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Overcoming immunological barriers in regenerative medicine

Johannes L Zakrzewski¹, Marcel R M van den Brink^{2,3}, and Jeffrey A Hubbell^{4,5}

¹Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

²Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

³Department of Immunology, Memorial Sloan-Kettering Cancer Center, New York, New York, USA

⁴Institute of Bioengineering, School of Life Sciences and School of Engineering, École

Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

⁵Institute for Molecular Engineering, University of Chicago, Chicago, Illinois, USA

Abstract

Regenerative therapies that use allogeneic cells are likely to encounter immunological barriers similar to those that occur with transplantation of solid organs and allogeneic hematopoietic stem cells (HSCs). Decades of experience in clinical transplantation hold valuable lessons for regenerative medicine, offering approaches for developing tolerance-induction treatments relevant to cell therapies. Outside the field of solid-organ and allogeneic HSC transplantation, new strategies are emerging for controlling the immune response, such as methods based on biomaterials or mimicry of antigen-specific peripheral tolerance. Novel biomaterials can alter the behavior of cells in tissue-engineered constructs and can blunt host immune responses to cells and biomaterial scaffolds. Approaches to suppress autoreactive immune cells may also be useful in regenerative medicine. The most innovative solutions will be developed through closer collaboration among stem cell biologists, transplantation immunologists and materials scientists.

Regenerative therapies aimed at restoring or replacing dysfunctional tissues and organs rely on a wide array of approaches that involve transplantation of cells, materials or combinations of cells and materials. If the cells are of autologous origin, the immunological barriers to transplantation are relatively low, although manipulations such as genetic modification can lead to immune responses. But successful transplantation of cells, tissues or organs that are allogeneic—derived from a donor who is not genetically identical to the recipient—must overcome numerous immunological barriers involved in allorecognition that collectively induce rejection of ‘non-self’ while tolerating ‘self’. Transplantation of solid organs or allogeneic cells into an immunocompetent individual usually elicits a host immune response against the donor cells. In addition, when allogeneic immune cells, particularly T cells, are transplanted into an immunocompromised recipient, the donor cells

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Correspondence should be addressed to J.L.Z. (zakrzewj@mskcc.org).

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can react against the recipient. The latter phenomenon, known as graft-versus-host disease, occurs frequently after allogeneic HSC transplantation¹. The development of clinical strategies to avoid immunological incompatibility by matching recipient and donor and to induce tolerance to 'non-self' by suppressing the host immune system is one of the great accomplishments of modern medicine, and as a result, solid-organ and allogeneic HSC transplantation are now part of mainstream clinical practice.

Restoring native tissues and organs through regenerative medicine faces many challenges, including directed stem-cell differentiation, achieving mechanical integrity of the graft, vascularization and innervation. If the cells used are allogeneic (or autologous cells engineered to express immunogenic therapeutic proteins), the immunological challenges are similar to those of conventional transplantation in that it is necessary to induce tolerance to immunogenic antigens (Fig. 1). In this Review, we consider whether established and experimental treatment modalities for inducing tolerance to transplantation antigens would be useful in the context of regenerative medicine. We also discuss innovative concepts developed specifically for regenerative therapies, such as approaches from the field of biomaterials and strategies that mimic evasion of the immune response by tumor cells or processes of peripheral tolerance. Finally, we propose areas for future research.

Allorecognition

The mechanisms involved in immunological recognition and rejection of transplanted donor cells are collectively known as allorecognition. Allogeneic donor cells interact with and activate cells of the adaptive immune system, including T lymphocytes, B lymphocytes and natural killer (NK) cells. The strength of this immune system response depends on the extent of disparity between the donor's and the recipient's transplantation antigens, i.e., cell-surface antigens that serve to differentiate 'self' from 'non-self'. The antigens most relevant to transplantation belong to a protein family known as major histocompatibility complex (MHC)². MHC class I molecules are found on the surface of almost all human cells (except red blood cells) and present peptide fragments of proteins expressed in the cell. Because these include peptides derived from pathogens, MHC class I molecules contribute to the defense against infectious disease. MHC class I molecules displaying particular peptides are recognized by T cell antigen receptors (TCRs) expressed on CD8⁺ cytotoxic T lymphocytes. TCR recognition of peptide–MHC class I molecules on a cell results in CD8⁺ T cell killing of that cell. MHC class II molecules are expressed only on specialized antigen-presenting cells, such as dendritic cells, macrophages and B cells; presentation of non-self antigen on MHC class II molecules leads to activation of CD4⁺ T helper cells.

At the molecular level, allorecognition can occur directly, semidirectly or indirectly (Fig. 2a)³. T cells can recognize allogeneic MHC-peptide complexes (both MHC class I or MHC class II) on allogeneic cells (direct) or on autologous cells (semidirect). Allorecognition is indirect if peptides derived from allogeneic transplantation antigens are displayed on autologous MHC class II molecules expressed on autologous antigen-presenting cells. Like T cells that respond to an infection, T cells that encounter allogeneic peptide–MHC (signal 1) are activated only if they also receive a costimulatory signal (signal 2) (Fig. 2b). Receipt of signal 1 in the absence of signal 2 induces antigen-specific T cell tolerance⁴. Professional

antigen-presenting cells such as dendritic cells are particularly adept at providing both signals to T cells and therefore have important roles in allorecognition and transplantation immunology³.

In contrast to allorecognition by T cells, MHC molecules have an entirely different role in allorecognition by NK cells. In these cells it is the absence of self MHC class I molecules that triggers activation, and expression of self MHC class I protects against NK cell-mediated killing. Thus, MHC-disparate cells (i.e., donor cells whose MHC alleles are not identical to those of the recipient) are a potential target of NK cells, and knockdown or ablation of MHC expression on donor cells—which might prevent T cell responses—is not a solution to the problem of allorecognition. In recent years it has become increasingly apparent that the role of NK cells in transplantation immunology is more complex and can also involve immune system regulatory functions, promoting suppression of both autoimmune and alloimmune responses⁵.

Central versus peripheral tolerance

Immunological tolerance to self is established and maintained by a variety of mechanisms that can be classified as central or peripheral. Central tolerance arises by clonal deletion of newly developed autoreactive T cells in the thymus. The vast majority of T cells released from the thymus are therefore tolerant to self and not to allogeneic antigens. But the process of central tolerance is not completely effective, and autoreactive T cells emerge from the thymus daily. That autoimmune diseases are rare attests to the effectiveness of peripheral tolerance, which takes place in lymphoid organs and tissues other than the bone marrow and the thymus, and acts to suppress or delete autoreactive lymphocytes. For example, immunosuppressive T cells known as regulatory T cells⁶ (generated either in the thymus or from mature naive CD4⁺ T cells in the periphery) prevent autoimmunity and chronic inflammation and therefore hold considerable promise as a means of inducing tolerance in regenerative medicine. Other cell populations with regulatory or suppressive properties include subpopulations of B cells, NK cells, NKT cells, dendritic cells and macrophages as well as myeloid-derived suppressor cells and mesenchymal stromal cells⁷. In addition to these cell types, particular molecular pathways involved in lymphocyte activation (CD28, CD40, CTLA-4) or exhaustion (programmed cell death protein; PD-1) can suppress T cell function and induce peripheral tolerance.

Lessons from transplantation

Transplantation of solid organs requires life-long immunosuppression because the host immune system remains intolerant to the allogeneic tissue. In contrast, in allogeneic HSC transplantation, the host immune system is eventually replaced by an immune system of donor origin. After an initial period with a high risk of immunological complications, such as graft-versus-host disease, successful HSC transplantation generates an immune system that is tolerant to both host and donor (as a result of both central and peripheral tolerance mechanisms), and immunosuppressive agents can usually be withdrawn within a year after transplantation. However, tolerance-inducing strategies are necessary before dual tolerance has been established, and many methods for promoting tolerance have been clinically

validated in the settings of solid-organ and HSC transplantation (Table 1). We describe these strategies and discuss which of them might be most relevant in the context of regenerative medicine.

Pharmacological approaches

Drugs that are commonly used to prevent or treat allograft rejection or graft-versus-host disease include immunosuppressive agents that affect T cell function, such as the calcineurin inhibitors cyclosporin A⁸ and tacrolimus^{9,10}, and the anti-metabolite mycophenolate mofetil^{11,12}. The potential utility of these agents in regenerative medicine is limited by considerable drug toxicities, broad immunosuppression (inhibition not only of alloreactivity but also of protective antimicrobial and antitumor immunity) and narrow therapeutic windows. However, other drugs may be more suitable. For example, although the mammalian target of rapamycin (mTOR) inhibitor sirolimus¹³ has a problematic side effect profile (including hypertension, headache, nausea, diarrhea and renal toxicity), this immunosuppressant displays two important beneficial properties: promotion of anti-tumor activity and expansion of regulatory T cells¹⁴. Other promising drugs are those that inhibit NF- κ B^{15–17} and histone deacetylase¹⁸ activity. Preclinical evidence suggests that targeting these pathways can tip the immunological balance in favor of allograft tolerance while preserving protective immunity.

Cell therapies

Preclinical studies in animal models of allogeneic transplantation and autoimmune diseases, as well as some clinical evidence, show that adoptive transfer of antigen-presenting cells, regulatory T cells, NKT cells or mesenchymal stromal cells may have tolerogenic effects, suggesting that they could provide an alternative to pharmacological immunosuppression in the context of regenerative medicine. However, it is important to recognize that any allogeneic cell is inherently immunogenic and, if injected into an immunocompetent recipient, risks elimination by alloreactive host T cells and NK cells. It may therefore be necessary to reduce this risk through pharmacological or antibody-based immunosuppression, at least during the initial phase after transplantation.

Dendritic cells are powerful antigen-presenting cells and have traditionally been used to activate the immune response against antigens such as a tumor-associated antigens¹⁹. Nevertheless, infusion of immature or 'semi-mature' dendritic cells (the latter characterized by increased expression of MHC and costimulatory molecules in the absence of proinflammatory cytokine production)²⁰ was shown to promote transplantation tolerance in several preclinical studies, including prevention of kidney allograft rejection in a rhesus macaque model²¹ and treatment of lethal graft-versus-host disease in mouse HSC transplantation models²². The tolerogenic properties of immature dendritic cells have been attributed to a number of possible mechanisms, including lack of efficient co-stimulation that results in T cell anergy or inhibition of clonal T cell expansion, as well as promotion of regulatory T cells²³.

Induction of antigen-specific tolerance by antigen-presenting cells is not limited to dendritic cells. B cells genetically modified to express a tolerogenic IgG-antigen fusion protein have

shown efficacy in experimental models of autoimmune diseases, such as encephalitis or uveitis²⁴. And adoptive immunotherapy using donor NK cells²⁵ or host NKT cells²⁶ reduced expansion of alloreactive donor T cells and ameliorated graft-versus-host disease without impairing graft-versus-tumor effects in mouse HSC transplantation models. NKT cells have the important advantage that tolerization to alloantigens can be achieved using autologous cells, whereas the evidence to date suggests that dendritic cells and NK cells must be of donor origin.

Regulatory T cells have been at the center of attention of transplantation immunologists for more than a decade. Extensive data from animal studies show that a polyclonal population of donor regulatory T cells can induce tolerance to alloantigens, provided that it contains sufficient numbers of alloantigen-specific regulatory T cells to suppress donor alloreactive effector T cells^{27–30}. Although purified antigen-specific regulatory T cells are generally more effective than polyclonal regulatory T cells, as yet there are no standardized protocols for efficient, good manufacturing practice (GMP)-compliant generation of large numbers of antigen-specific regulatory T cells. But GMP-compliant isolation and expansion of polyclonal regulatory T cells is already feasible³¹, and this approach could make banking of MHC-typed polyclonal regulatory T cells for off-the-shelf use in the setting of autoimmunity or alloimmunity a reality in the near future.

Ex vivo-expanded polyclonal regulatory T cells have been tested in clinical trials. The results of four trials in type 1 diabetes and graft-versus-host disease have been reported³², and several trials comparing regulatory T cell immunotherapy to conventional immunosuppressive regimens are under way^{32,33}. The available clinical evidence to date suggests that regulatory T cell therapy is feasible, safe and does not compromise protective immunity. A few trials have demonstrated efficacy in acute graft-versus-host disease^{32,33}.

Antigen-specific tolerance can be induced not only by transplanting regulatory T cells but also by manipulating endogenous regulatory T cells. For example, administration of low-dose interleukin 2 (ref. 34) or azacitidine^{35,36} increased endogenous regulatory T cell numbers (but not conventional T cells) and ameliorated graft-versus-host disease. This approach may be particularly relevant to regenerative medicine because controlled chemokine or cytokine release by polymeric delivery systems designed to release bioactive agents as the polymers degrade may enable recruitment of regulatory T cells to the site of implantation of tissue-engineered products. In one example, intragingival injection of a CCL22-releasing polymer reduced inflammation in a mouse model of periodontitis, indicating that therapeutic control of regulatory T cell migration can be achieved³⁷. These methods have the advantage of avoiding sorting and manipulation of regulatory T cells *ex vivo*.

Mesenchymal stromal cells found in the bone marrow and connective tissues throughout the body comprise heterogeneous sub-populations that are poorly defined. Indeed, cultured mesenchymal stromal cells are usually characterized not by cell-surface markers but by their behavior and morphology³⁸. In addition to their potential to differentiate into various mesenchymal cell types^{38,39}, which is being exploited for tissue engineering, mesenchymal stromal cells also exhibit immunomodulatory properties. The underlying mechanisms are

still under investigation, but there is evidence that immunosuppression induced by mesenchymal stromal cells is not MHC-restricted. If true, this would allow for off-the-shelf therapy using allogeneic cells³⁹. Encouraging preclinical and clinical studies have revealed beneficial effects of mesenchymal stromal cells in solid-organ transplantation^{40–42}, and these cells have been used with variable success in several clinical trials to prevent or treat graft- versus-host disease^{43,44}. Although more mechanistic studies and larger clinical trials are needed, there is no doubt that mesenchymal stromal cells are a promising option for regenerative medicine owing to their capacity to directly promote tissue regeneration while potentially dampening immune responses to allogeneic cells.

Hematopoietic chimerism

As mentioned above, allogeneic HSC transplantation can establish tolerance to transplantation antigens of donor origin. This enables subsequent transplantation of a solid organ from the same donor without the need for long-term immunosuppression⁴⁵. However, the overall toxicity of such procedures is substantial. To address this, protocols using nonmyeloablative, reduced-intensity regimens, sometimes in combination with thymic irradiation, have been developed to achieve stable mixed hematopoietic chimerism^{46,47}. Mixed chimerism is a state in which immune cells of donor and host origin coexist and—probably owing to elements of central and of peripheral tolerance⁴⁸—are tolerized to both donor and host antigens. These conditioning regimens are less toxic than the fully myeloablative regimens used for HSC transplantation to treat a high-risk malignancy. However, the risk of acute adverse events during the early post-transplantation period, as well as the burden of possible late effects (such as endocrine disorders and organ dysfunction) that can persist lifelong, is still considerable and may not be justifiable in regenerative therapies for less-serious diseases.

Antibody drugs

Monoclonal antibodies may provide a safer alternative to high-risk conditioning regimens because, compared with chemotherapy, the side effects are generally milder and mostly related to allergic reactions, avoiding major organ toxicities. Data from mouse HSC transplantation models indicate that injection of an antibody targeting the HSC antigen c-Kit (CD117) can temporarily clear HSC niches in the bone marrow⁴⁹. Administration of this antibody together with monoclonal antibodies that neutralize host lymphocytes with the potential to reject allogeneic HSCs prepares the bone marrow for donor HSC engraftment while inducing a transient state of transplantation tolerance with minimal organ toxicity^{49,50}. The use of antibodies to prevent allograft rejection entails both lymphodepletion (antibodies designed to deplete T cells and NK cells) and lymphosuppression (antibodies designed to suppress lymphocyte function)⁴⁹.

Various antibody drugs for reducing alloreactivity in the context of an intact host immune system have been tested in both preclinical and clinical transplantation studies. The majority of these agents target cytokines such as tumor necrosis factor- α and interleukin 2 (refs. 51–53) or costimulatory pathways. Costimulatory blockade can be achieved by monoclonal antibodies neutralizing CD28 or CD40 (refs. 54–56), or by infusion of cytotoxic T lymphocyte antigen 4 (CTLA4)-Ig^{57,58}, an inhibitory factor that prevents ligation of CD28

by CD80 or CD86, thereby preventing CD28 signaling. Although interleukin 2 receptor blockade suppresses one of the pathways involved in activation of alloreactive T cells, this approach alone is not sufficient to induce transplantation tolerance⁵⁹. Combination of interleukin 2 receptor blockade with costimulation blockade or costimulation blockade alone, on the other hand, allows toxicity-sparing, efficient and predictable suppression of alloreactive T cell responses, and these approaches are generally less immunosuppressive than depletion of T cells or the use of conventional immunosuppressant agents. Recent evidence indicates that costimulation blockade can decrease the number of regulatory T cells⁶⁰, underscoring the importance of further investigation to develop optimized, rational immunomodulatory approaches. Nonetheless, owing to their overall favorable safety profile, targeted immunosuppressive therapies have attracted the interest of the regenerative-medicine field.

Emerging strategies beyond transplantation

The experience of solid-organ and HSC transplantation offers a rich knowledge base of great value in regenerative medicine. In addition, a survey of recent developments in other fields reveals a diverse array of promising approaches to prevent the rejection and encourage the engraftment and long-term survival of transplanted cells. These include biomaterials approaches, considerations related to the site of transplantation, mimicking immune-evasion mechanisms upregulated by tumors, and harnessing mechanisms of antigen-specific peripheral tolerance through cellular, materials and molecular engineering.

Biomaterials for blocking immune recognition

Biomaterials have been used to physically separate donor cells from the host immune system, most notably in mouse models of type 1 diabetes mellitus. The Edmonton protocol for treatment for type I diabetes uses allogeneic cells rather than the technically complicated procedure of transplanting the whole pancreas. Islets of Langerhans isolated from donor pancreases are embolized into the hepatic portal circulation under immune system suppression with tacrolimus and sirolimus, and with initial treatment using daclizumab (which neutralizes the interleukin 2 receptor on T cells)^{22,61}. Currently, 44% of patients treated with the Edmonton protocol and its derivatives are insulin-independent three years after transplantation.

Researchers are now developing biomaterials-based methods to achieve similar clinical outcomes with less immunosuppression. Encapsulation of islets in hydrogels to restrict contact between donor islets and the host immune system was first attempted in the early 1980s (ref. 62). The pore size of the hydrogel was large enough to allow diffusion of small molecules and signaling proteins, including insulin, but small enough to block passage of antibodies, complement, host antigen-presenting cells (which could collect antigen) or T cells (which could kill encapsulated host cells by cytolytic mechanisms). In principle, encapsulation should reduce the need for immunosuppressive drugs after transplantation of allogeneic or even xenogeneic islets. In one study, an encapsulation approach restored normoglycemia for more than one year in mice with autoimmune diabetes that were transplanted with xenogeneic adult porcine islets together with CTLA4-Ig and anti-CD154 (CD40 ligand)⁶³. A more sophisticated device, which contained hydrogel-encapsulated islets

within polymer film membranes, prevented rejection of xenogeneic rat islets and restored normoglycemia in pigs with chemically induced diabetes for more than two months with no chemical or biomolecular suppression of the immune system⁶⁴. In those experiments, the eventual loss of glycemic control may have been due to growth of the recipient animal but not of the donor islet mass, which suggests that the duration of graft function could have been extended.

Biomaterials sending inward and outward signals

In addition to physically blocking immune recognition, biomaterial matrices and scaffolds can provide signals to transplanted islets to enhance their survival and morphogenetic signals to the surrounding tissue to enhance implant integration. For example, collagen IV has been used to provide survival signals from the extracellular matrix in degradable polymer scaffolds containing non-encapsulated syngeneic mouse islets; this enhanced islet function and reduced beta cell apoptosis in mice with chemically induced diabetes, accelerating restoration of normoglycemia⁶⁵. In another example of exploiting signaling by the extracellular matrix, restoration of normoglycemia after syngeneic islet transplantation was enhanced using fibrin matrices functionalized with fibronectin domains presenting platelet-derived growth factor, which increased angiogenesis from the host into the scaffold⁶⁶.

Selection and modulation of the transplant site

The strength of the immunological barrier to allografting depends on the anatomical site. The central nervous system, for example, is considered 'immune privileged', and transplantation into this site may be achieved with relatively mild suppression of the immune system. The eye is an extension of the central nervous system, and its anterior chamber (targeted by transplanting upon the iris) was used as a transplantation site for islet allografts in nonhuman primates, enabling long-term restoration of normoglycemia with anti-CD154 immunotherapy alone⁶⁷. A recent study of the mouse lymph node as a transplantation site for islets, hepatocytes and thymus tissue⁶⁸ demonstrated that similar immunosuppression regimens are likely to apply to lymph node transplants as to transplants in other sites, which is surprising given that the lymph node is highly enriched with both antigen-presenting cells and T cells.

In some cases, local interventions may be better than systemic interventions to dampen the response of the immune system to a cellular or tissue-engineered construct. For example, nanoparticles small enough to be swept into the lymphatics draining an injection site were used to deliver the immunosuppressive drugs tacrolimus and rapamycin to cells in the lymph node that drained an allogeneic skin transplantation site in the mouse, enabling prolongation of graft survival without systemic immunosuppression⁶⁹ (work by J.A.H. and colleagues), but did not prevent ultimate immunological rejection. The lymphatics and lymph nodes draining an allograft may be a particularly interesting target for immunosuppression that could reduce the need for systemic intervention. Responses of the immune system against allografts could also be decreased with locally administered immunomodulatory cells. For example, co-transplanting mesenchymal stromal cells and non-encapsulated islets in nonhuman primates with chemically induced diabetes prolonged graft function⁷⁰.

Mimicking tumor immune evasion

The development of tolerance-induction approaches in regenerative medicine may also find inspiration in the mechanisms by which tumors evade the immune system. Tumors modulate their local environment to induce nonspecific local immune suppression and specific tolerance to tumor-associated antigens that would otherwise elicit anti-tumor CD8⁺ cytotoxic T cell responses. For example, by expressing the lymphoid homing chemokine CCL21, tumors can alter their stroma to resemble lymph-node stroma in certain respects, including the presence of high endothelial venules and fibroblastic reticular cells that normally promote T cell peripheral deletion in the lymph node⁷¹. Supporting this notion, tumors that express CCL21 at levels similar to those in the lymph node maintained a more immunosuppressive microenvironment and could drive systemic immune tolerance, whereas tumors in which CCL21 expression was knocked down were more readily killed by host immune cells⁷¹. This observation suggests the possibility that CCL21 expression and lymphoid neogenesis might be useful to induce T cell tolerance to transplanted cells in tissue-engineered constructs. Similarly, tumors expressing the lymphangiogenic growth factor vascular endothelial growth factor-C (VEGF-C) enhanced tumor tolerance by increasing antigen scavenging and cross-presentation by lymphatic endothelial cells⁷², which in turn can activate CD8⁺ T cells without costimulation, leading to deletional tolerance. Even in conditions of normal tissue homeostasis, lymphatic endothelial cells scavenge and cross-present peripheral tissue antigens, leading to CD8⁺ T cell deletion in maintenance of peripheral tolerance⁷³.

Mimicking peripheral tolerance mechanisms

Another promising strategy is based on the process by which antigen is collected from apoptotic debris during the normal maintenance of peripheral tolerance. Antigen from apoptotic debris is collected by antigen-presenting cells in the absence of inflammatory signaling such that they (cross-) present it (signal 1) in the absence of co-stimulation (signal 2), providing tolerogenic antigen presentation. Harnessing this mechanism, researchers have chemically conjugated exogenous antigens to splenocytes through a process that induces splenocyte apoptosis. The cell-antigen adducts can induce tolerance, including regulatory T cell responses, to the conjugated antigen (Fig. 3a)^{74–77}. This approach can also be used for endogenous antigens (Fig. 3b)⁷⁵. When donor splenocytes were chemically treated as above to induce chemical modification and apoptosis, but no exogenous antigen was added, infusion of the treated cells into allogeneic mice allowed long-term function of the islet allograft in models of chemically induced diabetes⁷⁴, especially when the islets were transplanted in a biomaterial scaffold⁷⁸. The concept of conjugating antigen to apoptotic debris recently received initial clinical validation in a study in which peptides identified as antigenic in multiple sclerosis patients were chemically conjugated to autologous peripheral blood mononuclear cells, which were reinfused in patients in stable remission; the treatment diminished the numbers of autoreactive T cells, consistent with induction of tolerance⁷⁹.

Acellular delivery of antigens for tolerization is also being explored. In one study, antigens associated with 500-nanometer negatively charged polymer microparticles that had been administered intravenously were taken up by splenic marginal-zone macrophages that expressed the scavenger receptor MARCO, inducing antigen-specific tolerance in a mouse

model of experimental autoimmune encephalomyelitis. In principle, this approach could be applicable to a wide range of protein antigens, including those involved in allogeneic cell transplantation⁸⁰ (Fig. 3c). Antigen has also been targeted in various molecular forms to dendritic cells to induce its tolerogenic presentation owing to the lack of inflammatory signals to induce costimulation. For example, antigen has been fused to an antibody targeting the scavenger receptor DEC205, which is present on dendritic cells that are particularly effective in cross-presentation (CD8 α^+ dendritic cells)⁸¹. This technology has been demonstrated with partial success in mouse models of experimental autoimmune encephalomyelitis⁸¹ and autoimmune diabetes⁸², suggesting that it is generalizable. The dendritic cell asialoglycoprotein receptor, another dendritic cell scavenger receptor, has also been used to target antigen to dendritic cells to induce tolerance in nonhuman primates⁸³. Biomaterials approaches to induce a pseudocellular signal 1 (i.e., on a cellular mimic) have also been attempted. Conjugating peptide-MHC complexes to nanoparticles directly stimulated cognate T cells and generated regulatory T cell responses capable of blunting preexisting CD8 $^+$ T cell responses in a mouse model of autoimmune diabetes⁸⁴.

Finally, a study demonstrated a means of combining the biological potency of antigen delivery on apoptotic cell debris with the logistical simplicity of tolerization using protein rather than cellular reagents: antigens are engineered to bind cell populations *in vivo*, such as erythrocytes, that circulate and are eventually cleared through apoptosis as the cells age⁸⁵ (work by J.A.H. and colleagues; Fig. 3d). The reasoning is that as the erythrocytes age and are cleared by normal tolerogenic mechanisms, tolerance will simultaneously be induced to the exogenous antigen. Exogenous xenoantigens expressed on the surface of transgenic erythrocytes escaped immunodetection⁸⁶. In a mouse model of autoimmune diabetes, treatment with a beta cell antigen fused to an antibody that binds glycophorin A (specifically present on erythrocytes) led to deletion of beta cell-reactive T cells and prevention of diabetes⁸⁵. Tolerization using this approach was also induced to cells expressing xenoantigens, which do not benefit from any central tolerance mechanisms in the model organism⁸⁵, suggesting relevance for cellular transplants that express exogenous transgenes (e.g., to replace a defective or missing gene in congenital disease). This method should in principle be applicable to many protein antigens, including transplantation antigens.

Future directions

Several preclinical studies indicate that central tolerance to transplanted cells can be induced by intrathymic injection of alloantigens^{87–90}. Although there is no clinical precedence for intrathymic injections, minimally invasive image-guided intrathymic injections can be safely performed in mice⁹¹ (work by M.v.d.B., J.L.Z. and colleagues), suggesting that image-guided intrathymic injections will be clinically feasible, at least in children and young adults, who have large thymuses that have not yet undergone age-associated involution. Pending clinical validation, there is no doubt that the antigen specificity of such an intervention combined with the complete absence of immunosuppression would make this an attractive strategy for regenerative medicine.

Another possible approach to inducing central tolerance is to promote ectopic generation of tolerized T cells in an implanted artificial microenvironment that has been manipulated to

induce tolerance to both donor and host. A recent animal study suggests that this can be accomplished by injecting allogeneic thymic tissue fragments into lymph nodes, transforming them into organs that support development of T cells⁶⁸. Alternatively, it may be feasible to implant a tissue-engineered thymic organoid composed of the epithelial and hematopoietic cell types required for development of functional and tolerized T cells^{92–94}. This technology is still in its infancy, but it holds promise as a method of overcoming immunological barriers in future regenerative therapies.

Induced pluripotent stem cell (iPSC) technology makes it possible to produce autologous, specialized cells or tissues directly from patient cells^{95–97}. Although this would remove allogeneic barriers, it may not be a perfect solution to all of the immunological barriers confronting regenerative therapy (Fig. 1). A few studies have suggested that iPSC-derived cells can be immunogenic even when donor and recipient are genetically identical^{98–100}. This may be due to retention of developmental antigens, acquisition of xenogenic epitopes, or expression of aberrant antigens over the course of long-term cultures used to generate iPSCs and differentiated cells^{101,102}. Nonphysiologic *in vitro* differentiation can also alter the expression of molecules involved in immune recognition in a way that would trigger immune system reactions against the cells after transplantation, such as NK cell-mediated killing of cells expressing low levels of self MHC class I molecules¹⁰¹. Possible solutions to these problems include the development of mild immunosuppressive regimens (e.g., monoclonal antibodies targeting NK cells and/or T cell subsets) sufficient to induce tolerance to autologous iPSC-derived cells. However, any level of immunosuppression would likely increase the risk that rare pluripotent cells in an iPSC-derived population will form teratomas. Another solution might be direct conversion of somatic cells such as fibroblasts into other lineages, bypassing the pluripotent stage and the expression of embryonic antigens¹⁰³. If iPSC-derived or directly reprogrammed cells are combined with genome editing or expression of therapeutic transgenes, the exogenous biomolecules may be immunogenic.

The field of regenerative medicine is expanding rapidly, driven by an ever-increasing diversity and complexity of cell sources, biomaterials and designs. After years of research and development in academia and industry, promising strategies are moving into clinical trials in macular degeneration, spinal cord injury, diabetes, cardiovascular disease and other areas. Overcoming immunological barriers is therefore among the most pressing issues in the field. Recent advances and potential avenues for future research provide reason for optimism. If the past is any indication of what is to come, progress on induction of transplantation tolerance is likely to keep pace with the immunological challenges presented by emerging regenerative therapies.

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Figure 1.

The likelihood of encountering immunological barriers depends on the type of cells and tissues used in regenerative therapies. Allogeneic cells of any type are at highest risk for rejection and require tolerance-inducing interventions. Observations from solid-organ transplantation indicate that even in an allogeneic setting the tissue source may also matter, as it is easier to induce tolerance to some allogeneic tissues (e.g., liver) than to others (e.g., skin). Autologous cells can be immunogenic if they have been genetically engineered to express an exogenous protein. Allogeneic cells derived from embryonic stem cells or autologous cells derived from induced pluripotent stem cells may acquire immunogenic features during prolonged culture. Decellularized allografts used as tissue-engineering scaffolds are not expected to encounter substantial immunological barriers because the immunogenicity of extracellular matrix proteins is generally low.

Figure 2.

Molecular basis of T cell allorecognition and activation. **(a)** Allorecognition occurs in one of three ways when T cells interact with allogeneic target cells or peptides. In direct allorecognition, T cells are activated directly by allogeneic cells (antigen-presenting cells (APCs) or any cell expressing allogeneic MHC). T cell receptor activation is triggered by recognition of the complex comprising determinants of the allogeneic MHC class I or MHC class II molecule loaded with peptide (the peptide origin is irrelevant). In semidirect allorecognition, allogeneic MHC class I or MHC class II molecules are acquired and MHC-peptide complexes displayed by autologous APCs. The peptide origin is again irrelevant. For indirect allorecognition to occur, allogeneic transplantation-relevant proteins must first be processed by autologous APCs. Subsequently, peptides derived from these allogeneic antigens are cross-presented by autologous MHCII on autologous APCs. T cell receptor activation is triggered by recognition of the complex comprising determinants of the autologous MHCII molecule loaded with an allogeneic peptide. **(b)** T cell activation by antigen-presenting cells requires two types of signals. Signal 1 is received by the TCR when it interacts with a cognate peptide-MHC complex. Signal 2 is received by costimulatory molecules (e.g., CD28) when they interact with cognate ligands (e.g., CD80 or CD86). Molecules that inhibit T cell activation and/or induce T cell anergy include cytotoxic T cell lymphocyte antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1). Programmed death ligand 1 (PD-L1) is the main ligand for the PD-1 receptor.

Figure 3.

Antigen-specific tolerance induction by apoptotic, polymeric or protein carriers. **(a)** Autologous splenocytes are treated with ethylene carbodiimide in the presence of exogenous antigen, which results in the production of apoptotic cellular debris that displays the exogenous antigen but not activation signals that induce costimulation by antigen-presenting cells. Intravenous infusion of these apoptotic antigen ‘carriers’ results in T cell tolerance to the exogenous antigens. **(b)** Donor splenocytes can be treated as in **a** in the absence of exogenous antigen. In this scenario, T cells are tolerized to endogenous donor MHC molecules and other endogenous alloantigens. **(c)** Polymeric microparticles can be treated as in **a** in the presence of exogenous antigens to conjugate the antigen to the microparticle; alternatively, exogenous antigens can be encapsulated within the microparticles. After intravenous infusion, the microparticles are taken up by macrophages that express the scavenger receptor MACRO, and this induces tolerance to the exogenous antigens. **(d)** Exogenous antigens can be fused to antibody fragments (e.g., single-chain variable fragment, scFv) or other peptides that are specific for proteins expressed on the surface of red blood cells (RBCs). After intravenous infusion, the fusion proteins bind RBCs. Subsequent normal clearance of aged RBCs generates apoptotic RBC debris carrying the exogenous antigen but lacking activation signals. As in **a** and **b**, these apoptotic antigen ‘carriers’ induce T cell tolerance.

Table 1

Strategies for inducing immunological tolerance in HSC or solid-organ transplantation

Approach	Mechanism	Target	Refs.
Pharmacotherapy	Inhibition of signaling pathways	mTOR ^a	13,14
		Tyrosine kinases ^a	104
		NF-κB ^a	15–17
		HDAC	18,105
		JAK ^a	106
		PKC ^a	107
	Inhibition of neovascularization	VEGF	108
	Inhibition of the mevalonate pathway	HMG-CoA reductase ^a	109
Immunotherapy	Lymphocyte depletion	CD2 ^a	110,111
		CD20 ^a	112
		CD52 ^a	113
	Cytokine inhibition	TNF-α ^a	51
		IL-2 receptor ^a	52,114
		IL-6 ^a	115
		IL-7 receptor	116,117
	Blockade of costimulatory molecules	CD28 ^a	54,57,118,119
		CD40 ^a	120
		ICOS	121
	Tolerogenic cell therapies	Regulatory T cells ^a	27,28,30,122
		Dendritic cells ^a	22,123
		MDSCs	123
		NK cells ^a	25
		NKT cells	26
		MSCs ^a	43,44

HDAC, histone deacetylase; JAK, Janus kinase; PKC, protein kinase C; ICOS, inducible T cell costimulator; MDSCs, myeloid-derived suppressor cells; MSCs, mesenchymal stromal cells.

^aClinically validated approaches.