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Organ transplantation in rodents: Novel applications of long-established methods

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Abstract

Rodent models of solid organ transplantation have been used for many decades. Standardized operative techniques resulting in highly reproducible survival rates have been developed for several organs. This allowed scientists to investigate many clinically relevant problems, test new drugs and establish novel treatment regimens. Recently, many studies used these models to explore novel issues such as graft modification by pharmaceutical, surgical or genetic engineering methods, post-transplant regeneration, leukocyte trafficking or interactions between the innate and allo-specific arms of the immune response. The results from these studies clearly facilitate a more complex and comprehensive understanding of existing problem.

The long-established methods of rodent organ transplantation, combined with the newest achievements in surgical techniques, biotechnology and imaging, will remain indispensable tools of transplantation biology.

Keywords

rodents; experimental transplantation; microsurgery; gene therapy

1. Introduction

Rodent - primarily mouse and rat - models of solid organ transplantation have been used for decades. With improving surgical techniques, the list of transplant related issues that could be studied in experimental settings has been greatly expanded. Standardized operative techniques and highly reproducible survival rates have allowed scientists to investigate many clinically relevant problems, test new drugs and establish novel treatment regimens.

The present state of transplant biology, both as basic and clinical science, would have been very unlikely to be achieved without lessons learned from rodent transplant models. The progress in rodent organ transplantation has occurred in several ways over the last few decades.

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New organs have been successfully transplanted [i.e., trachea, lung], and existing procedures have been perfected. Fully vascularized models have become more widely used [heart, liver], and orthotopic positioning for previously heterotopically transplanted organs has been achieved [liver, small intestine] [Figure 1]. In addition to these primarily technical developments, previously established rodent models provide unique opportunities for approaching new problems, or allowing a more complex exploration of some well-studied ones. The main objective of this review is to discuss some of these novel applications, and emphasize the usefulness of rodent models in modern transplant research.

2. Historical perspective

Many aspects of transplant biology have been investigated with the help of rodent transplant models over the last few decades, but the effort was concentrated on several issues. The area of organ preservation - which continues to be of paramount importance in clinical transplantation even today - was an extensively researched field. Syngeneic rodent transplant models were instrumental in developing and comparing preservation solutions and agents. Probably the most notable, the University of Wisconsin solution [UW], was introduced in 1987 by Belzer and associates. Numerous studies performed in rodents revealed that its use permits extended hypothermic preservation time. In return, the implications of UW on the logistics of clinical transplantation, particularly liver transplantation, have been tremendous. It became possible to operate semi-electively, to improve patient selection and to admit patients from distant locations.

Another major clinical problem, which has received enormous attention in previous years, is ischemia/reperfusion injury [IRI]. Most aspects, including the signaling pathways and molecules related to IRI have also been identified and explored in rodent models. The different roles of crucial components of injury such as cytokines, adhesion molecules, vaso-active molecules or chemokines have also been clarified in these models.

The immune response to grafts mounted by the host is at the very heart of transplant biology. Allogeneic combinations of rodents were used extensively to perform mechanistic studies on allograft rejection, which lead to our basic understanding of both acute and chronic graft rejection. The recognition of cell types as well as the regulatory processes involved was based, to a significant extent, on knowledge from rodent experiments. Development and testing of new immunosuppressive drugs and compounds were also an essential area where the use of rodent transplant models proved indispensable. Tolerance, both as a biological phenomenon as well as the agents [e.g., monoclonal antibodies to co-stimulatory molecules] that would allow its development in clinical settings, were also extensively studied in these models. Various animal models based on antibody mediated co-stimulatory blockade against CD28, CD80, CD154, and CD40 have led to varying degrees of donor-specific hypo-responsiveness.

3. Novel applications

While the classical subjects of transplant biology outlined above remained the focus of experimental transplantation, in recent years several new approaches have been developed. The combination of these new applications with rodent transplant models facilitate a more complex and comprehensive understanding of existing problems, and may promote the establishment of new treatment methods [Figure 2].

3. 1. Supplements to preservation solutions

The efforts to further improve preservation conditions are continuously ongoing, and a large variety of ingredients were introduced into UW preservation solution. Some recently applied compounds such as trophic factors [1], caspase or calpain inhibitors, S-adenosylmethionine

[SAM] [2], insulin [3], or fructose-1, 6-biphosphate [FBP] [4] are targeting different pathways. While these studies provided promising results, none of these modifications to UW solution composition have found their way into routine clinical practice. Rodent models will be essential in further establishing the usefulness of these compounds.

3. 2. Modifying the donor or the graft

In recent years, various approaches have been developed to preserve cellular metabolic pathways and prevent inflammation either by treating the graft itself, or by treating the donor prior to graft procurement. A wide array of strategies including pharmacotherapy [immunosuppressive, anti-inflammatory and chemotherapy drugs, cytokines, vasoprotective agents, monoclonal antibodies, and antioxidants], irradiation [gamma or ultraviolet [UV] irradiation of the graft], cell transfer [bone marrow cells, blood, splenocytes, DC, and lymphocytes], and temporary controlled-warm ischemia [ischemic preconditioning] have been used for donor/graft treatment. Several new approaches from these possibilities are discussed here.

a. Pharmaceutical targeting of inflammatory pathways—5-amino-4-imidazole carboxamide riboside [AICAR] has been administered to improve the ability of the heart to recover from IRI, and recently, it was found useful for protection against hepatic injury in both steatotic and non-steatotic liver transplantation. AICAR improves NO synthesis and reduces oxidative stress, and it appears to be a promising strategy [5].

Growth factors such as insulin-like growth factor [IGF], cardiotrophin-1 and fibroblast growth factor [FGF] protect against IRI in the heart via the activation of phosphatidylinositol-3-OH kinase [PI3K]-Akt and p42/p44 extracellular signal-regulated kinases [Erk 1/2] [6]. This pathway promotes cellular survival, through recruitment of anti-apoptotic protection pathways, including activation of protein kinase C [PKC] and mitochondrial Raf-1, which has been shown to inactivate the pro-apoptotic factor, Bad [7]. Activation of the PI3K-Akt or the Erk 1/2 pathway also inhibits the conformational change in Bax required for its translocation to the mitochondria as well as caspase 3 and caspase 9 activation [8]. Thus targeting these pathways may ameliorate non-specific inflammation.

b. Ischemic preconditioning—A limited period of ischemia followed by normothermic reperfusion facilitates graft protection by reducing the pathological damage from a subsequent, longer period of ischemia [9]. This preconditioning effect -studied most extensively in myocardial tissue- is related to ATP-sensitive potassium channels. These channels are induced by falling levels of intracellular ATP. The resulting changes in the cardiac action potential prevent further ATP depletion protecting the cell from irreversible damage. Preconditioning has been studied in other organs as well. In the mouse liver, 10 minutes of ischemia followed by 15 minutes of reperfusion resulted in improved survival of subsequent prolonged periods of ischemia. The mechanism is inhibition of apoptosis through down-regulation of caspase-3 activity. Preconditioning effects can also be induced by hyperthermia and by compounds inducing heat shock proteins such as NO donors, adenosine receptor agonists, endotoxin derivatives, or opioid receptor agonists. The underlying mechanisms may be complex, for example, thermal preconditioning has been shown to impair leukocyte rolling [10].

c. Depletion of antigen presenting cells [APC] in the graft—The most frequent approaches to deplete grafts of dendritic cells [DC] include gamma irradiation, cytotoxic drugs, photosensitizer + UV radiation, and antilymphocyte antibodies. Donor or graft treatment of endocrine allografts for selective elimination of DC may result in indefinite allograft survival [11]. Low-dose UV pretreatment of human islets has been shown to reduce immunogenicity by preventing the expression of co-stimulatory molecules [12,13]. The studies for solid organ

transplantation show less impressive results. This might be partially related to the fact that DC function is required for the development of tolerance induction via regulatory T cells [14].

d. Genetic engineering and gene transfer studies—Organ transplantation offers a distinctive opportunity to evaluate the effects of genetic modification. The treatment can be performed *ex vivo*, resulting in localized expression of the gene of interest, while minimizing systemic hazards. Over the years, advances in vector development have allowed the clinical progression of this form of therapy to become more attainable. The most important directions of gene therapy in transplantation are graft protection and immunomodulation. Rejection of the graft in the immediate post-transplant period has been prevented through the transfer of immunomodulatory molecules in addition to tolerance inducing approaches. Chronic graft rejection may be similarly addressed through permanent tolerance induction or alternatively, through the introduction of molecules to resist chronic graft damage.

The complex cascade of mediators associated with IRI presents multiple targets for gene therapy. Graft cytoprotection is aimed at maintaining cellular functionality. It can be achieved by antioxidants, membrane stabilizers, and antiapoptotic molecules. The most investigated approach is the modulation of the heme oxygenase [HO]-1 system. Over-expression of heme oxygenase-1 or adenoviral-mediated expression of copper-zinc superoxide dismutase protects rat livers from ischemia/reperfusion-induced tissue damage and apoptosis [15]. HO-1 overexpression has been shown to increase the viability of grafts after prolonged cold storage in a series of transplant models by reducing IRI, inflammation and apoptosis. As a long-term effect, accelerated transplant arteriosclerosis, the main manifestation of chronic rejection, has also been reported to decrease by the induction of HO-1 expression [16]. Most experimental models inducing HO-1 have used metalloproteins [FePP and CoPP] or gene therapy. CO, a by-product of HO-1 metabolism, has been shown to reproduce the protective effects of HO-1 in several transplant models, and has the advantages of sufficiently penetrating the cell membrane at cold temperatures. Down-stream products of HO-1 metabolism such as bilirubin and biliverdin may also be useful to protect graft cells against IRI.

Similarly, over-expression of anti-apoptotic genes via Bcl-2 adenoviral vectors has protective effects on liver grafts. Expression of anti-apoptotic proteins such as Bcl-xL and A20 may even have long-term effects by mitigating antibody-induced transplant atherosclerosis as suggested in cardiac allografts. Endothelial cells expressing caspase-resistant Bcl-2 displayed increased resistance to apoptosis and cytotoxic T cell-mediated lysis. The blockade of leukocyte adhesion has also been accomplished in grafts by genetic engineering, and proved to be beneficial in reducing IRI. Anti-sense oligonucleotides to intercellular adhesion molecule [ICAM]-1 lead to a reduction of neutrophil accumulation in rat cardiac allograft. Intratracheal adenoviral administration of IL-10 reduced lung IRI, or IL-13 expression by adenoviral gene in liver graft resulted in an increase survival rates, suggesting the increased expression of anti-inflammatory cytokines ameliorate the effects of IRI [17].

Developing immunosuppressive and tolerogenic strategies is another important area of gene therapy using experimental organ transplantation models. Immunosuppression is often achieved via the blockade of the different components and pathways of antigen recognition. Using the principle of co-stimulatory blockade, alternative approaches to antibody treatment were also developed. Rats genetically modified to express CD40Ig using adenoviral vectors were able to maintain liver allografts indefinitely. Expression of CD40-Ig in rat cardiac transplants has also been shown to delay acute rejection [18,19]. *Ex vivo* treatment of rat islets with AdCTLA4-Ig protected the islets from alloimmune destruction in spontaneously diabetic BB rats.

T cell activation can be inhibited by preventing DC maturation as immature DC are poor stimulators of naïve T cells, and may induce alloantigen-specific hyporesponsiveness. Rat DC were transfected with an adenoviral vector encoding a kinase-defective dominant negative form of IKK2 [dnIKK2] to block NF- κ B activation. Since NF- κ B is central to DC maturation, dnIKK2-DC acquired potent regulatory properties, inhibiting naïve T cell proliferation toward allogeneic stimuli. Pretransplant infusion of allogeneic donor dnIKK2-DC prolonged the survival of a kidney allograft from the same allogeneic donor, without the need for immunosuppressive therapy [20].

Engagement of Fas [CD95] on the surface of T cells by Fas ligand [CD95L] leads to the induction of apoptosis in activated T cells. This process may be involved in the killing of CD4 T cells. DC genetically engineered to express CD95L on their surface are able to inhibit alloreactive T cell proliferation in vitro, and prolongation of cardiac graft survival following in vivo administration has also been demonstrated in rodents[21]. In a different approach based on the known immunosuppressive properties of soluble MHC antigens, recipient lymphocytes were genetically modified to overexpress or release donor-specific class I antigens. This, in turn, suppressed the immune response in rat recipients of cardiac grafts [22].

As a general observation, viral vector technology has proved to be better than non-viral vectors at delivering therapeutic genes to graft cells. Toxicity of viral vectors, however, delay the full realization of gene-based therapy in transplantation. With improving gene delivery methods, the results may improve, and the use of rodent models for screening is expected to remain at the forefront of research. The most commonly used viral vectors in transplantation are summarized in Table 1.

3.3. Leukocyte trafficking

There is an ever-increasing interest in understanding how the location and migration of different cell types following transplantation affect the development of the immune response. Transplant models allow the study of spatial and time-dependent distribution of cells by providing access to the graft, blood, primary and secondary lymphoid, and other organs. The rodent transplant models are essential in understanding the anatomic choreography of alloantigen presentation and T cell response, and have recently been used in establishing tolerance by altering the expression and distribution of the molecules controlling T cell migration. Allografts are infiltrated by neutrophils, macrophages and lymphocytes in a complex series of events following transplantation. Elevated levels of proinflammatory cytokines such as tumor-necrosis factor- α , interleukin-1 β [IL-1 β] and interferon- γ are a result of IRI. These cytokines initiate the differentiation of DC, and regulate their migration from the graft to secondary lymphoid tissues to present alloantigen to T cells. Additionally, allograft endothelial and parenchymal cells release different chemokines, and the expression of the endothelial adhesion molecules is up-regulated. These highly coordinated cellular processes eventually lead to the host T cell response [23,24].

New imaging methods have been applied to transplant models, and these techniques allow visualization of leukocytes to facilitate the study of cell migration. Bioluminescence imaging [BLI] can be performed in vivo in cardiac transplantation using luciferase-green fluorescent protein transgenic mice [beta-actin promoter or CD5 promoter]. Light intensity emitted from the recipient animals can be measured daily, and compared with the accumulation of inflammatory cell infiltration and structural changes of green fluorescent protein-positive cardiomyocytes by sequential immunohistochemistry. In vivo BLI can visualize migration and proliferation of passenger leukocytes in both syngeneic and allogeneic recipients [25].

Molecular imaging methods in general are expected to have major impact on transplantation biology. In addition to help with targeted biopsy, they will allow the non-invasive evaluation

of organs for IRI, or the detection, quantification and monitoring of complex immune processes such as acute and chronic rejection [26].

3.4. Postransplant regeneration

End-stage organ failure is a major cause of post-transplant death worldwide, and re-transplantation remains the current standard of care. Many steps associated with the transplantation process including procurement, preservation, the actual surgery, the immune attack and immunosuppression inflict damage on the organ. The injuries may also induce restorative and regenerative processes, and the long-term function of a transplanted organ depends on a balance between damage and repair. The unique opportunity provided by the transplant models leads to several new observations.

Regeneration and graft recovery are initiated from the resident parenchymal cells of the transplanted organ. Rodent ischemia and transplant models have been used to demonstrate that cytokine-dependent pathways in IRI damaging the graft may also play a significant role in initiating the cell cycle. Jun B, c-fos, c-myc and c-jun are induced during liver regeneration, and are associated with the initiation of DNA synthesis, are also increased after prolonged ischemic times [27]. TNF- α , IL-1 β and IL-6 induced as part of the IRI related inflammatory cascade also mediate liver regeneration. Further significance is implied by the severely defective hepatocyte replication in mice genetically deficient in these cytokines following partial hepatectomy.

Direct evidence for the complex role of TNF- α and IL-6 in post-transplant regeneration has also been demonstrated by a recent study. Implantation of small liver grafts may result in liver injury and defective regeneration. Partial 30% liver transplantation was performed in C57BL/6 wild-type mice [control group], and in three groups with down-regulation of the TNF- α pathway including TNF receptor 1 knockout mice, and mice undergoing Kupffer cell depletion either by gadolinium chloride or pentoxifylline pretreatment. Animal survival rates were significantly improved in the treatment groups compared with control, and liver injury was reduced. Liver regeneration occurred only in the treated groups accompanied with extremely high IL-6 and IL-10 levels. The robust protective effect of pentoxifylline was, however, not detected in IL-6 $[-/-]$ mice, and protection could be restored by r-IL-6. Thus, interruption of TNF- α signaling or depletion of Kupffer cells [its main source] reduces graft injury, and improves survival and regeneration [28].

Another example of common pathways between injury and regeneration following transplant related damage is the cell cycle surveillance mechanism. When defects in DNA synthesis and chromosome segregation are detected, the cycle progression is blocked at different decision points. The checkpoints are controlled by cyclin-dependent kinase [cdk] inhibitors, especially p21. Ischemic injury to the kidney results in marked induction of p21 mRNA in nuclei of both distal and proximal tubule cells. Mice homozygous for a p21 gene deletion displayed more profound damage following ischemia than p21 $[+/-]$ controls. Widespread cell death in these mice, on the other hand, was associated with increased cell cycle activity, suggesting that cell injury induces pathways that compete between cell death versus coordinated cell cycle control required for graft recovery [29].

Stem cells and progenitor cells also have a distinctive role in post-transplant regeneration. These cells originate from local pools and as well as from the circulation [30]. Their nature, regulation and long-term fate after transplantation are of significant importance.

For a local response, the presence of pluripotent progenitor cells, typically detected in embryonic tissues, is crucial in adult organs. In last few years, these cells have been described in several organs. The presence of nontubular cells expressing stem cell antigen-1 [Sca-1] that

are enriched for beta1-integrin, and show minimal expression of surface markers typical from bone marrow-derived mesenchymal stem cells, has been described in adult mouse kidney. Gene profiling showed enrichment for many genes of developmental molecules and self-renewal pathways, and clonal-derived lines of these cells may differentiate into myogenic, osteogenic, adipogenic, and neural lineages. When injected directly into the renal parenchyma after IRI, the cells adopt a tubular phenotype and potentially could contribute to kidney repair [31].

Similarly, bipotential clonal cell lines isolated from healthy liver of 8–10-week-old C57BL/6 mice were described to differentiate into hepatocytes and bile duct cells [cholangiocytes]. Cell lines were established from primary hepatocyte cultures, from isolated colonies, and by cloning of the hepatocyte-enriched suspension. Cells of the clonal cell lines expressed cytokeratin 19, A6 antigen, and alpha6 integrin, and a large panel of hepatocyte functions. They were shown to differentiate in clusters of hepatocytes and bile ducts in albumin-urokinase-type plasminogen activator/severe combined immune-deficient mice [32].

The renin-angiotensin system [RAS], that regulates blood pressure and fluid homeostasis, recently was also implicated in both IRI and liver regeneration after partial hepatectomy. Angiotensin-converting enzyme [ACE] inhibitors [captopril and enalapril] and angiotensin II [Ang-II] type 1 receptor blockers [losartan and candesartan] were able to downregulate cellular adhesion molecules [e.g., ICAM-1], inhibit the synthesis of proinflammatory cytokines and chemokines [[e.g., TNF α , cytokine-induced neutrophil chemoattractant-1 [CINC-1]] and reduce IRI [33]. ACE inhibitors [lisinopril, captopril and enalaprilat] also stimulate hepatocellular proliferation during liver regeneration after experimental partial hepatectomy [34]. Additionally, candesartan, a potent Ang-II type 1 receptor antagonist, induces hepatocyte growth factor, the most potent mitogen for mature hepatocytes [35]. Further research is needed to establish whether RAS modulation can be advantageous for hepatic regeneration.

In addition to local sources, reparative cells may also generate from the recipient's bone marrow. A recent study using green fluorescent protein [GFP]-positive recipient BM cells and non-GFP-expressing cardiac allografts in C57BL/6 BM-GFP chimeric recipients, detected GFP positive cells in the grafts by confocal microscopy. BM-derived recipient cells were recruited to areas of vascular injury with recipient endothelial cells and smooth-muscle-cells in the setting of ongoing alloimmune recognition at 14 days post-transplantation. Immunosuppression, tacrolimus in this study, did not seem to have an effect on the frequency of repopulation [36].

Several mediators of these processes have been identified including the CXC chemokine stromal-cell-derived factor 1 [SDF-1], required for stem cell homing. SDF-1 expression was found to be up regulated immediately after myocardial infarction, and intra-myocardial injection of SDF-1 plasmid in mice resulted in increased accumulation of labeled stem cells [37]. Another important factor in stem cell mobilization is endothelial nitric oxide synthase [eNOS]. Mice deficient in eNOS show reduced VEGF-induced mobilization of endothelial progenitor cells and impaired regeneration [38].

Other factors with known stem cell mobilization ability such as granulocyte colony stimulating factor [G-CSF] have also been investigated. In a recent study using rat female-to-male full and partial liver grafts and G-CSF, neither stem cell mobilization nor induced liver regeneration enhanced the incidence or rate of stem cell derived hepatocytes. This suggests that despite the plasticity of stem cells, their capacity to transdifferentiate into parenchymal cells and the regulation of this process is far from being fully elucidated.

3.5 Transplant models exploring interactions between innate and adaptive immunity

The significance of the interactions between the innate and adaptive arms of the immune response is increasingly recognized. Although many results came from tumor and autoimmune disease models, rodent transplant models have been instrumental for the investigation of the interactions between IRI and subsequent alloimmunity. Several “bridging” mechanisms have been described in liver, kidney and heart models.

Toll-like receptors [TLRs] are a family of pattern recognition receptors activated by specific components of microbes and self cell associated molecules such as basement membrane components, cytoskeletal proteins or nucleic acids. TLRs are expressed in different cell types, primarily in APC, including macrophages, DC and B cells. Activation of these receptors results in the expression of genes required to control infection and injury, including the production of inflammatory cytokines and chemokines, complement products, and the recruitment of neutrophils to the site of injury. Increased TLR4 expression in Kupffer cells was demonstrated shortly after reperfusion in rats undergoing orthotopic liver transplantation with cold preserved syngeneic organs, and TLR4 deficient mice were found to be less prone to IRI following hepatic ischemia [39,40]. Additionally, in vivo experiments demonstrated that TLR4 is required for the initiation of IRI in the liver. TLR4, which is constitutively expressed both in cardiomyocytes and the vasculature, has a similar role myocardial ischemia as hypoxia markedly increases its expression, and TLR4 negative mice displayed decreased infarct size [41,42].

Additionally, studies in transplant related IRI allowed the identification of endogenous ligands, and highlighted the significance of the activation of TLRs by these molecules. Heat shock proteins [HSPs] are intra-cellular molecular chaperones of aberrantly folded, denatured proteins involved in cytoprotection and adaptation for cell survival in response to stress. Following profound stress [e.g., cell necrosis], HSPs may be released extracellularly in a cytokine-like manner. The immunostimulatory and inflammatory activity of these molecules are mediated through TLR2 and TLR4 [43,44].

The primary mechanism by which TLRs affect the adaptive immune response is altering DC maturation. The tissue damage associated with IRI leads to the release of the endogenous ligands described above, and engagement of TLRs on DC induces activation and maturation. As suggested by liver transplant studies performed in rats, IRI-induced maturation profoundly modifies the distribution, phenotype and function of DC. DC then acquire a chemokine receptor profile during maturation that promotes the migration of these cells from inflamed tissue to secondary lymphoid tissue, and enables them to stimulate naive T lymphocytes [45]. Immature DC are incapable of initiating T cell responses, but have been shown to promote the expansion and differentiation of regulatory T cells and thereby tolerance, suggesting that the extent of graft injury may influence alloimmunity and subsequent survival [46,47].

The role of NK cells, yet another important arm of innate immune response, following solid organ transplantation remains unclear. Recently, it has been demonstrated in a rat model of orthotopic liver transplantation that NK cells of recipient origin infiltrate the grafts early after transplantation. When the intra-graft expression of chemokines known to attract NK cells was investigated, specific profiles for different grafts have been established. CCL3 was significantly increased only in allografts few hours post-transplant, while CCL2 and CXCL10 expression were higher in both syngeneic and allogeneic grafts. CXCL10 and CX3CL1 were upregulated in allografts by day 3 post-transplant, but not in syngeneic transplants. These changes resulted in the accumulation of IFN- γ producing NK cells, and promoted alloantigen specific T cell responses. The lack of NK cell accumulation allowed prolonged graft survival, suggesting that these cells have an important role in connecting innate and adaptive immune responses following transplantation [48].

The interactions between innate and allogeneic immune responses have major implications for graft survival. Evidence suggests that these effects are mutual: in addition to the impact of graft damage on T cell responses and tolerance mentioned above, T cells have also been shown to be involved in early non-specific inflammation via cytokines. Thus, both short and long-term graft function and survival are affected, underlining the importance of complex transplant models in this area of research.

3. 6 Transplantation of embryonic organs

Growing organs in situ by implanting developing animal organ anlagen or primordia is a new approach representing a possible solution to the problem of human donor organ shortage. Renal and pancreatic anlagen from animal embryos transplanted into animal hosts undergo differentiation and growth, become vascularized, exhibit function, and support life in otherwise anephric or diabetic hosts. These anlagen can be transplanted across concordant or highly disparate barriers. They can be stored in vitro prior to transplantation, up to for 3 days in UW preservation solution [49]. The anlagen can be transplanted into the respective organ, but also survive, develop and function when placed into the omentum [50].

This approach is still highly experimental, and both basic science and technical challenges need to be addressed. Its success depends on obtaining the anlagen at defined windows during embryonic development to eliminate the risk of teratogenicity and reduce immunogenicity, while preserving maximum growth potential. Characteristics for different organs as well as for different species are being investigated. Additionally, optimal timing for post-transplantation procedures such as ureteroureterostomy must also be optimized.

4. Summary and present perspective

Rodent transplant models have limitations. The inherent differences in the genetics, physiology and immunology between rodents and humans can make clinical translation difficult. Observations or protocols established in rodents typically require reevaluation in higher species. Technical issues may also be restrictive, in particular when assessing graft function. Heterotopically transplanted grafts [such as the heart] do not have the same vasculature and function as native or orthotopic organs. Intestinal transplantation in rodents in most cases also results in partial, non-functional grafts. Fully vascularized liver transplantation in rats can be performed but is very challenging in mice, and non-arterialized grafts are less functional. These limitations may also affect regeneration and long-term function.

Despite these drawbacks, rodent transplant models are extremely useful and informative. They provide essential clues for understanding the time-dependent development and spatial distribution of the allogeneic immune response and tolerance. It is expected that these models will further help to elucidate to interactions between the innate and allospecific arms of immune response. As long-term function of transplanted organs remains a major challenge in transplant biology, regeneration studies performed in rodent models could lead to a more complete understanding of this process. A new emerging field is regenerative medicine that promises to repair damaged organs through regeneration provided by transplanted cells, stimulation of endogenous repair mechanisms, or implantation of bioengineered tissue.

The long-established methods of rodent organ transplantation, combined with the newest achievements in surgical techniques, biotechnology and imaging, will remain indispensable tools of transplantation biology.

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Figure 1.
Intraoperative picture of a murine orthotopic small bowel transplant.

Figure 2.
Changing focuses in rodent organ transplantation over the years.

Table 1

Commonly used viral vectors for gene transfer in organ transplantation.

	Characteristics	Advantages	Drawbacks, complications
Oncoretrovirus	Single-stranded RNA	Relatively high titers Broad cell tropism Viral genome integration leading to long-term expression of transgene No toxic effects on infected cells	Risk of insertional mutagenesis Degradation of virus particles by complement Only infect dividing cells Possibility of formation of replication-competent retrovirus
Foamy virus	Complex retrovirus	Relatively high titers Stable gene expression due to viral integration Resistant to complement-mediated lysis Can be pseudotyped	Risk of insertional mutagenesis Serum conversion to human foamy virus
Lentivirus	Single-stranded RNA	Relatively high titres Can infect nondividing cells Expanded cell tropism by pseudotyping Stable gene expression due to integration of viral genome	Possible serum conversion to HIV-1 Insertional mutagenesis Presence of regulatory proteins may cause immune response Possibility of replication-competent virus
Herpes simplex (HSV)	Large enveloped double-stranded DNA virus	No integration into host cell genome Long-term episomal expression of transgene in neuronal cells Cytopathic effects in cancer cell	Inflammatory and toxic reactions Complicated genome and propagation
Adenovirus (Ad)	Nonenveloped virus with linear double-stranded DNA genome	Very high titers High levels of transient gene expression Can infect nondividing cells	Inflammatory and cytotoxic host immune responses Preformed neutralizing antibodies Not suitable for long-term expression Complicated vector genome
Adeno-associated virus (AAV)	Small nonenveloped single-stranded DNA genome	Wide cell tropism, infection of nondividing cells High titers Nonpathogenic, nontoxic	Difficult purification process Helper virus may be required for propagation Preformed neutralizing antibodies