Concise Review: Immunologic Lessons From Solid Organ Transplantation for Stem Cell-Based Therapies

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Abstract

Clinical organ transplantation became possible only after powerful immunosuppressive drugs became available to suppress the alloimmune response. After decades of solid organ transplantation, organ rejection is still a major challenge. However, significant insight into allorecognition has emerged from this vast experience and should be used to inform future stem cell-based therapies. For this reason, we review the current understanding of selected topics in transplant immunology that have not been prominent in the stem cell literature, including immune responses to ischemia/reperfusion injuries, natural killer cells, the adaptive immune response, some unresolved issues in T-cell allorecognition, costimulatory molecules, and the anticipated role of regulatory T cells in graft tolerance.

Introduction

The immune response to allogeneic cell therapies is a major challenge to translation of stem cell biology [1, 2]. For purposes of this review, allogeneic stem cell-based therapies include cell products derived from pluripotent embryonic stem cells or adult stem cells, both undifferentiated and differentiated, all of which will produce some immune recognition responses. Mesenchymal stem or stromal cells (MSCs) are an exception in that allogeneic MSCs do not induce classic rejection responses, and in fact MSCs are immunomodulatory and are being explored for the ability to turn down some of the cytotoxic responses to solid organ transplantation discussed in this review [3]. Cell transplant experience with pancreatic islet transplantation is a reminder that suppression of allogeneic responses to cellular grafts is far from straightforward. Stem cell biologists are aware that immunogenicity of transplanted stem cells and their differentiated derivatives is dependent on the relatedness of donor and host, the state of differentiation, manipulations of cells in culture, the anatomic site of delivery, and the particular cell type [4–6]. Nonetheless, there is not a consensus on methods to monitor immunogenicity after cell transplantation or after solid organ transplantation [7, 8], and investigators are faced with choosing an immunosuppression regimen empirically for novel allogeneic cell transplant trials.

The purpose of this review is to present and organize a large amount of information about the allogeneic immune response that has emerged from decades of experience in clinical solid organ transplantation. Although solid organ transplantation has yielded a large body of knowledge about immunogenicity and rejection, much of this information from the clinical experience has not been addressed in the stem cell literature. Here we discuss selected relevant issues that have emerged from clinical organ transplantation (especially liver transplantation) to motivate anticipation of similar problems in clinical application of allogeneic stem cell-based therapies. We specifically do not address acute rejection, and we do not review all of transplant immunology. Instead we point to specific major challenges in organ transplant immunogenicity that we hope will serve as a resource for understanding the immune response to allogeneic stem cell-based therapies.

Immune Response to Ischemia/Reperfusion Injuries

Ischemia/reperfusion (I/R) injuries may complicate stem cell therapies at the time of donor procurement and at the time of grafting. Both warm ischemia and cold ischemia are factors in solid organ transplant outcome. Ischemia, the lack of oxygen and nutrient supply, complicates liver transplantation, as it results in consumption of glycogen and ATP in liver sinusoidal endothelial cells (SECs), Kupffer cells, and hepatocytes. Kupffer cells respond by producing reactive oxygen species and proinflammatory cytokines, such as tumor necrosis factor-α and interleukin-1α (IL-1α), that recruit and activate recipient CD4 T cells and neutrophils upon reperfusion [9, 10]. Infiltrating CD4 T cells produce interferon-α, feeding back to activate Kupffer cells and stimulating hepatocyte cytokine release [11]. Given the rapid kinetics of reperfusion injury, it is unlikely that naïve CD4 T cells are involved in this process; rather, (antigen nonspecific) effector T cells that can be activated by an inflammatory milieu in the absence of cognate antigen are the likely mediators of immune damage in this setting [12]. In support of this role, liver-resident CD4 T cells of the effector memory phenotype (CXCR3+CD62L^{low}CD4+) have been identified at reperfusion, and abrogation of CD4 T-cell receptor-mediated activation with blocking CD4 antibodies confirms that activation of naïve CD4 T cells is not essential for I/R injury [13]. Furthermore, I/R induces passive release (necrotic cells or damaged extracellular matrix) or secretion (from stressed cells) of endogenous damage-associated molecular pattern molecules such as high-mobility group box 1, hyaluronic acid, ATP, DNA, and others, recognized by pattern recognition receptors, mainly Toll-like receptor-4 [14]. Damage to hepatocytes and SECs with microvascular perfusion defects increases adhesion of neutrophils and platelets in the sinusoids, Kupffer cell and SEC swelling, and sinusoidal narrowing, potentially perpetuating ischemia to a degree of complete absence of blood flow after reperfusion ("no reflow") [14]. Importantly, although both the innate and adaptive immune systems are involved in I/R injury, the underlying cascades leading to injury are not allogeneic processes. I/R-triggered innate immune activation in the liver is self-limiting, with IL-4, IL-10, and IL-13 playing major roles in curtailing the process [15–17]. Animal models of I/R using syngeneic organ transplantation confirm that damage of I/R injury is attributable to the procurement, storage, and graft-reperfusion procedures [18].

The implications of I/R injury for cell therapies have not been widely explored. For example, cell grafts delivered immediately after thawing may require manipulation to remove necrotic/apoptotic cells from the graft, or manipulation to alter the secretory profile of graft cells in response to the I/R injury of cryopreservation, for optimal clinical outcome. Importantly, the activation state of both the transplanted cells as well as the recipients' immune systems must be taken into account in the design of studies directed at characterizing the immunogenicity of cell grafts. For example, low-level killing of cultured neuronal progenitor cells by natural killer (NK) cells is significantly enhanced by preactivation of the NK cells by IL-15 [19].

NK Cells in Organ Transplant Rejection

Classic cytotoxic T-cell rejection responses are the most studied part of transplant immunology, and most immunosuppressive drugs target T cells. NK cells are relatively unaffected by standard immunosuppression regimens and so are an ongoing challenge to allogeneic grafts.

Transplantation itself, as noted above, results in tissue damage and inflammation, and consequently, upon reperfusion of the graft, the recipient immune system encounters a plethora of soluble and cell surface danger- and stress-signal molecules. With their array of activating receptors specific for stress-related cellular events, NK cells are uniquely equipped to detect and react to the damage initiated by I/R injury. NK cell activation is a function of the sum total of all activating and inhibitory signals received by the cell. The two major components of this system are inhibitory signals delivered by major histocompatibility complex (MHC) class I molecules, and activating signals delivered by molecules upregulated in response to stress and specific to the NK cell lineage [20–22]. The activating NK cell receptor NKG2D recognizes stress- or pathogen-derived ligands, whereas inhibitory killer immunoglobulin-like receptors (KIR receptors) recognize self but not allogeneic human leukocyte antigen (HLA)-A, -B, or -C (similar to the CD94/NKG2A heterodimer that interacts with the HLA-E and -Ib molecules) [23]. NK cell subsets express either KIR receptors or CD94/NKG2A, but because of the low polymorphism of the ligand for NKG2A, HLA-E, alloreactivity is seldom displayed [24]. In contrast, KIRs are highly polymorphic, and the developmental selection process usually ensures that MHC recognition is diverse and that at least one NK cell subset will recognize and react to the absence of any single MHC class I molecule [25–27].

Generation of stem cell banks with immunologically diverse stem cell lines and specific MHC genotypes is a strategy that has attracted a lot of attention, so that potential recipients will have access to cells with high likelihood of high-degree MHC matching [28–30]. Despite their role in transplant biology, KIR molecules have not yet been addressed in this context. Although NK cell-mediated organ damage might be limited, the role of these cells as initiators or perpetuators of adaptive immune responses, well known in other contexts [31–34], has not received much attention in the transplant setting. The stress sensor system of NKG2D-mediated activation has the potential to override MHC class I inhibition in vitro, suggesting the importance of understanding the consequences of I/R injury on NK cells [19, 35]

Similar to the MHC system, the repertoire of KIR genes is inherited, and certain genetic patterns are disease-associated. For example, patients who carry one activating KIR receptor have a 36% risk of cytomegalovirus infection and reactivation after kidney transplantation versus a 20% risk with more than one receptor [36]. The pressure on pathogens exerted by the highly potent NK cell responses is inhibited by several viruses (human cytomegalovirus, adenovirus, and vesicular stomatitis virus) by selective downregulation of activating NKG2D ligands [37–39]. The clinical challenge of infection in the setting of immune suppression after transplantation may be as important in the setting of cell therapies as it is in organ transplantation, further highlighting the need to understand NK responses for optimal transplant therapies [40].

The Adaptive Immune Response

Despite the targeting of T cells in most induction and maintenance immunosuppressive therapies, acute and chronic T-cell-mediated transplant rejection still accounts for a large part of transplant morbidity and graft loss. Allofactors are increasingly recognized as a heterogeneous, organ-specific group of targets. Mismatched MHC is only one such factor, along with autoantigens, proteins with a naturally high degree of variability, and proteins with developmentally restricted expression. The importance of non-MHC allofactors is highlighted by observations of rejection phenomena in HLA-identical sibling transplants [41–44].

The contribution of low-level, chronic T-cell responses in transplant rejection is incompletely understood, and the lack of monitoring tools to detect low-level organ damage from these responses is a real gap in the therapeutic armamentarium available to transplant physicians. Consequently considerable effort is directed at identifying accessible (peripheral blood) biomarkers that detect low-grade chronic immune responses, early organ damage from them, and in concert, over- or under-immunosuppression. Ideally such monitoring tools for the integrity of stem cell-derived transplants will emerge from studies in solid organ transplant recipients.

Fundamental Questions in T-Cell Allorecognition

Incredible detail about allorecognition is available from decades of clinical organ transplant experience, but fundamental questions that still plague solid organ transplantation will likely impact stem cell therapies. The study of allorecognition is limited by the practical difficulties in accessing information about a human graft over its lifetime. The existence of alloreactive T cells is a puzzling gap in the normally efficient thymic T-cell selection during development that excludes T-cell clones reactive with self-peptide-self-MHC. The high frequency of direct allorecognition reactions (1%–10%), much higher than the percentage of T cells that responds to foreign peptides presented on self-MHC during indirect recognition [45–47], deserves further study. Recently, two unexpected groups of T cells have been found to contribute to this pool: T cells specific for minor histocompatibility complex molecules (immunogenic non-MHC proteins with natural genetic variability) and virus-specific cross-reactive T cells [48–53]. The

structural bases of interactions of self-peptide-self-MHC selected T cells with allo-MHC molecules are not fully characterized, but the role of the peptide presented by the allo-MHC has been described: in cases with minimal genetic disparity between donor and recipient, the presented peptide itself seems to be involved during TCR-MHC binding, but if more distantly related, the presented peptide may serve mainly to maintain MHC conformation [54–57].

Issues as basic as the relative contribution of direct (presentation of allopeptides by antigen-presenting cells [APCs]) versus indirect (presentation of allopeptides by self-APCs) allostimulation in rejection responses are difficult to quantify. Furthermore, the mechanisms underlying rejection of one organ (or one cell type) may be fundamentally different from those underlying rejection of other organs [58] (Fig. 1). With most data gleaned from animal models, the following concepts are generally accepted: both direct and indirect priming can give rise to responses capable of transplant rejection. Direct presentation is thought to be dominant during the first weeks or months after transplantation, after which the donor APCs die off. Then, the driving force for longer-term rejection is indirect presentation [59–62].
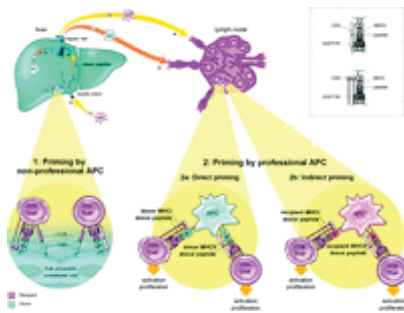
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Figure 1.
Nonprofessional and professional, direct and indirect presentation of antigen-initiated alloimmune responses. 1: Auto- and allocellular interactions can contribute to transplant alloresponses. In the liver, vascular endothelial cells can function as nonprofessional APCs. In direct presentation of allopeptides (2a) professional APCs are donor-derived dendritic cells that present allopeptides and interact with T cells. Indirect presentation is by recipient APCs that take up debris from the graft and present allopeptides (2b). In T-cell priming by nonprofessional APCs, recipient T cells migrate into the donor organ and interact with MHC and costimulatory molecules (CD80) presented by vascular endothelial cells activated by interferon-γ. 2a: Direct presentation. Donor-derived APCs (blue) migrate out of the organ into secondary lymphoid organs, where they interact with recipient CD4 or CD8 T cells. The MHC molecules are of the donor genotype (allo) and present allopeptides (shown in blue). 2b: Indirect presentation. Recipient APCs (pink) circulate to the donor, where they phagocytose debris of apoptotic or necrotic donor cells (blue). The APCs migrate out of the donor organ into the draining lymph node, where they interact with recipient CD4 or CD8 T cells. The MHC molecules on these APCs are of the recipient genotype, and they present allopeptides (blue). The inset shows an enlarged, labeled version of the components of the presentation complex. Abbreviations:

APC, antigen-presenting cell; MHCI, major histocompatibility complex class I; MHCII, major histocompatibility complex class II.

Similarly, the contribution of intragraft allostimulation (by nonprofessional APCs such as vascular endothelial cells) versus secondary lymphoid organs with professional APC allostimulation (both direct and indirect) may also impact cellular grafts. Very few human data are available, but antigen-presenting function has been reported for human endothelial cells: studies using primary human umbilical vein endothelial cells cocultured with allogeneic T cells demonstrate CD8 T-cell proliferation of mostly CD8+CD45RO+ (memory) rather than naïve cells, and endothelial cell (EC) expression of MHC II, with CD4 T-cell proliferation observed only after CD8 coculture [63]. (Murine literature in this area is not addressed here.) Other studies suggest that human ECs mediate a specific form of tolerogenic capacity via induction of regulatory T cells (Tregs) [64–66]. Surprisingly, (unlike in mice) PD-L1 is not involved in the generation of Tregs by human vascular endothelial cells, and intercellular adhesion molecule-1 can promote the generation of highly functional alloreactive Tregs characterized by high levels of surface HLA-DR [67, 68]. These studies suggest that rejection by nonprofessional APCs (most likely ECs) may be operative after cellular grafts as well, in addition to the role of secondary lymphoid organs during initiation of alloreactivity. Depending on the graft site and cell type, cellular grafts may be variably "protected" by host endothelial cells.

Secondary Lymphoid Organs in Allorecognition

Two distinct views about the role of secondary lymphoid organs in allorecognition have been put forward, and clarification of the function of lymphoid organs has implications for developing optimal immunosuppressive strategies. (a) Priming of alloresponses by vascular endothelial cells is a potentially continuous process, as these cells are, in contrast to donor APCs with limited viability, available for interaction with and activation of T cells for the life span of the graft. In addition, vascular endothelial cells are not only potential initiators of immune responses but also subject to immune effector functions of the cellular and humoral immune responses [69, 70]. (b) In contrast, exclusive priming of effector T cells in secondary lymphoid organs by professional APCs implies that various dendritic cell subsets are involved with specific functions and distributions throughout the body [71–74]. Adaptive immune responses are also potentially initiated in newly formed intragraft tertiary lymphoid organs, but the contribution of these sites to alloresponses is not known [10, 75–77]. The choice of immunosuppression regimen may also change the relative importance of the cell types and locations of the alloresponse over the lifetime of the organ, with different organs (cell types) initiating different alloresponse patterns.

Costimulatory Molecules

A critical factor during initiation of T-cell responses is the appropriate supply of a second signal provided by costimulatory molecules, as the interaction of costimulatory molecules and T cells determines the quality and quantity of the immune response. The requirement

for costimulatory molecules differs between CD4 and CD8 T cells, and naïve and memory T cells. During initiation of the immune response, CD8 and CD4 T cells interact simultaneously with the same APCs (professional, nonprofessional, or semiprofessional) (Fig. 1). In addition to the essential MHC I and II molecules, an array of costimulatory receptors and ligands are presented by the APCs and their interaction partners on T cells [78–80]. The most prominent costimulatory molecules are of the B7 family: CD80 (B7-1) and CD86 (B7-2), both of which interact with CD28 and CTLA-4. Importantly, interaction of CD80 or CD86 with CD28 generates a stimulatory signal for the T cells, whereas interaction with CTLA-4, which has a 5–10 times higher affinity for CD80 and CD86, results in inhibitory signals to the T cell, with reduced IL-2 secretion and G_1 arrest. Absence of costimulation in the presence of MHC stimulation results in permanent T-cell unresponsiveness (anergy) [81].

The requirement for costimulatory signals is not uniform among T cells. Although naïve CD4 T cells have a higher dependence on costimulation than CD8 T cells, memory T cells are relatively independent of these signals and are therefore less stringent in their activation requirements [82, 83]. In the transplant setting, memory T cells specific for several groups of antigens may exist: allopresensitization via blood products, viral infections, or the general shift in the T-cell compartment toward a memory phenotype with aging (antigen-inexperienced T cells with memory phenotype) [84, 85]. Together, these factors may result in a shift of responsiveness in the T-cell compartment toward a state where lower levels of costimulation, for example on incompletely activated APCs in low-inflammation environments, are sufficient to elicit T-cell responses. These factors are also important when considering the possible "threshold" of inflammation necessary for the initiation of the immune response. Unfortunately, because of short life span and absence of underlying infections, animal models have limited predictive value.

Given the critical but transient requirement for the interaction of costimulatory molecules for the generation of alloresponses, costimulatory molecules are targets of new immunosuppressive and immunomodulatory regimens. These drugs are potent suppressors of T-cell activation (for example, belatacept, a B7-specific fusion protein, inhibits the CD28 interaction with CD80 and CD86) but have limited effectiveness with the recall memory responses [86, 87]. To complicate the picture, immunosuppression itself creates fundamental changes in the compartments of the adaptive CD4 and CD8 T cells, as these populations have a natural tendency to "fill" the compartment, such that T-cell depletion during induction therapy stimulates replenishment, partially achieved by proliferation of the leftover T cells, in a process called homeostatic proliferation [88–90]. Once again, homeostatic proliferation is not the same for all cell types. The CD8 T-cell compartment recovers faster than the CD4 compartment, and whether regulatory T cells expand during homeostatic proliferation is unsettled [90]. Additionally, homeostatic proliferation results in phenotypic shifts such as the conversion of naïve T cells into cells with a functional memory phenotype, relatively independent of costimulation, a process that has been suggested as a barrier to transplant tolerance [91].

The Humoral Adaptive Immune Response in Allotransplant Rejection

Donor-specific antibodies (DSAs) are anti-donor HLA antibodies present at the time of transplantation or generated de novo. The incidence and clinical consequences of DSAs are organ-dependent and may significantly impact outcome, including survival [92–94]. The mechanisms by which DSAs mediate graft injury are not fully understood; complement activation and subsequent C4d deposition are monitored for pathologic diagnosis, since other mechanisms such as NK cell-mediated damage and antibody-dependent cell-mediated cytotoxicity are more difficult to identify [95, 96]. In general, antibody reactivity with the vascular endothelium induces/enhances cellular activation with subsequent expression of adhesion molecules that, together with complement, attract immune cells, including platelets, macrophages, NK cells, and others. The severity of the resulting damage, especially microvascular damage and ultimately graft necrosis, again are organ-type-specific. Not all DSAs are equal: donor-specific HLA antibodies of the IgG3 class are associated with rejection versus other subclasses of IgG, and de novo anti-MHC class II antibodies are associated with worse outcome than anti-MHC class I antibodies [97]. Interestingly, B cells have been described to form functional ectopic tertiary lymphoid tissues in transplanted organs, with the same microarchitecture as secondary lymphoid organs, supporting germinal center reactions contributing to rejection [98, 99].

Transcriptional profiling of liver transplant recipients who developed operational tolerance (absence of graft rejection despite withdrawal of immunosuppressive therapy) revealed a predominant influence of two cell types of the innate immune system in development of tolerance. NK cells and a subset of $\gamma\Delta$TCR+ T cells are present in increased numbers in liver recipients with operational tolerance [100–102], leading to the conclusion that operational tolerance in this setting is distinct from normal (not transplanted) individuals because operational tolerance is an active process rather than just a recognition of the transplant as "self." On the other hand, the transcriptional signature of kidney recipients suggests that operational tolerance of these grafts involves B cells and genes involved in lymphocyte trafficking and cell cycle control, again showing how immune mechanisms vastly differ from organ to organ [102–104]/cell to cell. These studies suggest that allorecognition is not only a one-sided hurdle to be overcome for transplant survival; operational tolerance is a form of immune recognition and requires immune recognition to be established.

Tregs in Transplantation

Tregs have reached the attention of the stem cell transplant community, and their manipulation will likely play an important role in the short-term development of novel cell therapies. Although other cell types play regulatory roles in negative regulation of immune responses, the only cells that consistently exert active tolerizing functions on the immune response (inhibition of immune effector mechanisms by a fully developed population of effector cells) are CD4 regulatory cells or Tregs. Consequently, new

clinical protocols are being developed to avoid deletion of Tregs with T-cell immunosuppressive therapies, to expand Tregs and infuse them peritransplantation, or to induce them after transplantation. The long-term results of these approaches are highly anticipated for the potential to fundamentally improve the lives of transplant recipients.

Conclusion

Clinical trials using allogeneic stem cell-based therapies are an increasing feature of translational regenerative medicine, using a variety of adult stem cell sources and, less frequently, embryonic stem cells. Immunosuppression for patients in these trials is largely empiric and graft survival often cannot be directly assessed. Consequently, information about rejection or tolerance of allogeneic stem cell grafts is important for informing the design of stem cell-based trials and therapies. Several decades of solid organ transplantation has resulted in tremendous insights into the mechanisms of rejection and tolerance. This experience can serve as a context for interpreting the immune response to cellular grafts and, in the long term, the design of optimal immunosuppressive regimens for recipients of allogeneic stem cell-based therapies.