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Novel Approaches to Immunomodulation for Solid Organ Transplantation

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Abstract

Despite significant advances in the field of transplantation in the past two decades, current clinically available therapeutic options for immunomodulation remain fairly limited. The advent of calcineurin inhibitor–based immunosuppression has led to significant success in improving short-term graft survival; however, improvements in long-term graft survival have stalled. Solid organ transplantation provides a unique opportunity for immunomodulation of both the donor organ prior to implantation and the recipient post transplantation. Furthermore, therapies beyond targeting the adaptive immune system have the potential to ameliorate ischemic injury to the allograft and halt its aging process, augment its repair, and promote recipient immune tolerance. Other recent advances include expanding the donor pool by reducing organ discard, and bioengineering and genetically modifying organs from other species to generate transplantable organs. Therapies discussed here will likely be most impactful if individualized on the basis of specific donor and recipient considerations.

INTRODUCTION

Redundancies in human immune defense systems and polymorphisms of human leukocyte antigens (HLAs) are evolutionarily advantageous in protecting humans against severe infections. In transplantation, however, the same redundancies present a significant barrier to the development of simple and efficacious therapies against rejections. Currently available antirejection immunomodulation largely relies on global immunosuppression. Such nonspecific immunosuppression leads to loss of defense immunity against life-threatening infections such as cytomegalovirus and BK virus, the latter being a common cause of kidney allograft failure independent of rejection. It also renders transplant recipients more susceptible to malignancies and metabolic side effects that increase their overall cardiovascular mortality and/or impair their quality of life. The ideal immunomodulation would therefore act only on cells with specificities against the donor and promote acceptance of the allograft as self, a state known as operational tolerance. Such a strategy would minimize side effects and toxicities of general immunosuppression while inhibiting allograft rejection. Additional therapies targeting nonimmunological injuries such as ischemia reperfusion injury would further maximize utilization of marginal organs and expand the donor pool.

Achieving all of the above is unlikely with a single therapeutic modality. Rather, a combinatorial approach of modulating both the recipient immune system and the donor organ would most likely be necessary. Components of such a strategy are outlined below.

RECIPIENT IMMUNOMODULATION

Novel Pharmacological Immunosuppressive Therapies: Beyond Calcineurin Inhibitors

With the widespread use of calcineurin inhibitors, acute rejection rates and 1-year allograft survival have both drastically improved. However, their impact on long-term allograft survival has not been as impressive. This is largely due to their adverse metabolic side effects including posttransplant diabetes, nephrotoxicity, and dyslipidemia leading to allograft injury (1). Therefore, immunosuppressive therapies that are not based on calcineurin inhibitors are urgently needed.

Costimulation blockade. Costimulatory signals, also known as signal 2, are required for T cell activation, along with signal 1, which is recipient T cell receptor recognition of alloantigen peptides presented in the context of self major histocompatibility complexes (MHCs) (2). Signal 1 in the absence of signal 2 induces a state of anergy rendering T cells refractory to further antigenic stimulation. Several costimulatory interactions have been identified, including CD28-B7, CD40L-CD40, and ICOS-ICOSL (inducible costimulator–ICOS ligand), which provide targets for costimulation blockade.

CD28 is a costimulatory molecule expressed by all T cells except a subset of CD8 memory T cells (3). It binds to B7 molecules, CD80 or CD86, on antigen-presenting cells (APCs). B7-CD28 signaling in T cells leads to upregulation of genes involved in T cell activation. B7 can also bind to and signal through CTLA-4, a coinhibitory molecule expressed on activated T cells and FoxP3+ regulatory T cells (Tregs). This binding is of a higher affinity than that of B7-CD28 and can signal bidirectionally, resulting in a negative regulation of immune responses in both T cells and APCs. CTLA-4-Ig (belatacept) is a fusion protein of the extracellular domain of CTLA-4 and a portion of the Fc domain of immunoglobulin G1 (IgG1). It functions by (*a*) blocking B7 binding to CD28 on T cells and (*b*) mimicking CTLA-4-B7 negative signaling in APCs. It has been shown to prolong allograft survival (4) and, in some cases, induce tolerance (5). The BENEFIT Trial compared the efficacy of belatacept to a cyclosporine-based maintenance immunosuppression regimen (6).

Despite an increase in early acute rejection rates, belatacept maintenance resulted in fewer side effects, better kidney allograft survival at 7 years, and decreased development of de novo donor-specific antibodies (DSAs), which is the main culprit of antibody-mediated rejection (ABMR).

Blocking CD40-CD40L interaction has been widely tried in small and large animal transplant models. Murine anti-CD40L given in combination with donor-specific transfusion to fully mismatched heart allograft recipients results in 100% long-term allograft survival (7). Combining MR1 with CTLA4-Ig without donor-specific transfusion also leads to long-term acceptance of fully mismatched heart or skin allografts. Multiple nonhuman primate (NHP) kidney transplantation studies targeting either CD40 or CD40L have shown significant benefit in allograft survival (4, 8). Interestingly, anti-CD40L showed better efficacy than anti-CD40. This is thought to be due to additional blockade by anti-CD40L of CD40L-CD11b interaction on innate immune cells, reducing their allograft infiltration (9). The efficacy of anti-CD40L has also been demonstrated in pig-to-NHP xenogeneic transplantation (10). It is thought to be mediated by several mechanisms including increased Treg function and inhibition of effector and follicular T cell function, germinal center formation, B cell maturation, antibody production, and class switching (11). Despite these promising results in preclinical models, clinical development of CD40-CD40L blocking agents for human use has been stalled, largely by the high affinity of the older generation of anti-CD40L to platelets leading to life-threatening thromboembolic events (12). Newer agents without the Fc receptor for platelet binding are currently in development and/or in early clinical trials (13).

Desensitization. Access to donor organs can be limited due to the presence of preformed anti-HLA antibodies. This state, known as allosensitization, disproportionately effects multiparous females and previous transplant recipients. Early desensitization therapies were composed of intravenous immunoglobulin and plasmapheresis but failed to achieve significant long-term benefit (14, 15). B cell depletion using anti-CD20 monoclonal antibodies such as rituximab is effective in reducing antibody levels and increasing transplantation rate in allosensitized recipients. Limited success was noted in a single-center trial with 59 highly sensitized recipients, with 37% of such recipients developing ABMR and graft survival being 84% at 2 years (16). Downstream to B cells, targeting long-lived plasma cells using anti-CD38 (17) or proteosome inhibitors (bortezomib, carfilzomib) has only shown a transient decrease in antibody levels (18). Anti-CD38 further depletes Tregs and increases the risk for T cell–mediated rejection. However, in a model of sensitized NHP kidney transplantation, the combination of costimulation blockade with carfilzomib resulted in sustained suppression of DSAs and prolonged graft survival (19). Another novel agent to promote transplantation across a positive crossmatch is imlifidase, an endopeptidase that cleaves IgG. It is shown to precipitously reduce DSAs, although a rebound typically occurs within a week. Therefore, imlifidase may be a useful substitute for plasmapheresis in the acute setting, but a combination with more durable treatments would be necessary to reduce the future risk of ABMR (20).

Therapies for ABMR. DSAs have deleterious effects on allograft survival due to both acute and chronic ABMR. The above-mentioned strategies targeting various aspects of B cells and plasma cells have had minimal success in preventing functional decline of renal allografts measured by glomerular filtration rate. Terminal complement inhibition such as with eculizumab has also been investigated in ABMR, especially in those refractory to conventional therapies. While effective in reducing DSAs, it has shown no improvement in overall graft outcomes (21). Anti-IL-6 therapy has recently emerged as a promising treatment for both acute and chronic ABMR, showing a significant reduction of glomerulitis, peritubular capillaritis, C4d deposition, and DSAs, as well as stabilization of glomerular filtration rate (22). IL-6 promotes expansion and activation of T and B cells, and controls the differentiation of naïve B cells and plasmablasts into mature

antibody-secreting plasma cells. Furthermore, it promotes proinflammatory signaling in innate immune cells such as monocytes, macrophages, and natural killer (NK) cells. Beyond the immune landscape, IL-6 also promotes fibrosis via collagen synthesis in fibroblasts and differentiation of myofibroblasts. Therefore, the effectiveness of anti-IL-6 therapy can be attributed to its overarching effects on both immune and nonimmune cells. This example highlights the need for therapeutic targets beyond T and B cells. Preclinical data also indicate that NK cells play a role in ABMR by promoting endothelial damage and microvascular inflammation. This may be due to non-DSA-mediated activation of NK cells from "missing self," where donor-recipient HLA class I mismatch leads to the absence of NK cell inhibition by killer cell Ig-like receptors (23). Therefore, future therapies targeting NK cells have the potential to change the landscape of ABMR treatment.

Cellular Immunomodulatory Therapies

We review the pros and cons of three approaches using cellular therapies for modulating alloimmunity.

Peripheral modulation by infusing a single suppressor cell population. Various suppressor cell populations have been thought to dampen transplant immune responses (24). Some are known to exert antigen-specific suppression whereas others suppress nonspecifically. A typical regimen is one or more intravenous infusions of the intended cell preparation to transplant recipients. The best-studied suppressor cell population supported by the strongest preclinical data is Tregs (25). Several non–mutually exclusive mechanisms mediate immunosuppression by Tregs, including contact-dependent and contact-independent mechanisms of suppressing effector T cells and APCs, and promoting other suppressor cells such as regulatory B cells and myeloid-derived suppressor cells. Other suppressor cell populations investigated in clinical trials include regulatory macrophages, tolerogenic dendritic cells (DCtols), and mesenchymal stromal cells (MSCs).

Regardless of which suppressor cell population is used, three critical unanswered questions limit their clinical applications. First, should the recipient or the donor be the source of such cells? Recipient cells are more readily available post transplantation, provided that the effects of ongoing immunosuppression on the desired cells can be dealt with. Donor cells are more difficult to obtain, particularly in the setting of deceased donor transplantation, and may be subjected to alloimmunity once infused, but immunosuppression is not an interference.

Second, what is the appropriate number of cells to infuse? This is probably the most difficult question to address, as not only might the answer be different in different individuals with different cell types and safety concerns (e.g., the possibility of over-immunosuppression, or sensitization to donors if cells of donor origins are used), but also the answer would dictate the need for ex vivo cultures, expansions, and quality control. In the case of Tregs, available data suggest an advantage of donor-specific Tregs over polyclonal Tregs (26), supporting several ongoing efforts in ex vivo expansion of donor-specific Tregs (27, 28). The more manipulated a cell product is, the more cumbersome the quality control process will be before it can be safely infused in humans. In the case of Tregs, phenotype and function of post-culture cell products are typically examined by their high fidelity in expression of the transcription factor FoxP3 and in vitro suppression of T cell proliferation. Treg-specific demethylated region has also been used as a surrogate for their stability.

Third, how can we ensure that the cells function as intended once they are infused, and if so, that they will continue to function? Answers to these questions currently rely entirely on extrapolation from preclinical data, although a more accurate understanding in humans will have critical implications in the dosing, redosing, and possibly intentional elimination of these cells. Regardless, existing data suggest that they at best will reduce, but not eliminate, alloimmune

responses. Consequently, if tolerance is the goal, this type of therapy alone will likely promote, but not achieve, the goal.

Peripheral modulation by engaging multiple suppressor cell populations in vivo. Given independent mechanisms of immunosuppression by the various suppressor cell populations, it follows that combining them in an appropriate cell ratio may achieve a synergistic effect. However, combining multiple ex vivo–generated cellular products is logistically impossible. An alternative approach is to simultaneously engage them in vivo. Infusion of donor apoptotic cells is one such approach. Apoptotic cell clearance is a process that a human body performs billions of times a day to ensure proper cellular turnover and host homeostasis, and therefore has evolved elaborate and redundant mechanisms to ensure its noninflammatory nature. As discussed in the preceding section, whenever donor instead of recipient cells are used, one potential parallel mechanism of function of these cells is providing donor antigens to the recipient under anticipated immunoregulation of the suppressive functionality of the said cells. But if death by apoptosis, rather than any desired live cell function, is the only attribute needed, the production and quality control of such a product could be significantly simplified.

In our lab, through a decade of research, we found that treating cells with ethylene carbodiimide (ECDI) generates a donor cellular therapy with this precise attribute (29). In their rapid apoptosis after their injection, the treated cells simultaneously mobilize several recipient suppressor cell populations, including regulatory macrophages, Tregs, and DCtols, as well as myeloid-derived suppressor cells, to collectively establish a network of immunoregulation (30). It is important that cells used for ECDI treatment express both donor MHC class I and II in order for tolerance to be donor-specific. This consideration favors the choice of potentially cryopreservable and ex vivo expandable donor cells for this purpose. ECDI donor cells, in their death by design, can be considered a donor "decoy" that can be given periodically and repetitively to reinforce donor-specific immune inhibition. Needless to say, it is of paramount importance that under no circumstance could ECDI donor cells be sensitizing. In stark contrast to the early practice of donor-specific transfusion, or infusions of other forms of live donor cells such as donor DCtols and donor MSCs, ECDI treatment provides the "discipline" to donor cells that is essential in preventing them from becoming sensitizing. This attribute has been demonstrated in various rodent models and in NHP models (31). It is important to note that this strategy, by mobilizing multiple recipient suppressive cell populations, is ultimately a peripheral tolerance strategy, and can therefore be subjected to disruption by various unexpected host immune activation events such as infections.

Central modulation by inducing donor-recipient bone marrow chimera. Central deletional tolerance is the most robust tolerance for transplantation. Its principle was clearly demonstrated by early kidney transplants in patients with multiple myeloma who required a bone marrow (BM) transplant to cure their underlying hematological malignancy as well as a kidney transplant to cure their end-stage myeloma kidney disease. In this case, if the BM and the kidney were from the same donor, once the BM engraftment took place, the subsequent kidney transplant would require no immunosuppression. However, in the absence of a need for allogeneic BM transplant to cure cancers, the challenges of this type of approach are (*a*) how to minimize recipient preconditioning and yet still promote effective donor BM engraftment, and (*b*) once the BM chimera is established, how to definitively eliminate the risk of graft-versus-host disease (GVHD). Recent advances in this space have demonstrated the efficacy of facilitating cells (32), most likely a combination of various suppressor cell populations (33), in promoting effective donor BM engraftment, although the risk of GVHD remains. Another interesting advance in this regard is the possibility of cotransplanting thymic tissues along with solid organs (34), as well as the ability to generate chimeric thymus using induced pluripotent stem cells (iPSCs) (35). However, several important questions remain

unanswered, including the role of native thymus and choice of parameters in chimeric thymus construction. Nonetheless, these advances may eventually allow the establishment of donor-specific central tolerance without any risk of GVHD.

Noncellular Immunomodulatory Therapies

Extracellular vesicles (EVs) (36) and nanoparticles (NPs) provide unique advantages in overcoming limitations of cellular therapies.

Extracellular vesicles. EVs are lipid bilayer particles naturally released from almost all cells. They carry cell-specific bioactive cargo to mediate cell-cell communications. The function of EVs varies by their cellular origin; those released from immune cells range from being proinflammatory to being immunosuppressive. EVs derived from donor dendritic cells (DCs) carry donor MHCs and initiate immune response via semidirect recognition (37). Conversely, EVs from other cells, such as neutrophils (38), alternatively activated macrophages (39), or MSCs (40), have immunomodulatory and reparative functions. Notably, neutrophil EVs have anti-inflammatory and immunosuppressive effects on DCs and macrophages while blunting T cell activation and inducing Treg expansion (41). MSCs are multipotent stem cells that support self-renewal, proliferation, and differentiation of hematopoietic stem cells, and themselves have proangiogenic and immunomodulatory effects. These effects are in part executed by MSC-EVs carrying transcription factors, microRNAs, and lipids that have regenerative effects in models of kidney, heart, liver, and lung injuries. In a rat model of kidney ischemic injury, kidneys perfused with EVs from rat BM-derived MSCs during hypothermic perfusion showed reduced global ischemic damage (42). In an ex vivo human lung perfusion model, MSC-EVs in perfusates were shown to increase alveolar fluid clearance and improve airway and hemodynamic parameters (43). EVs and their cell of origin can be further engineered to increase their therapeutic cargo and to alter surface composition to enhance their stability and targeting (44). Currently, human BM MSC-derived exosomes are in a phase I trial for the treatment of rejection in abdominal solid organ transplant patients (NCT05215288).

Nanotechnology. Nanochannel membranes have been incorporated into implantable devices to promote constant and extended drug release in both systemic and local fashions. Additionally, liposome encapsulation of drugs such as rapamycin and tacrolimus provides a pharmacokinetic advantage over their current formulations. Using NP-based drug delivery technologies, immunosuppression agents can also be specifically delivered to the allograft and lymphoid organs. For example, MECA79 is a monoclonal antibody that binds to peripheral node addressins expressed on high endothelial venules in lymph nodes. MECA79-coded NPs can be loaded with tacrolimus and have been shown in a murine heart transplant model to improve graft survival without detectable systemic tacrolimus levels (45). Similarly, lymph node–directed delivery of anti-CD40L by NPs was also more effective in promoting heart allograft acceptance compared to systemic delivery (46). In addition, NPs have been developed as a modality for induction of transplant tolerance (47). Coupling of donor antigens to NPs can replace apoptotic donor cell infusions in murine models of transplantation tolerance (48). These particles can be further modified (e.g., with phosphotidylserine) to promote regulatory cell expansion (49).

DONOR OR DONOR ORGAN IMMUNOMODULATION

Modifying Existing Organs

Several approaches have been experimentally explored to modulate existing organs with a goal of benefiting the transplant outcome.

Ex vivo machine perfusion. Historically, static cold storage (SCS) is the gold standard for organ preservation, as lowering temperatures to below 10°C is thought to reduce metabolism and maintain organ viability. However, due to the increasing number of high-risk donor organs and longer travel times, there is an urgent need for improved methods for organ preservation. Marginal organs with significant cold ischemia time are often discarded. Reversal of procurement-related injury can increase utilization of these organs while reducing post-transplant complications such as delayed graft function, ischemic cholangiopathy, early rejection, and graft loss.

Ex vivo continuous machine-based perfusion is a new organ preservation modality that has been investigated using various temperatures (hypothermic versus normothermic) with both oxygenated and non-oxygenated fluid perfusates. In comparison to SCS, hypothermic machine perfusion (HMP) reduces the incidence of delayed graft function and improves graft survival in kidney transplantation (50). In liver transplantation, HMP results in a lower incidence of recipient early allograft dysfunction, biliary complications, and ischemic cholangiopathy (51). Beyond the metabolic advantage of reducing organ ATP demand, HMP also has several immunomodulatory effects. In liver perfusion models, a decrease in production of hypoxia inducible markers [HIF-α and ARNT (aryl hydrocarbon receptor)] is observed, suggesting that oxygenation demands are better met with HMP than with SCS (52). Furthermore, a number of inflammatory cytokines [TNFα (tumor necrosis factor α), IL-1β, and IL-8], chemokines, and adhesion molecules [ICAM-1 (intercellular adhesion molecule), MCP-1 (monocyte chemoattractant protein-1), and P-selectin] are also lower in HMP. This is likely due to a reduction in proinflammatory autocrine and paracrine signaling in organs subject to HMP.

Normothermic machine perfusion (NMP) is a novel platform that provides near-physiological organ preservation with circulation of warm oxygenated perfusate through the vasculature. This enables continuation of normal cellular metabolism and ATP repletion while allowing for organ viability and quality assessment. The typical perfusate is nutrient-rich and erythrocyte-based; however, synthetic oxygen carriers have demonstrated equal efficacy. Additional variables that can be modulated include temperature, arterial pressure, and volume repletion. The effect of NMP on donor resident leukocytes remains to be investigated, as do methods for reducing leukocyte infiltration after reperfusion. Cytokine filters during porcine kidney NMP have been used to reduce levels of IL-8 and IL-6 (53), while leukocyte filters during pig lung NMP have been used to reduce T cell infiltration post transplantation (54). Due to its normothermic nature, NMP is also conducive to organ reconditioning requiring cellular activities. Examples of reconditioning include (*a*) gene therapy with interfering RNA (iRNA) or lentiviral vectors, (*b*) immune cell modulation by cytokine administration or direct cell therapies, (*c*) EVs with anti-inflammatory and immunosuppressive functions, (*d*) NP-based endothelial cell targeting via anti-CD31 and anti-ICAM-1 conjugation, and (*e*) combinations of biological agents such as prostaglandin, prostacyclin, and thrombolytics.

The opportunities for therapeutic interventions with minimal to no risks to the recipient are enormous. Further investigations are needed to standardize and implement machine perfusion protocols more widely.

Senolytics. Senescent cells accumulate in various tissues with aging and are characterized by expression of tumor-suppressor proteins, which regulate G1/S cell-cycle checkpoint and prevent malignant transformation of aging cells. These senescent cells adopt a senescence-associated secretory phenotype (SASP) leading to the production of inflammatory cytokines, proteases, and growth factors that induce senescence in the surrounding non-senescent cells (55). Chronic diseases such as diabetes, cardiovascular disease, and chronic kidney disease, together with biological aging, contribute to a process called "inflamm-aging" (56). This is characterized by low-grade

chronic inflammation that accelerates cellular senescence. These chronic diseases are often present in both transplant recipients and donors, thus compromising the function and life span of the transplanted organ. ATM (ataxia-telangiectasia mutated) kinase inhibitors and JAK (Janus kinase) inhibitors are senolytic drugs that prevent the release of SASP-associated factors (57).

Additionally, older organs have an exaggerated response to ischemia/reperfusion injury by releasing cell-free mitochondrial DNA, which amplifies DC-mediated Th1/Th17 alloimmune response (58). In this regard, dasatinib (D), a Src/tyrosine kinase inhibitor, and quercetin (Q), a natural flavonoid that binds to BCL-2, were investigated in murine cardiac transplantation with allografts from old donors (58). D+Q selectively eliminated senescent cells, reduced cell-free mitochondrial DNA and age-associated inflammation, and prolonged cardiac allograft survival. Organ discard, specifically of older organs, remains a significant challenge. Therefore, senolytics offer a unique opportunity to expand the donor pool.

Engineering New Organs

Several approaches have been experimentally explored to engineer new organs with a goal of expanding the organ source while still benefiting the transplant outcome.

Tissue engineering. Demands for transplantation of flat tissues such as skin and bladder have stimulated tissue engineering as a promising approach for generating organ substitutes. Such advances as "organ-on-a-chip," organoids, and various scaffold materials have accelerated attempts to design tissue-level architecture. Engineered organs can be designed to be genetically identical to the recipient or, through biomodification or encapsulation, significantly lower their immunogenicity. For instance, in islet transplant, biocapsule-like "nanoglands" can be generated using biocompatible polymers; this enables housing of pancreatic islets in a growth factor–rich matrix to promote neovascularization (59). Broadly speaking, there are two major approaches of organ engineering: one that involves engineering the smallest components of a tissue and assembling them into a larger construct, and another that involves repopulation of a macroscopic organ scaffold with appropriate cells.

Technologies for engineering organ-specific tissue range from simple microfluidic flow chambers to complex 3D structures known as microphysiological systems (60). Polydimethylsiloxane, a soft silicone polymer, has been utilized to generate the kidney endothelial-epithelial interface, or a cord of hepatocytes to mimic the liver sinusoidal transport, or even complex lung exchange barriers (61). Hydrogel is another biomaterial that has been used for casting scaffolds that mimic the architecture of tissues and organs (62). These materials have been further incorporated in bioprinting, a 3D reconstruction with anatomical information extracted from patients' imaging data.

Whole-organ scaffolds are made by decellularizing donor organs and are subsequently repopulated with the patient's own cells to generate a functional organ (63). This process yields a structure that preserves the native spatial distribution of cells and extracellular matrix, and provides the microenvironment for appropriate organization of the seeded cells. This technique has had a moderate degree of success for generating functional organs. For example, when rat organs were generated this way, kidneys produced urine (64), hearts pumped (65), and lungs oxygenated (66). Advances in stem cell technology, specifically in iPSCs, can make it possible to generate specific cells in large quantities using patients' own cells for the purpose of recellularization. However, significant advances are still needed for generation of transplantable organs.

Gene editing for xenotransplantation. Cross-species transplantation can meet the challenge of organ shortage while possibly overcoming HLA sensitization barriers. Domesticated pigs

have anatomical and physiological similarities to humans despite their considerable phylogenetic distance. However, pig-to-human transplantation has historically presented formidable immunological challenges. Recent advances in genetic engineering have successfully reduced these challenges by removing genes encoding immunogenic proteins (e.g., αGal) and altering genes regulating the complement cascade. GGTA1 gene knockout led to the production of pigs without cell surface αGal. Insertion of the human gene encoding α1,2-fucosyltransferase (H transferase) modifies donor cell surface proteins (67) to protect against both hyperacute rejection and delayed xenograft rejection (68). H transferase expression on pig endothelial cells limits monocyte adhesion and activation, as well as NK cell–mediated endothelial cell lysis (69). Another immunological barrier in pig-to-human transplantation is the incompatibility between pig complement regulatory protein and human complement proteins, resulting in uncontrolled complement activation. Insertion of genes expressing human CD46 (a membrane cofactor protein) (70), CD55 (a decay-accelerating factor), and CD59 (a membrane inhibitor of reactive lysis) in the pig genome reduced complement activation in a pig-to-baboon transplant model and prolonged graft survival to 92 days (71). Similarly, pig and human coagulation systems also suffer from genetic incompatibility and predispose recipients to consumptive coagulopathy. CD39 is a critical thromboregulatory protein that inhibits platelet aggregation via the production of adenosine monophosphate and diphosphate. Therefore, transgenic expression of human CD39 can also be beneficial (72). Recent advances in gene editing technologies have made multiple simultaneous donor genetic modifications feasible, and have resulted in the recent first pig-to-human heart (73) and kidney (74) transplantations. These advances will likely be a critical turning point for xenotransplantation.

SUMMARY

The future of organ transplantation is exciting, with advances in immunosuppression, cell-based therapies, tolerance induction, machine perfusion, and conditioning of donor organs. These therapies can combat organ shortage, limit the side effects of lifelong systemic immunosuppression, and minimize graft rejection. To widely implement the various strategies discussed here, it is vital to understand that a "one size fits all" approach would likely not be advisable. Instead, an individualized immunomodulation approach based on thorough donor and recipient considerations would be needed to yield the best outcomes.

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