

Immune rejection: current understanding and new solutions

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Abstract. While organ transplantation has emerged as a successful treatment option for certain human diseases, failure to induce life-long graft tolerance remains the most significant obstacle to achieving optimal post-op outcomes. To better cope with this challenge, it is particularly important to understand and distinguish between the different mechanisms that induce graft rejection. After transplantation, host immune responses can be triggered by both stranger and danger signals. Genetic differences between individuals contribute to the identification of foreign entities by recipient immune cells. The recovery and preservation of organs lead to the cell stress or death, inducing inflammation and further aggravating graft damage. Herein, I aim to summarize the modern understanding of transplant immunology and compare the current medical scheme with innovative strategies. This review focuses on recent progresses in stem cell transplant and compiles technological breakthroughs to improve autologous iPSC therapeutics.

Keywords: Immune rejection, Organ transplantation, Transplant immunology, Stem cells.

1. Introduction

Organ transplantation has been recognized as an effective therapeutic approach for selected human diseases. This process involves the replacement of non-functioning organs and tissues with healthy ones called grafts. The graft is provided by the donor to the recipient, also called the host. When the donor and the recipient are the same species, the graft is allogeneic (allograft). When the donor and recipient differ in species, the graft is xenogeneic (xenograft).

Transplantation of solid organs, such as kidneys, livers, and hearts is commonly practiced in clinical settings. Other procedures including lung and pancreas transplants are becoming more prevalent¹. The total number of transplants performed in the United States has doubled over the past 25 years, yet there are still over 100,000 patients in the United States awaiting a life-saving transplant (*UNOS Data and Transplant Statistics | Organ Donation Data*, 2023). Based on federal government statistics, 17 patients die while waiting every day³. The demand for donors is both urgent and immense.

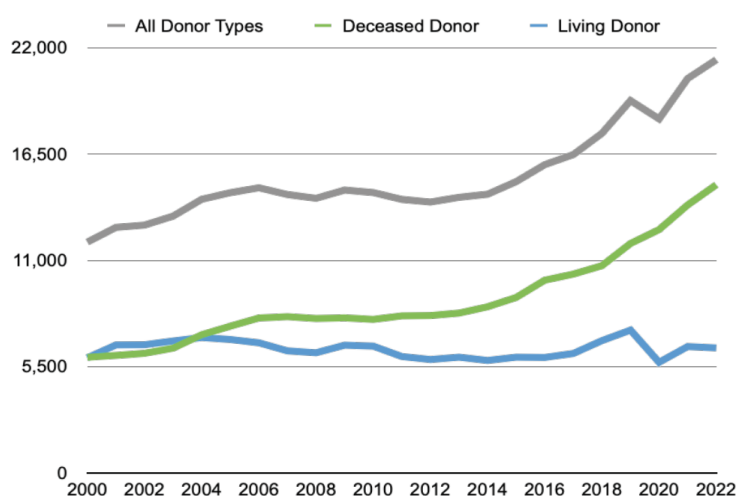


Figure 1 Trend of solid organ donations in the United States since 2000. Data from United Network for Organ Sharing. Available at <https://unos.org/data/>

Despite the increase in organ transplant procedures (**Figure 1**), immune rejection to allografts presents another barrier to achieving optimal transplant outcomes. The surgical process leads to tissue injuries and the release of danger signals, stimulating the immune system to respond to damages.

Additionally, the immune system recognizes the foreign nature of allografts, further rejecting the graft⁴. Immune rejection following organ transplantation is classified into three types based on the duration of onset: Hyperacute, acute and chronic¹. Hyperacute rejection, characterized by its rapid onset after the transplantation procedure, typically occurs within minutes to hours. Patients receive frequent screening for antibodies against potential donors to mitigate the risks of hyperacute rejection¹. Acute rejection manifests between one week to several months after transplantation^{5,6}. With the help of modern immunosuppressive strategies, less than 15% non-sensitized patients develop acute rejection⁷. On the contrary, chronic rejection, lacking effective treatment, has emerged as the primary cause of long-term organ failure post transplantation⁶. Recent investigations have shed light on the notable role of myeloid cells and macrophages to exert long-lasting effects on allografts⁸.

The Innate and adaptive immunity together mediates the defense responses against infectious microbes¹. Innate immunity reacts rapidly, within hours or days after the infection. Major components of the innate immune system includes the barrier epithelia that blocks entry of microbes; macrophages, mast cells and dendritic cells for detecting foreign microbes; white blood cells (leukocytes), natural killer cells and other cells that eliminate the invaded microbes and damaged host cells^{9,10}. Adaptive immunity is mediated by lymphocytes and consists of three distinct steps: Antigen recognition, lymphocyte activation and elimination. Immunologic memory occurs at the end of the primary response, leading to stronger and faster reaction at secondary exposure¹¹. Adaptive immunity can be either humoral or cellular. Humoral immunity is mediated by antibodies produced by B lymphocytes. B cells clonally express membrane-bound antibodies, each recognizing specific antigens. Helper T cells (CD4+ T cells) are also required to initiate response against protein antigens and stimulate the production of antibodies. Antibodies soon target the infectious microbes for elimination^{12,13}. Cellular immunity is mediated by T lymphocytes of distinct functions. Helper T cells secrete cytokines and membrane molecules to activate other cells for elimination. Cytotoxic T Lymphocytes (CTLs) directly kill infected cells and tumor cells. Regulatory T cells mainly inhibit immune response and maintain nonreactivity to self. T lymphocytes do not directly produce antibodies. Instead, they specifically recognize major histocompatibility complexes (MHCs) bound with peptides derived from foreign proteins¹⁴⁻¹⁶.

Stem cells are found in embryos and adult cells with the potential to differentiate into specialized/matured cell types (**Figure 2**). During the course of specialization/maturation, stem cells gradually lose their differential potency. Recent technological advancement has allowed the artificial reversal of maturation, inducing pluripotency in any cell type^{17,18}. Various stem cells have been reported to accelerate tissue regeneration and have promising clinical potential¹⁹. Currently, pluripotent stem cells (PSC) and adult stem cells (ASC) generated organoids are most highlighted in regenerative medicine²⁰.

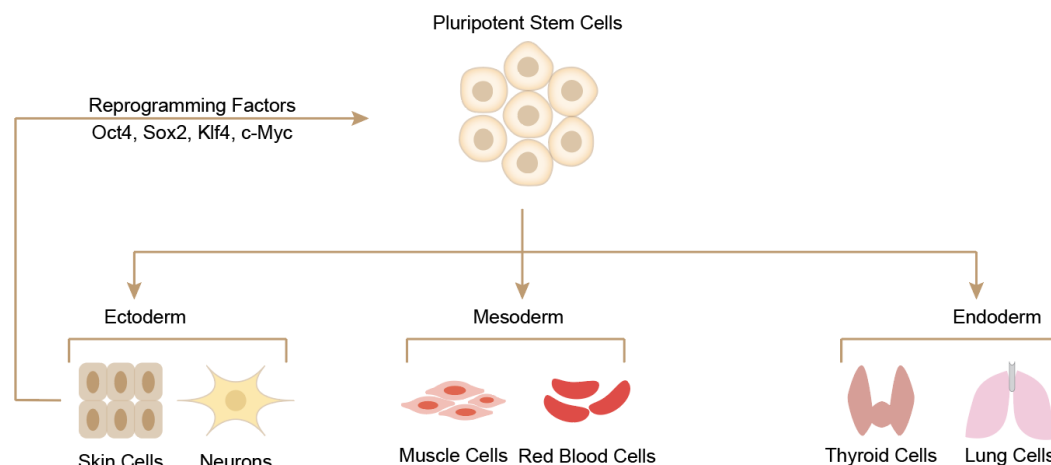


Figure 2 Pluripotent stem cell differentiation and reprogramming. Embryonic stem cells (ESCs) have the potential to develop into specialized cell types. Upon induction by reprogramming factors, adult somatic skin cells can be reverted to a pluripotent state.

2. Immune rejection

In the early 20th century, Alexis Carrel invented the three-point vascular anastomosis method, solving the problem of vascular suture and enabling solid organ transplantation^{21–23}. However, attempts to perform transplantation have failed successively due to limited understanding of the immune system. Until the 1930s, Gibson and Medawar performed skin transplantation, revealing significant rejection of allogeneic skin grafts with a woman who received both her own and her brother's skin²⁴. Hence, suppressing host immune rejection has become a prevailing method to ensure long-term patient and graft survival.

2.1 Types of rejection

Immune rejection following organ transplantation is classified into three types based on the duration of onset: Hyperacute, acute and chronic¹. Hyperacute rejection triggers local coagulation cascades through antibodies directed against allogeneic MHC molecules and blood group antigens, eventually resulting in thrombosis and infarction¹. Acute rejection can be cellular, humoral, or a combination of both^{25,26}. With the help of modern immunosuppressive strategies, less than 15% non-sensitized patients develop acute rejection⁷. In contrast, chronic rejection poses a considerable challenge. Current immunosuppressive medicines cannot effectively induce life-long graft tolerance. Thus, chronic rejection has become the leading cause for long-term organ failure⁶. Recent investigations have shed light on the notable role of myeloid cells and macrophages to exert long-lasting effects on allografts. These observations underscore the limitations of current immunosuppressive strategies in promoting long-term graft tolerance⁸.

2.2 Rejection mechanisms (STRANGER v.s. DANGER)

Current understanding of allograft rejection is mainly composed of two pathways: The STRANGER and the DANGER model⁴. The traditional Stranger model suggests that the immune system distinguishes between Self v.s. Nonself²⁷. Individual genetic differences, mainly due to the polymorphism of MHC, contribute to the identification of foreignness of the graft. MHC is one of the most important molecules that determine the compatibility of the graft with the host²⁸. Host T cells can directly recognize APC (Antigen Presenting Cells) presenting unprocessed donor MHC molecules. T cells also indirectly recognize donor MHC molecules processed by APCs or MHC-derived peptides in association with host MHCs²⁹. After allorecognition, T cells differentiate into either helper T cells or CTLs to mediate graft rejection³⁰. B cells require multiple signals to produce alloantibodies. First, naive B cells recognize allogeneic MHC molecules, process, and present peptides to T cells through the indirect recognition pathway³¹. Then, B cell interaction with T helper cells provides costimulatory signals. Next, T cells produce cytokines to promote B cell proliferation and differentiation¹². Plasma cells are the terminal differentiated forms of B cells, secreting high affinity antibodies that mediate graft rejection and immune damage¹³. Worthnotingly, residual immune cells in the allograft are capable of recognizing alloantigens from the host, exerting immunological functions, and culminating into the graft-versus-host disease³².

In addition to identifying nonself, the DANGER model suggests that the immune system responds to danger/alarm signals sent by damaged cells. Danger signals may be released following cellular exposure to toxins, mechanical damages, or other factors^{33,34}. Presently, machine-perfused preservation has significantly reduced organ damage compared to static cold storage³⁵. Nevertheless, perfusion results in ischemia, and cells release adaptation factors to accommodate the hypoxic environment^{36,37}. For example, HIF- α degradation is prevented in a hypoxic state, while increased expression of HIF- α activates innate immunity and promotes apoptosis^{38–40}. Ischemia also disrupts cellular homeostasis, redox status and nutrient supply. Such imbalance impairs the proper function of endoplasmic reticulum, resulting in improper protein folding⁴¹. Accumulation of misfolded protein in the ER induces several response pathways. Eventually, ER stress may activate the cell death pathway^{42,43}. Moreover, the following blood reperfusion further exacerbates graft damage. Activated

endothelial cells promote the secretion of inflammatory cytokines and chemokines. Neutrophils and other immune cells infiltrate the injured tissues upon intracellular releasing of DAMPs (Damage-associated molecular patterns; ^{44,45}. Furthermore, the immune system detects and eliminates dead cells. Apoptosis releases DAMPs that induce inflammation and stimulates adaptive immunity ⁴⁶. Therefore, the immune system responds effectively to the health state of the cells after transplantation (**Figure 3**).

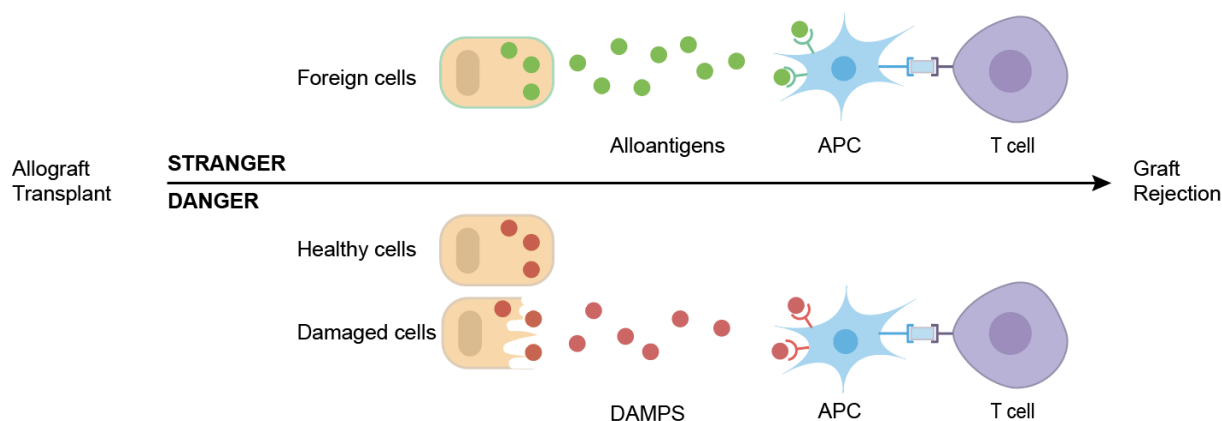


Figure 3 DANGER v.s. STRANGER model. Allograft transplant triggers immune rejection in two pathways collectively. Genetic differences between the host and the donor lead to allorecognition by T cells. Cellular damage releases DAMPs, inducing immune responses.

2.3 Immunosuppression

Early attempts to manage immune rejection involved whole-body irradiation and the administration of cortisones ^{47,48}. Azathioprine later emerged as an immunosuppressive drug for kidney transplants ⁴⁹. In 1969, Cyclosporine A (CsA) was isolated as a calcineurin inhibitor specifically targeting lymphocyte proliferation and activity ⁵⁰. Tacrolimus, sharing a similar mechanism to CsA, has a stronger inhibitory effect and lower toxicity ⁵¹. Sirolimus, also known as Rapamycin, is similar to Tacrolimus in structure yet different in mechanism. Sirolimus blocks IL-2 and suppresses T-cell activation ⁵². Mycophenolic acid (MPA), the active ingredient of Mycophenolate-mofetil (MMF), selectively inhibits lymphocytes proliferation by targeting Inosine-5'-monophosphate dehydrogenase, the rate-limiting enzyme in the classical synthesis pathway of guanine ⁵³. Later, tumor necrosis factor (TNF) produced by T cells was identified as the target of Methotrexate as an immunosuppressant ⁵⁴. More recently, Belatacept has received approval as an immunosuppressant drug that selectively blocks the activation of T cells ⁵⁵. Glucocorticoids, administered in conjunction with immunosuppressants, have demonstrated remarkable efficacy in controlling immune rejection (**Figure 4**) ⁵⁶.

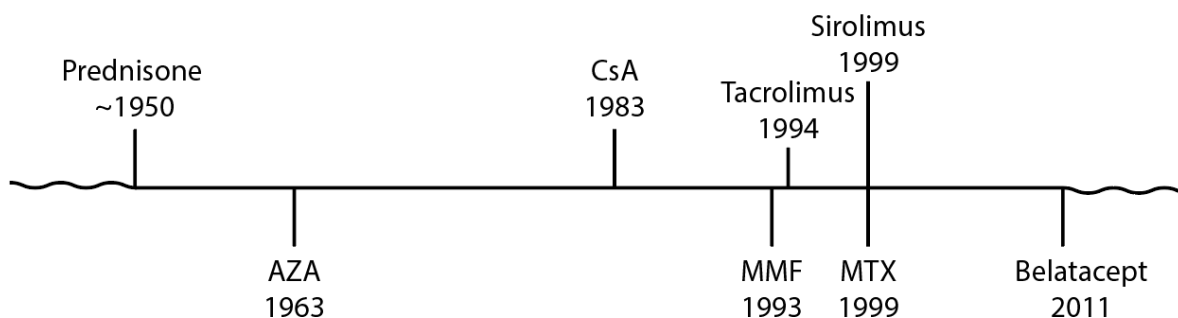


Figure 4 Approval of immunosuppressive medications. Clinically used immunosuppressants and their year of approval by the Food and Drug Administration (FDA) are displayed in a timeline.

3. Recent advancement in solid organ transplantation

3.1 Xenotransplantation

Xenotransplantation is a procedure that involves a nonhuman animal donor. The pursuit of xenotransplantation has been driven, in part, by the need to address the shortage of available grafts⁵⁷. Since 1905, clinicians have attempted xenotransplantation using various animal sources such as rabbits, chimpanzees, baboons, goats, and pigs. However, the clinical application of xenogeneic organs became prohibited in 1999 due to high risks of hyperacute rejection and the transfer of zoonotic diseases. More recently, xenotransplantation has regained clinical interest, particularly with the advent of CRISPR/Cas9 technology⁴. The selection of a suitable animal source for xenotransplantation requires careful consideration of multiple factors, including the risk of infection transmission, challenges in breeding, organ size, and other compatibility issues. Among the options, pigs have been determined as the preferred source due to their comparable organ size and physiology to humans⁵⁸. Nevertheless, xenotransplantation from pigs presents significant challenges, such as the presence of unknown epitopes and a different coagulation system. Humans present an antibody against α -1,3-galactose on the surface of pig cells, triggering hyperacute rejection hours after the transplant⁵⁹. Researchers have generated the GTKO (Galactosyltransferase Gene Knock-Out) pigs, lacking the enzyme to add galactose to the oligosaccharides to pig endothelium⁶⁰. Additionally, a group of scientists successfully knocked out 62 PERV (Porcine Endogenous RetroViruses) fragments, reducing the transmission to human cells to 1/1000 of before⁶¹. In 2021, a gene-edited pig kidney was transplanted into a patient who experienced cerebral death, without hyperacute rejection or transmission of porcine retroviruses⁶². Furthermore, the first cardiac xenotransplant was conducted in January 2022, when a gene-edited pig heart was transplanted into a patient with end-stage heart disease. The patient has survived for 2 and half months⁶³.

3.2 Stem cell induced organ transplant

Stem cell therapy bears great clinical potential due to its differential self-renewal potency. Mainly PSCs (Pluripotent Stem cells) and ASCs (Adult Stem Cells) are implicated in tissue and organ regeneration strategies. Moreover, PSC- and ASC-derived organoids offer an *in vitro* 3D structure and functional mimicry of organs, serving as a useful model for drug screening and disease modeling^{64,65}

PSCs

Embryonic Stem Cells (ESCs) and induced Pluripotent Stem Cells (iPSCs) are two types of PSCs that hold significant potential in the field of organogenesis. Both of them are capable of differentiating into various tissues.

- a) ESCs are derived from blastocysts, and can differentiate into different cell types depending on culture conditions⁶⁶. Clinical trials have recently demonstrated the safety and efficacy of ESC-derived therapies in treating conditions such as spinal cord injury and macular degeneration⁶⁷⁻⁶⁹. However, further clinical trials utilizing ESCs have been impeded by ethical concerns related to the use of human embryos. Besides, incomplete differentiation of ESCs may give rise to teratomas⁷⁰. Moreover, in murine models, immune rejection to differentiated ESCs has been observed, highlighting added drawbacks of ESC-based therapeutics⁷¹.
- b) iPSCs are artificial PSCs generated from fibroblasts by inducing transcription factors: Oct4, Sox2, Klf4, and c-Myc¹⁸. In contrast to human ESCs, iPSCs are derived from adult somatic cells, thereby avoiding the ethical concerns associated with using human embryos⁷². Furthermore, the use of autologous iPSC-derived therapies potentially reduces the risks of immune rejection⁷³. In 2017, Japanese scientists performed successful transplantation of iPSC-derived retinal cells to treat macular degeneration⁷⁴. However, the transcription factors to induce iPSCs are tumorigenic^{72,75-77}. This oncogenic property raises concerns about the

neoplastic development of cells derived from iPSCs⁷⁸⁻⁸¹. Moreover, the delivery of reprogramming factors involves a viral expression system, previously reported to induce immune and inflammatory responses in host tissues^{77,82}. Furthermore, in a recent data compilation report, only 11% of iPSC-related clinical trials explored its potential as a treatment approach⁸³. Therefore, iPSC-therapeutics lack satisfactory evidence and are significantly inadequate for clinical applications.

ASCs

ASCs have more restricted self-renewal and differential potential than PSCs⁸⁴. They localize at specialized structures called niches to maintain tissue homeostasis in developed organisms⁸⁵. Among different ASCs- based cell therapies, mesenchymal stem cells (MSCs) are the most highlighted in tissue regeneration. Harvested from adult tissues, MSCs exhibit the capacity to differentiate into various mesodermal lineages, including adipose, muscle, chondrocytes, and osteoblasts⁸⁶⁻⁸⁹. These cells play a vital role in tissue regeneration and repair due to their ability to secrete a range of growth factors that promote cell proliferation and angiogenesis. In addition to their regenerative potential, MSCs possess advantageous characteristics such as anti-inflammatory and immunomodulatory properties⁹⁰. Consequently, MSC-based therapeutics have achieved commercialization although several challenges persist^{91,92}. The efficacy of MSC-related therapies remains a subject of debate and further investigation⁹⁰. Moreover, allogeneic MSCs display detectable levels of human leukocyte antigen (HLA; human MHC) antigens^{93,94}. Cases of rejection and chronic immune response have been reported in both animal and human clinical trials⁹⁵⁻⁹⁷.

Developments to enhance stem cell therapeutics

c) Co-transplantation of activating factors

Stem cells differentiate in response to specific ligands and growth factors⁹⁸. Therefore, co-transplantation of activating factors is likely to increase the survival and efficacy of stem cell transplantation. For example, BMPs (Bone Morphogenetic Proteins) and their peptide precursors promote the osteoblastic differentiation of MSCs⁹⁹⁻¹⁰¹. Additionally, extracellular matrix proteins enhance the regenerative efficacies of stem cells. Collage matrix and fibrin improves the clinical efficacy of MSC-transplant¹⁰². Recombinant periostin and N-acetylated proline-glycine also accelerate angiogenesis¹⁰³⁻¹⁰⁵.

d) Nonviral delivery systems

As mentioned previously, the viral expression system to deliver reprogramming factors induce immune and inflammatory responses in host tissues^{77,82}. Logically, using non-viral systems would be more beneficial for reprogramming somatic cells^{106,107}. Current non-viral methods include electroporation, complexation and the incorporation of nanoparticles¹⁰⁸. Previous delivery of mRNA with electroporation was successful to induce pluripotency of somatic cells, yet multiple rounds of electroporation resulted in massive cell death¹⁰⁹. Complexions with cationic polymers effectively deliver mRNA to express reprogramming factors^{110,111}. More recently, nanotechnology has been exploited to generate iPSCs. First, Cap-NPs (Calcium phosphate NanoParticles) facilitated the delivery of reprogramming factors to generate virus-free iPSCs¹¹². Then, the cationic bolaamphiphile was developed for protein delivery and proven successful induction of human iPSCs¹¹³. CNPs (Chitosan Nanoparticles) were recently employed to deliver recombinant OCT4 protein, holding promises to convert human fibroblast into transgene-free iPSCs¹¹⁴. Recently, a GO-PEI (Graphene Oxide-PolyEthylenImine) delivery system was developed to allow direct conversion from somatic cells to induced neurons¹¹⁵.

4. Conclusion

Organ transplant has become the preferred treatment for certain human diseases. However, immune rejection impedes life-long graft tolerance and contributes to eventual organ failure. Over the past century, concerted scientific endeavors have fostered a comprehensive understanding of the human immune system, largely benefiting patient survival in need of organ transplant. While

introduction of immunosuppressive medications has effectively curtailed the incidence of acute allograft rejection, chronic rejection still poses a significant challenge to long-term graft survival. Encouragingly, recent advancements in bioengineered xenotransplantation and stem cell therapy brings new hopes in tackling this persisting impediment. Though requiring further and more in-depth clinical analysis, autologous iPSC transplant holds considerable promise in combination with biomaterial and nanotechnology. Overall, the progressive refinement of organ transplantation will continue to help the countless individuals in need of life-saving interventions.

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