney, heart, liver and lung) function, and the best therapeutic approach for the kidney.

The various research strategies undertaken in an attempt to improve transplantation outcome have been analysed in this expertise: induction of tolerance, understanding of the mechanisms involved in acute and chronic rejection, optimisation of immunosuppressant therapy, research into new, more specific immunosuppressant molecules, investigation of the cell and molecular mechanisms of ischaemia/reperfusion and research into new agents aimed at transplant protection, expansion of the donor pool to include "marginal" donors and attempts to define risk scores as well as complications anticipated with optimised treatments.

The transdisciplinary approach adopted for transplantation research, which is unique in medicine, applies to both clinical and basic research. This is a good example of translational research. In fact, transplant patient follow-up allows the complex, physiopathological processes of tolerance and rejection to be investigated right from the outset alongside the development of infectious, cardiovascular and metabolic complications together with cancer. Transplantation research thus increases knowledge in various medical disciplines. Transplantation is also an ideal model for assessing new immunosuppressant or immunomodulating treatments. Numerous molecules originally used in transplantation have since been applied to other domains. An understanding of the harmful phenomena surrounding organ recovery, preservation and subsequent implantation in the recipient should trigger considerable medical and surgical improvements.

A structured research development programme for transplantation would provide a relevant link between basic research, clinical research and therapeutic research.

FINANCING AND STRUCTURING TRANSPLANTATION RESEARCH

In France, clinical research into transplantation is mostly financed by the PHRCs (Programme Hospitalier de Recherche Clinique-Clinical Research Hospital Programme), foundations, funds targeted for more extensive projects, the Agence Nationale de la Recherche (ANR-National Research Agency), the Agence de la Biomédecine (Biomedicine Agency), Oseo Innovation contracts and patient associations. The role of various societies, sponsors and industrial partners should be emphasised. Financial support can also be obtained from regional councils or the CHUs (University Medical Centre). More recently, new resources have been the subject of tenders financed by ministerial departments in conjunction with research organisations and care establishments (AP-HP and CHU), and Réseaux Thématiques de Recherche et de Soins (RTRS-thematic networks for research and care)[9]. The aim of certain tenders is to forge links, which can be perpetuated over time.

Furthermore, a certain number of teams are currently involved in European research networks (*Reprogramming the Immune System for the Establishment of Tolerance, Xenome, etc.*)

[9] The research and care translational topic network dedicated to transplantation sciences (Centaure) is based on the mutual approach to design and logistic resources at three pivotal centres that are particularly innovative in the transplantation sector (Nantes, Lyons, Necker/Paris).

It should be stressed that transplantation is not individualised in the Instituts Thématiques (Thematic Institutes) recently created by Inserm. Moreover, the term "transplantation" does not appear as such in the various tenders put forward. The difficulty therefore resides in incorporating transplantation projects in tenders, which were not initially intended for this topic. Transplantation is, by definition, a transversal activity, ranging from basic immunology to the management of immunosuppression complications such as cancer or chronic infections, for instance. Furthermore, the topic of ischaemia/reperfusion, which is specific to transplantation, cannot be financed in the current context.

As regards financing and recognition of the "transplantation" discipline, the expert group recommends:

- To propose transplantation-specific topics in meetings during which European tenders are being drafted;
- To systematically include the topic of transplantation in national tenders relating to diseases leading to the loss of function in organs that would benefit from transplantation (e.g. cystic fibrosis and lung transplantation);
- To individualise the transplantation item within national research institutes;
- To recognise the specific features of a university diploma in transplantation medicine (DESC–Additional Specialist Study Diploma) in order to off-set the lack of vision in relation to this activity, which is carried out exclusively in public hospitals. Transplantation should also be included in initial and continuous training programmes for physicians.

With regard to structuring transplantation research, the expert group proposes to promote:

- Support for multicentre projects allowing significant cohorts to be reached and clinical research activity to be structured at regional or national level. To this end, it is important to have financing in place to facilitate assistance with the design and implementation of clinical studies;
- The composition of registers containing details of related complications (cancer, nephrotoxicity and infections), which would allow thorough, stringent analysis of changes in treatment and treatment adjustments (combination of immunosuppressants and new therapeutic categories);
- The linking of French registers with European registers possessing relevant information; the development of simple procedures for accessing French and European data.

The expert group proposes to incorporate in transplantation research development specific topics that have been discussed in this expertise and which are subsequently discussed in greater detail.

Tolerance / rejection

Over the last 30 years, considerable progress has been achieved in the field of immunosuppressant treatments aimed at preventing or treating allograft rejection. The immunosuppressants currently used generally suppress immunity and are devoid of alloantigen-specific properties. These treatments are not entirely effective (effective for inhibition of acute rejection but not chronic rejection) and, in addition, generate over-immunosuppression responsible for the increased incidence of infection and tumours.

DEVELOP RESEARCH IN IMMUNE TOLERANCE

One of the aims of research is to induce a state of "operational immune tolerance", i.e. the absence of any pathogenic immune response to alloantigens expressed by the transplant without affecting the recipient's ability to take effective action against various exogenous antigens. Published data confirm that the possibility of establishing immune tolerance in transplantation is no longer a myth or an option exclusively reserved for the experimental transplantation sector, but is in the throes of becoming a clinical reality thanks to new immuno-intervention strategies.

The expert group recommends:

- Continued development of experimental strategies to establish allograft tolerance and promote the understanding of underlying cell and molecular mechanisms;
- Encouraging, with relevant resources, the development of new immunological markers allowing a state of immune tolerance to be diagnosed and followed up in animal models and clinical studies;
- The promotion of clinical protocols aimed at minimising immunosuppression as a prior, ethically acceptable step towards more ambitious protocols to eliminate immunosuppression; to prove the concept behind these immunosuppression protocols in targeted populations: patients with a low immunological, risk-particular case of liver transplantation;
- In the case of protocols using new immunosuppressants, the implementation of ancillary studies allowing the impact of these medicinal products on the establishment of tolerance to be discussed in greater detail (development of regulating cells, for instance);
- Systematic promotion of immunological monitoring in transplant patients via an organisational network focusing on immunological laboratories, possessing transplantation expertise and financed by dedicated tenders;
- The promotion of cell therapy protocols in man by creating regulatory conditions essential for their implementation.

PURSUE RESEARCH IN THE MECHANISMS OF ACUTE AND CHRONIC REJECTION

The incidence of acute rejection at 1 year post-transplantation varies from 5% (kidney) to 50% (lung). The onset of acute clinical and infraclinical rejection is associated with the development of chronic rejection with harmful repercussions on long-term graft survival. The effector mechanisms of the alloimmune response in acute and chronic rejection have not been fully elucidated.

The expert group recommends:

- The development of research in transplantation immunology aimed at promoting a better understanding of the mechanisms involved and the respective role of innate and adaptive immune responses, the role of T memory cells and humoral immunity in the alloimmune response;
- The development of non-invasive tests to increase the sensitivity and specificity of the methods used in clinical practice to monitor graft immunity status;
- As regards chronic rejection, the development and validation of markers for fibrosis, fibrogenesis or even nephrotoxicity or viral infection, which will allow the specific nature of this type of rejection to be refined;

- The validation of new anti-HLA antibody assay methods and the definition of graft access conditions and resulting immunosuppressant strategies.

PROMOTE RESEARCH AIMED AT OPTIMISING CURATIVE TREATMENT OF REJECTION

The curative treatment of acute rejection is relatively homogeneous at the present time. As regards acute cell rejection, high doses of steroids are required in the management of less severe forms and anti-lymphocyte antibodies in severe forms. Acute humoral rejection warrants non-standardised treatment combining steroids, plasma exchange, immunoglobulins and anti-CD20 antibodies.

Chronic rejection treatment is based above all on knowledge of the immunological and non-immunological mechanisms of rejection, allowing one or other of these components to be targeted and resulting in a change or reduction in immunosuppression.

The expert group recommends:

- The development of new immunosuppressants (lymphocyte anti-receptor antibodies) to avoid the use of high doses of corticosteroids;
- Standardisation of humoral rejection treatment (studies currently underway) and the provision of molecules with a real effect on plasmocytes;
- The development of molecules interfering with mechanisms involved in the advance of chronic rejection injuries (inhibition of growth factors, blockage of smooth muscle cell proliferation, etc.).

PURSUE RESEARCH IN NEW IMMUNOSUPPRESSANTS

Paradoxically, when advances made in immunosuppression triggered a reduction in the incidence of acute rejection during the first year of transplantation, the graft life-span was not significantly increased. In fact, current immunosuppressant therapies have poor control over the humoral response and T-lymphocyte memory response, thus remaining ineffective in preventing the advance of chronic rejection. The nephrotoxicity of calcineurin inhibitors is also an important factor in long-term kidney graft failure. Moreover, the average age of the transplanted patient population exposes recipients to even greater risk of cancer. The average age of the donors is also increasing, thus reducing initial graft quality.

The expert group recommends the development of new immunosuppressants with the following properties:

- New modes of action to complement existing modes of action: inhibition of memory lymphocytes, blockage of alloantibody synthesis and inhibition of antibody-mediated, chronic, active rejection, blockage of the progression of vascular and fibrotic lesions;
- A good benefit/risk ratio devoid of nephrotoxic effect and with good global tolerance;
- Anti-tumour properties or no pro-tumour effect;
- A potential for inducing tolerance or complying with effector cells involved in tolerance mechanisms.

USE NEW EFFICACY CRITERIA IN THE CLINICAL DEVELOPMENT OF IMMUNOSUPPRESSANTS

The main efficacy criteria for immunosuppressants over the last 15 years have been: the incidence of acute rejection, graft and patient survival and composite criteria including these principal parameters. At the present time with the reduction in the incidence rate for acute rejection, which affects less than 15% of primary transplant patients and excellent patient and graft survival, other evaluation criteria are required in order to develop new immunosuppressants and improve the benefit/risk ratio of long-term treatments.

The expert group recommends:

- The promotion of studies of 3 years' duration to develop a new immunosuppressant;
- The routine evaluation of renal toxicity by biological (kidney function and proteinuria) and histological (early markers of fibrosis) criteria; new tissue and blood biomarkers could be obtained from transcriptome analysis, proteomics and genomics;
- The routine testing of the antitumour properties of immunosuppressants; consideration of cancer and cardiovascular risks should become a major objective in the design of long-term immunosuppression;
- The initial inclusion of quality of life questionnaires during pivotal, prospective, randomised studies;
- The introduction of pharmacogenetic studies to individualise immunosuppressant treatments;
- The inclusion in prospective clinical studies of risk populations, hyperimmunised patients, elderly patients, diabetic patients and patients with chronic viral infection (HBV, HCV and HIV).

Ischaemia/reperfusion

It has now been clearly established that the ischaemia/reperfusion (I/R) syndrome during organ recovery, preservation and implantation in the recipient plays a key role in the development of early graft dysfunction and chronic rejection. The physiopathological process of I/R triggers a complex series of phenomena. An understanding of the mechanisms of physiological adaptation to ischaemia-induced stress is one of the most promising perspectives of research in terms of medical applications and the development of transplant preservation methods.

PURSUE THE PHYSIOPATHOLOGICAL CHARACTERISATION OF ISCHAEMIA/REPERFUSION SYNDROME

Recent advances in our understanding of the physiological mechanisms of I/R emphasise the predominant role of the production of radical species and inflammation probably responsible for the aggravation and, above all, persistence of this disease. I/R is also involved in the link between the lesions it generates and innate immunity via the maturation of dendritic cells. The molecular study of I/R has highlighted certain signalling pathways such as pro- or anti-apoptotic routes. However, we are currently a long way off from controlling all of the mechanisms involved in I/R and the particular features associated with warm and cold ischaemia.

The expert group recommends the development of physiopathological response focusing on the following points in particular:

- Characterisation of the physiopathological mechanisms involved in mitochondrial damage;
- Identification of the genes controlling the mechanisms involved in I/R-related damage (*microarrays*);
- Determination of the relationship that exists between cytoprotective genes and genes involved in inducing innate immunity (e.g. the coding gene for HSP70 involved in both phenomena);
- Definition of the role of I/R and innate immunity in the development of tolerance;
- Identification of the proteins and molecules involved in the signalling pathways required to activate the innate immune system;
- Recognition of the links between ischaemia/reperfusion-induced damaged, early graft dysfunction and chronic rejection.

It should be emphasised that I/R research in transplantation must be multidisciplinary, organised in networks and based on integrated biology tools with an approach involving genomes, proteomes and metabolomes. Relevant *in-vivo* experimental models must also be characterised for this type of study.

IDENTIFY ISCHAEMIA/REPERFUSION SYNDROME MARKERS

Currently, the efficacy of any strategy aimed at improving transplant preservation can only be evaluated with any degree of certainty after the transplant in question has been implanted and revascularised in the recipient.

The expert group recommends that organ viability markers be identified:

- During the phase preceding organ recovery given the impact of events affecting the donor's condition on graft outcome;
- During the early phase of I/R in an attempt to utilise organs that are not currently employed due to a lack of precise evaluation methods. The use of perfusion machines is beneficial for measuring graft viability markers.

The expert group recommends investigating markers to evaluate the long-term consequences of the I/R syndrome. Determination of such biomarkers will call on biochemistry or nuclear magnetic resonance spectroscopy, for instance.

PROMOTE RESEARCH IN TRANSPLANT STORAGE AND PRESERVATION

Preservation solutions, which are heterogeneous in terms of their composition and performance, authorise a preservation period of approximately 4-6 hours for the heart and lungs, 10-12 hours for the liver and 24 hours for the kidneys. For 10 years, the common approach geared towards improving the quality of organ preservation has been based on the inhibition of metabolism by hypothermia, the suppression of cell oedema thanks to impermeant agents and stimulation of energy metabolism during reperfusion. Numerous protective agents have thus been tested. However, the benefits observed in an experimental situation often manifest in the form of inconclusive results in a clinical setting. Validation of preservation fluids still faces methodological difficulties in terms of clinical evaluation:

heterogeneity of haemodynamic donor conditions, considerable variation in warm and cold ischaemia periods and heterogeneity of the clinical condition of recipients and immunosuppression protocols.

The approach using organs from elderly donors, marginal donors and non-heart-beating donors (having undergone warm ischaemia) challenges the relevance of existing perfusion methods.

The expert group recommends the promotion of research with particular reference to the following points:

- Evaluation of the protection afforded by pharmacological agents administered to the donor in an attempt to inhibit harmful molecules or to strengthen protective, metabolic pathways; the effects on all organs likely to be retrieved from the same donor must be evaluated; ethical considerations must be taken into account (when does pre-treatment start?);
- Clinical validation of surgical procedures such as transplant ischaemia pre-conditioning, reperfusion post-conditioning or conditioning of the recipient in an attempt to reduce preservation/reperfusion injuries;
- The use of potentially appealing gene transfer, which is not without side effects in animals and which poses problems of an ethical nature;
- The use of perfusion machines and perfusion conditions (temperature, pH, pressure, solution composition, etc.);
- The development of new generations of "metabolically active" preservation solutions by adding trophic or "immunomodulating" factors;
- Impact of preservation methods on the long-term fate of the graft.

Donor/recipient

There is a shortage of transplants for all solid organ transplantations. Several strategies have been developed to overcome this problem: re-evaluation of the pool of available donors, choice of surgical technique to optimise the number of organs for transplantation and the selection of recipients most likely to benefit from transplantation in terms of survival. Evaluation of these strategies is of paramount importance because the choice of donor/recipient match conditions the outcome of allo-transplantation. This problem has become even more pertinent since the use of transplants based on so-called extended criteria and which are also known as marginal donor organs.

ESTABLISH PROGNOSTIC SURVIVAL SCORES

The primary objective of organ transplantation is to improve patient survival. In some patients, heart, lung and liver transplantation did not improve their survival. Consequently, the available transplants should ideally be given to recipients who are most likely to benefit from them.

The expert group recommends:

- The development of prognostic scores to quantify the impact of the donor's characteristics on organ and/or recipient survival;

- Integration in these scores of donor characteristics and those relating to donor/recipient interaction (height, gender *mismatch,* etc.);
- Validation of these scores in large patient cohorts in different centres or even countries; the implementation of multicentre projects involving the use of French, European and international registers via the Biomedicine Agency.

OPTIMISE TRANSPLANTATION WITH A MARGINAL TRANSPLANT

By definition, a marginal transplant does not fulfil all of the criteria defining an ideal transplant.

In the case of using non-optimal transplants, the expert group recommends:

- The implementation of evaluation techniques to assess the condition of these transplants using biological (metabolic criteria, gene expression profiles) or clinical criteria;
- The provision of suitable, high-performance preservation methods;
- An evaluation of the impact of transplanting these transplants on transplantation success;
- Definition of the ideal recipient of a marginal transplant. This definition must incorporate demographic (age) and metabolic (matching of donor-recipient metabolic requirements) criteria. The experimental data raises the question of an increased immunological risk with a marginal transplant. Extensive clinical studies are required in order to answer this question;
- Optimisation of the management of transplant patients with a marginal organ. This involves evaluation of the preservation methods (shorter cold ischaemia periods) and immunosuppressive strategies pertaining to marginal transplants.

DEFINE THE POSSIBILITIES OF EXTENDING THE TRANSPLANT POOL ACCORDING TO THE ORGAN

In kidney transplantation, so-called marginal kidneys respond to a definition based on studies of registers, especially North American registers. The results obtained with these transplants are, by definition, inferior in terms of survival than those obtained with "optimal" transplants. Organ recovery from non-heart-beating donors is a potential, non-negligible source of transplants. The expert group recommends:

- Clinical research aimed at evaluating the best preservation strategy for various types of kidney transplants; evaluation of the results obtained nationally with these transplants;
- Evaluation of kidney transplant results based on ABO-incompatible, living donors in France.

As regards the lung, the choice of surgical technique is a crucial factor in determining the fate of the graft. In France, over 70% of lung transplants carried out involve transplantation of both lungs. Practices vary considerably from one centre to the next. The superiority of double-lung transplantation is not, however, evident in all these patients (patients over 60 years of age) or in all indications (pulmonary fibrosis). The expert group recommends:

- Consideration of the routine implementation of single-lung transplantations in patients over 60 years old or suffering from pulmonary fibrosis, for example, which, with a constant number of donors, could substantially increase the number of transplants.

In liver transplantation, marginal donors are defined by a higher risk of graft failure compared to "ideal" donors, or by the risk of transmission of an infectious, metabolic or tumour-related disease. The main sources for extending the selection criteria are represented by elderly donors, donors presenting with steatosis (fatty liver), bacterial or viral infection or a tumour and non-heart-beating donors. Several risk factors can also be associated with the same donor. Some surgical techniques also lead to marginal grafts, especially split or reduced liver procedures. The expert group recommends:

- The establishment of a liver transplantation risk score based on European cohort data;
- The development of algorithms for optimal allocation of marginal donors using a risk score based on the information contained in sufficiently large databases;
- Research intended to highlight the interactions between the donor's age and the recurrence of hepatitis C;
- Compilation of a list of transplants that cannot be split due to logistics or a lack of team awareness.

In heart transplantation, the main options for extending donor selection criteria are based on recourse to elderly donors, donors with moderate coronary lesions or donors with an infection or a tumour. An increase in the transplant ischaemia period could also boost the number of available transplants. It is also particularly important to carry out optimal resuscitation and to protect the myocardium during and after surgery. The expert group recommends:

- Research concerning post-conditioning in heart transplantation;
- Evaluation of the interest in using organs from non-heart-beating donors;
- Evaluation of organ perfusion machines (tested on kidneys); this technique seems to be capable of limiting the consequences of ischaemia and can be used to evaluate marginal transplants.

Post-transplantation complications

Organ transplantation is a procedure fraught with numerous complications. Immunosuppression used to prevent rejection increases both the risk and severity of surgical complication and post-surgical infections. Immunosuppressants that inhibit calcineurin have in common a nephrotoxic effect, which has harmful, long-term consequences. Finally, the risk of cancer is increased in transplant patients.

PREVENT AND RESTRICT INFECTIOUS, CARDIAC AND METABOLIC COMPLICATIONS IN THE TRANSPLANT PATIENT

Surgical complications must initially be reduced. This objective involves improving surgical techniques and using microsurgical methods.

Infection remains the major short- and mid-term problem. There is a link between the onset of infectious complications and the quality of surgical sequelae. All uncomplicated surgery reduces the risk of bacterial infection.

The expert group recommends:

- Evaluation of the impact of new immunosuppressants on the onset, type and severity of infectious complications, especially viral infections;
- Optimal evaluation in real time of the viral status of both the donor and the recipient, and optimisation of prevention (e.g. anti-HBV vaccination prior to transplantation); collaboration between high-performance transplantation centres and microbiology and virology laboratories.

Cardiovascular and dysmetabolic complications are becoming increasingly common in both the mid- and long-term. The risk depends not only on immunosuppression but also on HCV.

The expert group recommends:

- Improved evaluation of the risk in terms of immunosuppression;
- Research into mechanisms connecting HCV and diabetes;
- A multi-disciplinary approach for the long-term management of transplant patients (prophylaxis and treatment); collaboration between transplantation centres and cardiologists, nutritionists, endocrinologists and diabetologists.

PREVENT AND LIMIT NEPHROTOXICITY

Calcineurin-inhibiting immunosuppressants have in common a nephrotoxic effect due to vasoconstriction, which has harmful effects regardless of the type of organ transplanted. This effect manifests clinically as acute or chronic kidney failure.

Physiopathology of the nephrotoxicity of calcineurin inhibitors has not yet been fully elucidated. New mechanisms reported in the literature refer to epithelio-mesenchymatous and endothelio-mesenchymatous transition lesions.

The expert group recommends research into the mechanisms triggered by calcineurin inhibitors in order to manage nephrotoxicity more effectively in transplant patients.

PREVENT AND LIMIT CANCERS

Cancers represent one of the main, post-transplantation complications with delayed onset that impact upon the quality of life and survival of transplant patients. One of the primary predisposing factors for the onset of cancer is the pre-existing disease that initially led to transplantation: renal carcinoma in kidney transplantation and hepatocellular carcinoma combined with hepatitis B and C viruses in liver transplantation.

The development of cancer is also promoted by immunosuppressant treatment. Some immunosuppressants are more likely to cause cancer than others. Skin cancers and post-transplantation lymphomas frequently occur in transplant patients.

To reduce the risk of cancer, the transplantation teams have proposed various strategies to minimise immunosuppressant treatments and use new therapeutic categories. In addition to these pharmacological perspectives, other avenues can be explored in order to improve the survival and quality of life of transplant patients.

The expert group recommends:

- Exploration of new immunosuppressant therapeutic categories specific to the host-transplant relationship and less likely to trigger cell deregulation;

- Better identification of risk factors or factors of genetic predisposition;
- Setting up exhaustive registers, specifically dedicated to cancer, with pre-transplantation screening and targeted, post-transplantation follow-up;
- Educating patients and training transplantation teams.

APPENDIX 1

Inserm collective expertise: Methodology

An Inserm collective expertise[10] sheds scientific light on a given subject in the field of health on the basis of a critical analysis and synthesis of the international scientific literature. The collective expertise is implemented at the request of institutions wishing for access to recent research data pertinent to their decision-making process with respect to public policy. An Inserm collective expertise is to be considered as an initial stage that is necessary but most frequently not sufficient to result in decision-making. The conclusions of the collective expert review contribute to, but cannot replace, debate between the professionals involved or society debate if the questions addressed are particularly complex and sensitive.

At the request of an institution, the Inserm collective expertise may be accompanied by an 'operational' expertise addressing application of the knowledge and recommendations and taking into account contextual factors (existing programs, structures, players, training, etc.). The latter type of expert review elicits contributions from the players in the field able to respond to the feasibility aspects, representatives of the administrations or institutions responsible for promoting applications in the field involved, experts having contributed to the reviews, and representatives of patient associations. The sharing of varied cultures and experience enables a complementary approach to the collective expertise in an operational framework. Moreover, a variety of work (recommendations for good practices, public hearings, etc.) implemented under the auspices of the High Authority for Health (HAS) may follow an Inserm collective expertise.

Collective expertise has been an Inserm mission since 1994. Some sixty collective expert reviews have been implemented in numerous health fields. The Institute guarantees the conditions under which the expert review is implemented (exhaustiveness of the document sources, qualification and independence of the experts, transparency of the process).

The Inserm Centre for Collective Expertise organizes the various stages of collective expertise from the initial problem statement through to communication of the report, with the assistance of Inserm departments. The Centre team, consisting of engineers, researchers and a secretariat, implements the document searches, logistics and chairing of the expertise meetings. The team contributes to the scientific writing and to compiling the expertise products. Regular exchanges with other public organizations (EPST) implementing the same type of collective expertise have enabled similar procedures to be set up.

Problem statement

The problem statement phase enables definition of the institution's request, checking that accessible scientific literature on the issue raised is available and drawing up specifications which state the framework of the expertise (status report on the perimeter and main themes of the subject), its duration and budget, documented by a convention signed by the sponsor and Inserm.

During the problem statement phase, Inserm also organizes meetings with patient associations in order to ascertain the questions those associations wish to have addressed

[10] Inserm accredited label

and the data sources available to them. The information is incorporated in the scientific program of the expertise. For certain subjects, exchanges with industrial partners are indispensable in order to obtain access to complementary data not available in the databases.

Expertise monitoring committee and assistance unit setup

A monitoring committee consisting of the institution and Inserm representatives is set up. The committee meets several times during the expertise to monitor the progress of the review, discuss any difficulties encountered in addressing the issues, ensure compliance with the specifications and examine any new factors in the regulatory and political context pertinent to the ongoing review. The committee also meets at the end of the expertise for presentation of the conclusions and prior to compilation of the final version of the report.

For expertises addressing sensitive issues, an assistance unit is also set up and consists in representatives of the Directorate General of Inserm, scientific board, ethical committee of Inserm, communication department, human and social science researchers and specialists in the history of science. The role of that unit is to identify, at the start of the expertise, the issues liable to have strong resonance for the professionals involved and civil society, and to suggest hearings of professionals in related fields, representatives of civil society and patient associations. In short, the unit is responsible for measuring the perception that the various recipients may have of the expertise. Before publication of the expert report, the assistance unit pays special attention to the wording of the synthesis and recommendations, including, if necessary, the expression of the various points of view. Downstream of the expertise, the unit is responsible for strengthening and enhancing the circulation of the results of the expertise, for instance by holding colloquia or seminars with the professionals of the field and players involved or holding public debates with representatives of civil society. Those exchanges are to ensure enhanced understanding and adoption of the knowledge generated by the expertise.

Literature searching

The specifications drawn up with the institution are translated into an exhaustive list of scientific questions reflecting the perimeter of the expertise with the assistance of referral scientists in the field and members of Inserm. The scientific questions enable identification of the disciplines involved and construction of a key-word arborescence employed in the systematic searching of international biomedical databases. The articles and documents selected on the basis of their pertinence with respect to answering the scientific questions constitute the document base, which is forwarded to the experts. Each member of the group is asked to add to the document base over the course of the expertise.

Institutional reports (parliamentary, European, international, etc.), raw statistical data, associations' publications and other documents from the gray literature are also inventoried (non-exhaustive) in order to complement the academic publications provided to the experts. The experts are responsible for taking or not taking into account those sources depending on the interest and the quality of the information supplied. Lastly, a review of the main articles in the French press is supplied to the experts during the expertise in order to enable them to follow developments on the theme and the social repercussions.

Constitution of the expert group

The expert group is formed on the basis of the scientific skills necessary for analysis of the bibliography collected and on the basis of the complementarity of the group members' approaches. Since an Inserm collective expertise is defined as a critical analysis of the academic knowledge available, the choice of the experts is based on their scientific skills certified by publications in peer-review journals and their recognition by their peers. The expert recruitment logic, based on scientific skills and not on knowledge in the field, is to be stressed in that it is a frequent source of misunderstandings when the expert reports are published.

The experts are selected from the French and international scientific community. They are to be independent of the partner sponsoring the expertise and recognized pressure groups. The composition of the expert group is validated by the Directorate General of Inserm.

Several scientists outside of the group may be requested to contribute occasionally to a particular theme during the expertise.

Expert review implementation lasts between 12 and 18 months, depending on the volume of literature to be reviewed and analyzed and the complexity of the subject.

Initial expert group meeting

Before the first meeting, the experts receive a document explaining their mission, the scientific program (issues to be addressed), schedule, the expertise bibliographic database to date and articles more specifically addressing certain experts on the basis of the skills.

During the first meeting, the expert group discusses the list of issues to be reviewed and completes or modifies it. The group also examines the document base and proposes supplementary searches with a view to enriching that base.

Expert critical analysis of the literature

During the meetings, each expert orally presents a critical analysis of the literature with respect to the aspect allocated to the expert in his/her field of expertise and communicates the accepted facts, uncertainties and controversies with respect to current knowledge. The questions, remarks and points of convergence or divergence elicited by the group analysis are taken into consideration in the section that each of the experts compiles. The analysis report, consisting of various sections, thus constitutes the state of the art for the various disciplines pertinent to the issue under review. The bibliographic references used by the expert are cited in and at the end of each section.

Synthesis and recommendations

The synthesis summarizes the broad lines of the literature analysis and identifies the main findings and principles. Contributions from contributors outside the group may be summarized in the synthesis.

The synthesis is more specifically intended for the institution and decision-makers with a view to use of the knowledge presented therein. The wording of the synthesis is to take into account the fact that it will be read by non-scientists.

As of report publication, the synthesis is posted on Inserm's website. The synthesis is translated into English and posted on the NCBI/NLM site (National Center for

Biotechnology Information of the National Library of Medicine) and Sinapse site (Scientific INformAtion for Policy Support in Europe, European Commission site).

If requested by the institution, certain collective expertises include 'recommendations'. Two types of 'recommendations' are formulated by the expert group. 'Principles for action' based on a validated scientific reference system with a view to defining future public health action (mainly in screening, prevention and management) but which are not under any circumstances to be considered 'operational' recommendations insofar as no economic or political components have been taken into account in the scientific analysis. 'Research orientations' are also proposed by the expert group with a view to filling in the gaps in scientific knowledge observed during the analysis. Once again, these proposals cannot be considered 'priority' research without their being put into perspective. That is the task of the pertinent authorities.

Critical review of the report and synthesis by prominent 'readers'

For certain expertises addressing sensitive subjects, a critical reading memorandum is requested from several prominent 'readers' selected on the basis of the scientific or medical knowledge and managing or evaluating French or European research programs or having contributed to ministerial working groups. Similarly, the report and synthesis (and recommendations) may be submitted to figures with good knowledge of the 'field' and able to grasp the socioeconomic and political issues associated with the knowledge (and proposals) presented in the expertise.

Presentation of the conclusions of the expertise and debate

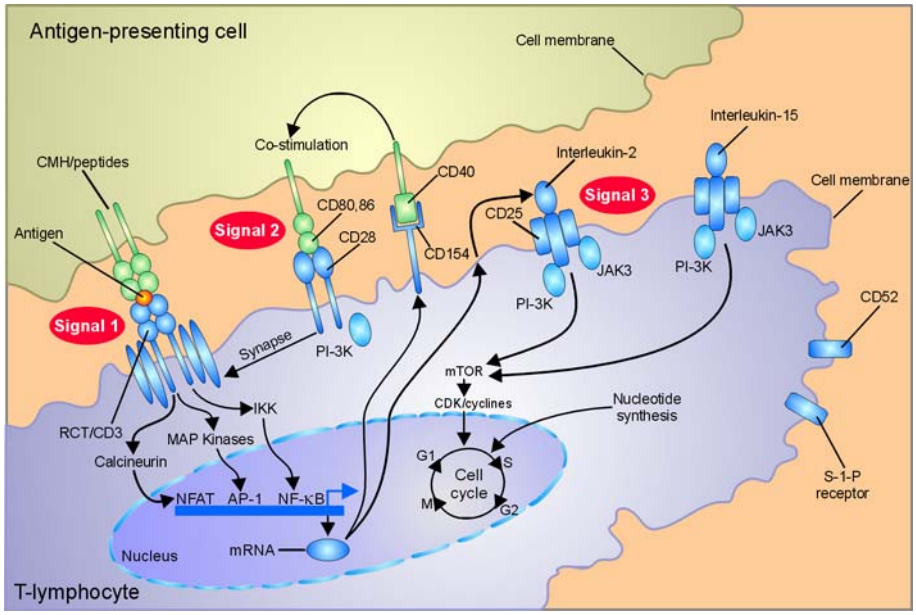
A seminar open to the various sectors involved in the subject of the expertise (patient associations, professional associations, unions, institutions, etc.) enables an initial debate on the conclusions of the expertise. On the basis of that exchange, the final version of the synthesis document incorporating the various viewpoints expressed is compiled.

APPENDIX 2

T-Lymphocyte Activation in Transplantation

In the graft and surrounding tissues, dendritic donor and recipient cells migrate to zones enriched with T-lymphocytes of the recipient's secondary lymphoid organs.

The antigen presented on the surface of the dendritic cells (professional antigen-presenting cells) binds to T-lymphocytes via the T receptor (TCR, T-*cell receptor*), which transmits signal 1. A second signal (signal 2) is triggered following the interaction between molecules CD80 and CD86 (co-activation molecules) present on the surface of dendritic cells and their CD28 receptor on the lymphocytes. The amplification of signal 1 by signal 2 allows the intracellular activation of several signalling pathways: calcium/calcineurin, MAPK and NK-kB. The ensuing activation of the transcription factors promotes the expression of new molecules by the T-lymphocyte including IL-2 (interleukin-2), CD154 and CD25. By binding to their specific receptors, IL-2 and other cytokines (IL-15) activate the Pi3K/mTOR (*mammalian target of rapamycin*) pathway, which initiates T-lymphocyte proliferation (signal 3).



Three T-lymphocyte activation signals (according to Halloran, 2004)

AP-1: activating protein-1; CDK: cyclin-dependent kinase; MHC: major histocompatibility complex; IKK: IkB kinase; JAK3: Janus kinase 3; mTOR: mammalian target of rapamycin; NFAT: nuclear factor of activated T cells; NF-kβ: nuclear factor-kβ; PI-3K: phosphoinositide-3-kinase; TCR:T-cell receptor; S-1-P: sphingosine-1 phosphate