Dear author,

Please note that changes made in the online proofing system will be added to the article before publication but are not reflected in this PDF.

We also ask that this file not be used for submitting corrections.

Trends in Molecular Medicine

CellPress

Review

Engineering Strategies for Allogeneic Solid Tissue Acceptance

Ana Rita Sousa,[1] João F. Mano,[1,*] and Mariana B. Oliveira[1,*]

Advances in allogeneic transplantation of solid organs and tissues depend on our understanding of mechanisms that mediate the prevention of graft rejection. For the past decades, clinical practice has established guidelines to prevent allograft rejection, which mostly rely on the intake of nontargeted immunosuppressants as the gold standard. However, such lifelong regimens have been reported to trigger severe morbidities and commonly fail in preventing late allograft loss. In this review, the biology of allogeneic rejection and self-tolerance is analyzed, as well as the mechanisms of cellular-based therapeutics driving suppression and/or tolerance. Bioinspired engineering strategies that take advantage of cells, biomaterials, or combinations thereof to prevent allograft rejection are addressed, as well as biological mechanisms that drive their efficacy.

Highlights

Systemic immunosuppressants have allowed the transplantation of life-saving organs. However, they cause deleterious effects and long-term graft failure. Strategies promoting allogeneic graft acceptance and the maintenance of immunological competence have been proposed.

Cell-based tolerance-inducing strategies that preserve immune competence have already extended human allograft acceptance, although toxic side effects or difficult reproduction of observed effects in humans have counteracted their clinical translation.

Localized tolerance/immunosuppression mediated through bioengineered setups comprising immunomodulatory biomaterials and/or tissue engineering-inspired tools already achieved allograft acceptance in murine models and are game changing in the field. Such advances have contributed to unveiling the complex interplay of immune cells and allogeneic transplants.

Strategies to Achieve Allograft Acceptance: Advances from Standard-of-Care Approaches

Allogeneic organ transplantation remains a common clinical choice to recover organ function in several pathologies. In 2018, approximately 147 000 solid organ transplants were performed worldwide[i]. Standard-of-care therapies to prevent allograft rejection rely on the lifelong systemic administration of nonspecific immunosuppressants, known for globally immunocompromising recipients. Due to associated risks, immunosuppression (IS) therapies are carefully tuned to prevent rejection while avoiding severe side effects. Such IS regimens [1] are also allied to donor selection comprising preoperative **ABO-matching** (see Glossary) and **HLA-matching** with allotransplant recipients. Although this combined approach has efficiently hindered hyperacute and acute rejection [1,2] (Box 1), it broadly fails in preventing chronic rejection, the last obstacle to long-term graft acceptance [3]. In October 2020, ~109 000 individuals were on the transplant waiting list in the USA, from which ~17 die every day while waiting for an organ donor[ii, iii]. The lack of treatment for chronic rejection directly relates with the steady cumulative half-life of kidney grafts, between 9 and 12 years, for the past 25 years [1]. Lastly, systemic IS leads to severe long-term morbidities, related to the immunocompromised condition itself (e.g., oncological diseases), and to potentially lethal drug-associated toxicities [3]. Reported limitations of systemic IS justify the high demand for alternative approaches, ideally not relying on recurrent medication, since therapeutic nonadherence is the main risk factor for short-term allograft failure [1].

In-depth understanding of the mechanisms orchestrating graft acceptance may leverage urgent advances in allogeneic transplantation. Promising efforts have focused on the induction of immunological tolerance that lead to host unresponsiveness to donor antigens, while preserving immunocompetence [2]. Examples include cell-based therapies relying on the administration of hematopoietic donor cells, which already achieved **operational tolerance** in humans (Figure 1) [2]. The adoptive transfer of tolerogenic cells or stem cells has also induced tolerance and/or localized suppression in preclinical and clinical studies [4–6] (Figure 2). Finally, bioengineered approaches comprising biomaterials, cells, or combinations thereof [7–9]

[1]Department of Chemistry, CICECO – Aveiro Institute of Materials, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

*Correspondence: jmano@ua.pt (J.F. Mano) and mboliveira@ua.pt (M.B. Oliveira).

b0.2 Box 1. Mechanisms of Allogeneic Recognition and Immune Tolerance

b1.3 **Allograft Rejection**

b1.4 Allograft rejection may be hyperacute, acute, or chronic. Hyperacute rejection mainly occurs in the first 48 hours post-
b1.5 transplantation and is mediated by pre-existing antibodies against donor antigens (mainly endothelium ABO antigens)
b1.6 [96]. Acute rejection mainly occurs 1-week post-transplantation. Rejection comprises direct allorecognition, where host
b1.7 alloreactive T cells directly recognize donor APCs in lymph nodes. Indirect allorecognition may also occur and involves
b1.8 the capture and alloantigen presentation to host alloreactive T cells by graft-migrating host APCs [96]. IS drugs are
b1.9 targeted at reducing alloreactive T cell activation. Therefore, the combinatorial use of IS and preoperative ABO and
b1.10 HLA-matching may be required to avoid acute rejection [97]. Finally, chronic rejection persists for months/years and is con-
b1.11 sidered a nontreatable condition [1,98]. Cellular-mediated chronic rejection involves indirect recognition of major and minor
b1.12 mismatched histocompatibility antigens of the donor [98]. Additionally, antibody-mediated chronic rejection drives organ/
b1.13 tissue fibrosis, which is ascribed as the major cause of late graft loss [1].

b1.14 **Mechanisms of Central and Peripheral Tolerance**

b1.15 Self-tolerance describes the ability of the human body to recognize self-produced antigens as harmless. Reported mech-
b1.16 anisms of self-tolerance indicate that HSC precursors arise from BM and migrate to thymus to follow T cell maturation.
b1.17 Such central tolerance mainly promotes: (i) self-MHC restriction, through positive selection, where thymocytes bind with
b1.18 low affinity to self-MHC molecules; (ii) self-tolerance, through negative selection, where self-reactive thymocytes are
b1.19 deleted. Additionally, peripheral tolerance mechanisms influence autoreactive T cell fate, mainly by: (i) clonal deletion;
b1.20 (ii) regulatory T cell (Treg)-mediated suppression; or (iii) clonal anergy [97]. Clonal deletion combines both intrinsic and
b1.21 extrinsic [Fas/Fas ligand (Fas/FasL)-mediated] apoptosis. The latter implicates the activation of T cell receptor (TCR),
b1.22 upregulating cell-surface death receptors (Fas molecule) on autoreactive T cells, when a proinflammatory response is
b1.23 no longer desired, driving cell apoptosis under autocrine/paracrine Fas/FasL binding [96]. Furthermore, Tregs act directly,
b1.24 either by inducing T cell apoptosis, or inhibiting the maturation of MHC-II expressing APCs, impairing their function. Indirect
b1.25 mechanisms involve inactivating T cells either by secreting anti-inflammatory cytokines or by depleting the microenviron-
b1.26 ment from proliferation-inducing cytokines [99]. Finally, proper T cell activation requires both interaction of T cell TCR/
b1.27 CD3 molecules (Signal 1) and co-stimulatory molecules such as CD28 (Signal 2) with the antagonists (CD80/CD86)
b1.28 present in APCs. Therefore, clonal anergy involves the antigen presentation in privation of such TCR co-stimulatory
b1.29 molecules (Signal 2) [96].

Glossary

ABO-matching: ABO blood group antigens exist at the surface of red blood cells and epithelial/endothelial cells. Preoperative ABO screening tests minimize likelihood of rejection.

Graft-versus-host disease (GvHD): donor mature and memory alloreactive $CD4^+/CD8^+$ T cells are attracted by inflammatory signals released from the recipients' epithelium, triggering recipient rejection.

Heterologous immunity: pre-existing naïve and memory alloreactive T cells might crossreact against allogeneic mismatched HLA-peptide complex, triggering allograft rejection.

'Hit and run' mechanism: MSCs transiently adopt an anti-inflammatory phenotype in response to acute proinflammatory triggers and vice versa. The ephemerality of this hit and run effect might be related to the fast clearance of MSC suspensions from the body, either from lack of cell adherence or allogeneic immune rejection.

HLA-matching: the HLA (or MHC) system is codified by a set of multiple genes that are inherited *en-bloc* from a single chromosome, forming a haplotype. Every individual inherits two different HLA haplotypes (one from each parent). Thus, any subject not having an identical twin has a 25% chance of finding a complete HLA-matched sibling. Preoperative HLA genotyping provides donor/recipient compatibility grade.

Immune evasive: *in vitro* cultured mesenchymal stem cells (MSCs) commonly exhibit low levels of HLA-I and absence of HLA-II or positive co-stimulatory molecules, establishing the paradigm of the 'immune privileged' MSCs. Recent evidence identifies 'MSC immune evasiveness', where MSCs primed with proinflammatory cues at an inflammation site start expressing HLA-I and HLA-II, raising its immunogenicity. The concomitant immunosuppressive phenotype promoted by the 'hit and run' effect might mask allogeneic MSCs, which help transiently evade the immune system. Q11

Induced Tregs (iTregs): conventional $CD4^+$ T cells might diverge *in vitro* into iTregs, being generally unstable due to loss of FoxP3 expression, which can lead to further *in vivo* differentiation into effector T cells after injection. Antigen-specific iTregs diverge from antigen-specific conventional $CD4^+$ T cells.

(Figure 3) may provide clues to unveil the mechanisms behind innovative acceptance-inducing strategies (Table 1, Key Table). The following sections critically review the impact of different strategies on the recipients' response, while addressing the reported biological mechanisms influencing their efficiency.

Cell-Based Therapies

Hematopoietic Chimerism

In general, hematopoietic chimerism is achieved through donor bone marrow transplantation (BMT) acting as a support for a secondary life-saving transplant. Donor hematopoietic cells/stem cells perform thymic and peripheric presentation of allogeneic antigens, contributing to both central and peripheral tolerance, usually culminating in the gradual withdrawal of IS [2,10]. Depending on **preparative conditioning** [11] and recipient response, two different conformations, full or mixed, may be achieved. Full chimeras generally imply toxic regimens guiding the complete replacement of host with donor bone marrow (BM). However, mixed hematopoietic chimerism scenarios support the coexistence of both host and donor hematopoietic populations and its occurrence may be transient or durable [10].

Achievement of sustained mixed hematopoietic chimerism was first reported in the 1950s to trigger tolerance to **major histocompatibility complex (MHC)**-mismatched skin allografts in free-martin cattle, independently of IS. Sustained mixed chimeras were considered for several years as a necessary condition for allograft tolerance [12]. However, the induction of sustained chimeras in HLA-disparate scenarios in humans has proven difficult [13]. Thus, most subsequential studies have focused on full chimerism, demonstrated to induce tolerance in MHC-mismatched scenarios [13,14]. For several decades, the success of chimerism-based procedures was

thought to depend on the permanent existence of donor chimeras. This concept, however, was challenged when allograft tolerance was observed in rats with short-lived donor chimeras [15]. Later on, transient mixed chimerism was proven to guide MHC-mismatched allograft tolerance in non-human primates [16]. Although controversial, the induction of mixed chimerism seems to significantly reduce the risk for **graft-versus-host disease (GvHD)** associated with full chimerism [13].

The efficacy of chimerism in preventing allograft rejection seems to be tissue-type dependent, benefiting from naturally tolerogenic liver and kidney transplant environments [17]. While mixed chimerism already induced tolerance to 60–70% of human kidney allografts, it has not been successfully transposed to pancreatic islet, lung, or heart allografts [18].

Full Chimerism

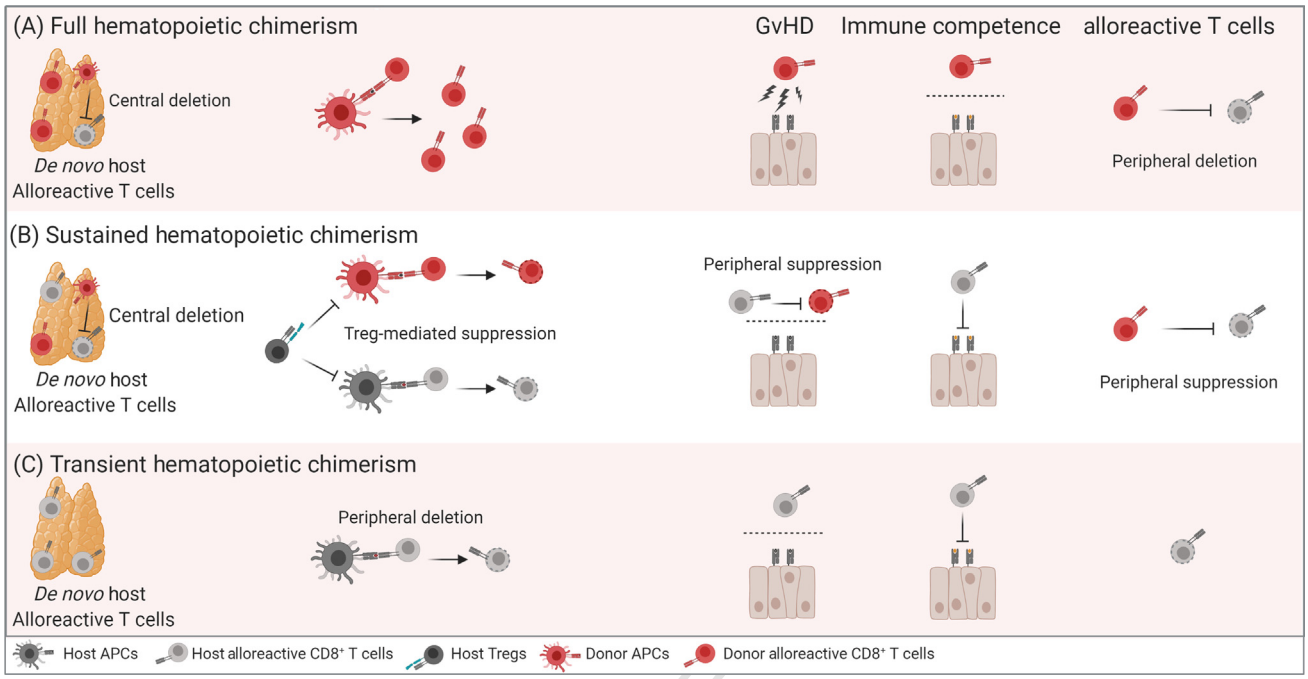
In full chimeras, donor hematopoietic precursors proliferate into the host BM and mature in the thymus. Mature dendritic cells (DCs) and antigen-presenting cells (APCs) from the donor further contribute to thymic negative selection (Box 1) [2,19], leading to the central deletion of new alloreactive T cells and self-reactive clones. Thus, newly generated donor/host-derived lymphocytes are tolerant to both the self and allogeneic antigens [19]. In full chimerism, tolerance comprises central deletion with minor peripheral deletion and regulatory T cell (Treg)-mediated suppression, since preparative conditioning deletes most host alloreactive T cells [2,20]. However, the donor BM also contains memory and mature hematopoietic cells (e.g., donor T cells) that may not be thymically selected, mounting GvH responses. Therefore, peripheral deletion of residual host alloreactive T cells that escaped conditioning has been speculated to occur [10] and undesirable GvHD may happen [2,21]. Another significant drawback of HLA-mismatched full donor chimeras is their inability to react against danger-associated antigens whose presentation is restricted to host-HLA molecules, compromising immune competence [22].

The high toxicity of pretransplant IS in full chimerism protocols has raised clinical concerns. To reduce toxicity, a nonmyeloablative preparative conditioning was developed to precede HLA-mismatched living-donor kidney transplantation (KT). Human recipients were administered 1 day later with cryopreserved hematopoietic stem cells (HSCs) enriched in FCRx, a bioengineered donor HSC product containing facilitating tolerogenic cell populations ($CD8^+/TCR^-$). The therapy hindered GvHD and enhanced the engraftment of HSCs [23] (NCT00497926[iv]). Since most recipients achieved full chimerism after 1 year, they were gradually weaned off IS [14]. However, 8 years after transplantation, two of 31 recipients developed GvHD, causing one death [24]. Although less toxic preparative regimens could minimize the recipients' burden, the persistent risk of GvHD in full chimeras continues to motivate the development of safer approaches.

Sustained Mixed Chimerism

In a human trial (NCT03292445[v]), sustained mixed chimerism was established in recipients of living donor HLA-matched kidney, along with a supportive enriched $CD34^+$ hematopoietic cell transplantation. A 10-day post-transplant conditioning included total lymphoid irradiation and anti-thymocyte globulin, with maintenance prednisone and cyclosporine. Most recipients achieved sustained mixed chimerism, ranging from 6 to 12 months, leading to IS withdrawal up to 5 years and no allograft rejection events [25]. Despite reducing the odds for GvHD and preserving immune competence, sustained mixed chimeras seem only to prevent rejection in HLA-matched allograft recipients [10,25]. The biological mechanisms behind this phenomenon are presented in Box 2. Efforts to induce transient chimeras have fueled expectations for safer strategies in HLA-mismatched scenarios.

Major histocompatibility complex (MHC): complex of polymorphic glycoproteins expressed at the surface of most blood cells/body tissues. MHC-compatible transplantation might avoid hyperacute/acute rejection.
Natural Tregs (nTregs): thymically derived regulatory T cells are polyclonal $CD4^+CD25^+FoxP3^+$Tregs with affinity for self-antigens, suppressing the immune response in a non-antigen-specific fashion. Their stable immunosuppressive function comprises multiple gene hypomethylation, namely on FoxP3 gene (one hallmark of Treg function). It is also possible to produce antigen-specific nTregs under antigen presentation in stringent conditions, however few antigen-specific nTregs are found *in vivo*.
Operational tolerance: a rare tolerance state where the recipient spontaneously maintains allograft function for >1 year in the absence of immunosuppression, mainly observed after (hypothesized tolerogenic) liver/kidney transplantation.
Preparative conditioning: conditioning deletes recipient (alloreactive) T cells, that would otherwise reject the new BM, before chimerism is induced.



Q1 Figure 1. Representation of Tolerance-Inducing Hematopoietic Chimerism Setups. (A) Full hematopoietic chimerism in HLA-mismatched scenario. Host hematopoietic cells are severely deleted due to preparative conditioning, making donor hematopoietic cells the predominant chimeric population. Remaining host hematopoietic stem cells may migrate to the thymus, where they will be negatively selected by donor hematopoietic cells. As a result, residual host alloreactive T cells are deleted, as well as donor alloreactive T cells due to thymic selection. As host antigens are being presented to the donor alloreactive T cells that escaped thymic selection, those may expand triggering graft-versus-host disease (GvHD), compromising immune competence, while impairing allograft rejection [10]. (B) Sustained mixed chimerism in HLA-matched scenario. After preparative conditioning, remaining host alloreactive T cells are centrally deleted. Regulatory T cells (Tregs) mediate suppression of alloreactive T cell clones early after transplantation. The balance between host-versus-graft (HvG) and GvH responses, seen in lymphocyte-enriched allografts (e.g., liver), might enable GvHD to be impaired by host alloreactive T cells and allograft rejection to be impaired by donor alloreactive T cells. After disappearance of donor chimeras, host alloreactive T cells will be peripherally deleted long-term. Host T cell clones also maintain immune competence to foreign antigens. (C) Transient mixed chimerism in HLA-mismatched scenario. Suppression is initially supported by Tregs, but the peripheral deletion of alloreactive clones is the main mechanism acting in the long-term, being mediated by peripheric persistent tolerogenic alloantigen presentation, avoiding GvHD (since donor chimerism is over) and allograft rejection, while maintaining immune competence. Abbreviation: APC, antigen-presenting cell.

Transient Mixed Chimerism

Promising results of transient mixed chimerism were obtained in a clinical trial comprising HLA-mismatched combined kidney and bone marrow transplantation (CKBMT), followed by low toxicity nonmyeloablative regimens (NCT00801632[vi]). All ten recipients developed mixed transient chimerism, which lasted from 2 to 3 weeks. Seven of those patients were weaned off IS, with four achieving up to 11 years of IS independence, while two developed chronic rejection [26]. Peripheral deletion was the main factor responsible for long-term tolerance (Box 2). Since chimerism is transient and short-lived, the donor alloreactive mature/memory cells are rapidly removed, reducing the risk of GvHD, showcasing an advantage over other chimerism typologies, while improving immune competence [2]. Interestingly, it has also been speculated that defined peripheric host APCs perform thymic presentation of donor antigens, mediating central deletion [27].

While transient mixed chimeras may impose the lowest risk for GvHD, their efficacy in inducing tolerance remains variable among recipients. Thus, developing biomarkers to stratify patients with predictive response to each of the chimerism-induction strategies may be indispensable for therapeutic decision. Designing tools to monitor recipient response post-transplantation

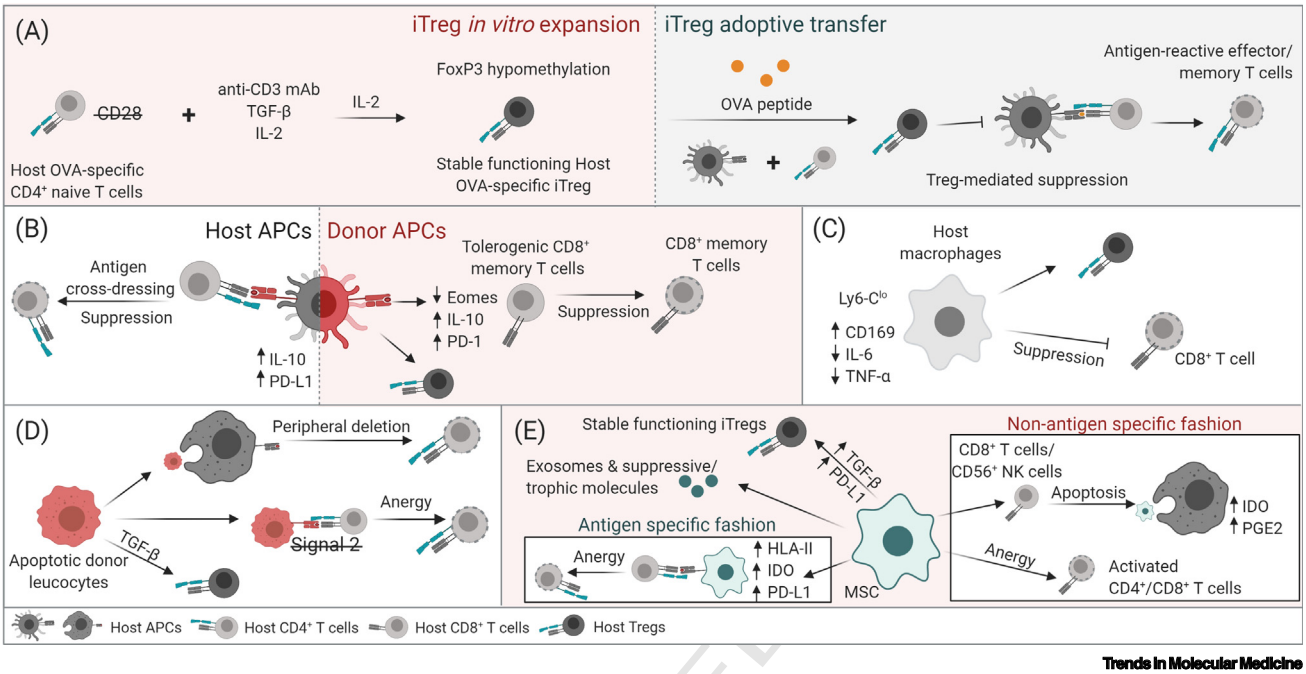


Figure 2. Allograft Acceptance Mediated by Tolerogenic Cells. (A) Induced regulatory T cells (iTregs). Incubation of ovalbumin-reactive (OVA) naïve $CD4^+$ T cells with anti-CD3 monoclonal antibody, TGF-β, and IL-2, without CD28 signaling, produced stable functioning host antigen-specific iTregs. Following adoptive transfer, iTregs suppressed antigen-reactive effector/memory $CD4^+$ T cells, previously activated by host antigen-presenting cells (APCs). (B) Tolerogenic dendritic cells (DCs). Adoptive transfer of donor tolerogenic DCs triggered the expansion of host Tregs and suppressed alloreactive $CD8^+$ memory T cells. Host APCs performed tolerogenic alloantigen cross-presentation, upregulating IL-10/PD-L1, suppressing alloreactive $CD4^+$ T cells. (C) Regulatory macrophages. *In vivo* induced regulatory macrophages downregulated Ly6C and IL-6/TNF-α and upregulated CD169, promoting graft-infiltrating Treg expansion and suppressing $CD8^+$ T cells *in vitro*. (D) Apoptotic donor leucocytes (ADLs). ADLs were mainly phagocytized by APCs, which performed tolerogenic alloantigen presentation, triggering peripheral deletion of $CD4^+$ T cells. ADLs triggered initial TGF-β-mediated expansion of Tregs and directly rendered $CD4^+$ T cells anergic through antigen presentation without Signal 2. (E) Mesenchymal stem cells (MSCs). MSCs mediated immunosuppression through indirect (e.g., secretome) and/or direct cell contact. MSCs directly induced stable functioning iTregs. Following non-antigen-specific fashion, MSCs rendered activated $CD4^+$/$CD8^+$ T cells anergic. Furthermore, $CD8^+$ T/$CD56^+$ NK cells induced perforin/Fas-mediated apoptosis of infused MSCs. Host APCs phagocytized apoptotic MSCs, propagating immunosuppression. Autologous tolerogenic antigen-pulsed MSCs also upregulated HLA-II, indolamine-2,3-dioxygenase (IDO), and programmed death-1 ligand (PD-L1), rendering antigen-specific $CD4^+$ T cells anergic. Adapted from references [4,6,32,33,39,41,43,44,55,58,62]. Color code: red, donor; gray, host; teal, host Tregs.

may be crucial to establish a minimum threshold of response correlating with engraftment success and capable of helping to predict the need for rescue therapy [28].

Tolerogenic Cells

Tolerogenic Leucocytes

The adoptive transfer of induced/*in vitro* expanded host Tregs [29–31], or other entities such as tolerogenic DCs [32] and macrophages [33], constitute a peripheral tolerance-inducing strategy.

Studies in human KT recipients showed that intravenously delivered tolerogenic cells (Tregs, macrophages, and DCs) minimized IS-related side effects [29], while infused host polyclonal **natural Tregs (nTregs)** had an adjuvant role (NCT02145325[vii]) [30]. Only a single study succeeded at achieving full IS withdrawal (≥2 years) in HLA-mismatched liver transplantation (LT) through the injection of inducible donor antigen-specific Tregs (UMIN-000015789) [31]. Although controversial [34], results from mice models report antigen-specific Tregs to prolong allograft survival when compared with polyclonal Tregs [35]. While most studies focused on facilitating *in vivo* [36] or *in vitro* [37] expansion of Treg cells, their production with stable epigenetic profile *in vivo* was crucial for potency [4]. Consistently, abolishing CD28 co-stimulation, promoting

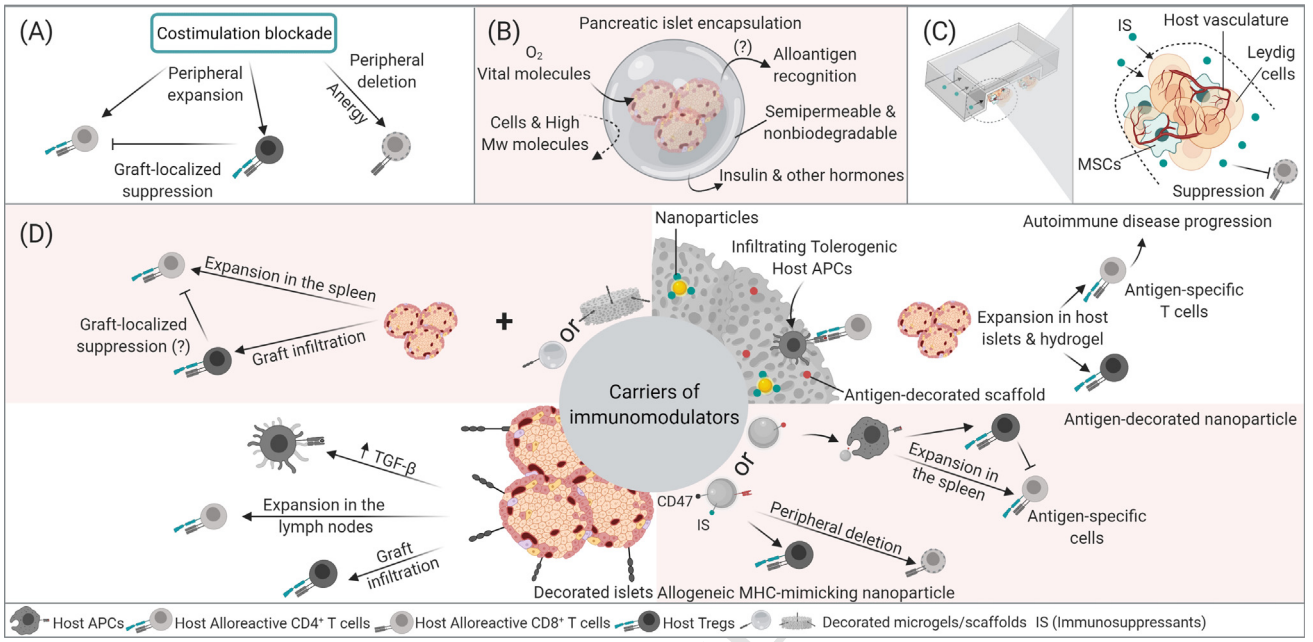


Figure 3. Strategies Encompassing Acellular and Bioengineered Cell Hybrids That May Promote Allograft Acceptance. (A) Co-stimulation blockade. Alloreactive cells were deleted or rendered anergic. Furthermore, residual alloreactive T cell expansion was locally kept in check by regulatory T cells (Tregs). (B) Immunoisolation. Islet encapsulation enabled the release of tissue-specific molecules and provided oxygen/vital molecules while hampering direct, but not indirect, immune recognition. (C) Carriers of immunomodulatory cells. A U-shaped macrodevice eluted CTLA4Ig into a cell reservoir accommodating mesenchymal stem cells (MSCs) and allogeneic Leydig cells. CTLA4Ig suppressed alloreactive T cells arriving from host vasculature. (D) Carriers of immunomodulators. Immunosuppressants were locally/transiently presented to cotransplanted islets (left panel). Decorated microparticles or scaffolds triggered graft-infiltrating host Treg expansion, speculated to control splenic alloreactive T cell proliferation. Alternatively, decorated islets implicated persistent alloantigen presentation and graft-infiltrating Treg expansion, which established localized tolerance in the long-term, despite alloreactive cell expansion in lymph nodes. Delivery of antigens/major histocompatibility complex (MHC)-mimics in scaffolds/nanoparticles (right panel). In autoimmune diabetes, tolerance was achieved using antigen-decorated nanoparticles phagocytized by antigen-presenting cells (APCs), triggering pancreas-infiltrating Treg expansion, which restricted effector T cell proliferation in the spleen, reverting diabetes. In another example, antigen-decorated hydrogels triggered the expansion of hydrogel-infiltrating tolerogenic APCs and promoted Treg proliferation. Since effector T cell expansion was not impaired in islets/hydrogel, diabetes progressed. Finally, a model of allotransplantation received polyethyleneimine (PEI)-poly lactic-co-glycolic acid (PLGA) nanoparticles functionalized with an allogeneic MHC-mimic, CD47, and immunosuppressants (IS), that triggered peripheral deletion of MHC-alloreactive T cells and promoted Treg expansion. Adapted from references [8,67,71,72,74,76–78,82].

the hypomethylation of FoxP3, produced stable functioning **induced Tregs (iTregs)** that impaired expansion of effector/memory antigen-reactive T cells. However, unstable iTregs were less suppressive in mice [4]. Interestingly, polyclonal Tregs were also found to have an adjuvant effect when combined with chimerism-induction therapies in non-human primates [18]. Infusion/*in vivo* induction of regulatory APCs is proposed as an alternative to Treg-mediated suppression.

Generally, the immunosuppressive phenotype of tolerogenic DCs involves the *in vitro* expression of low MHC-II and CD80/CD86 and high IL-10 and TGF-β [38], leading to Treg proliferation in mice kidney allografts [39]. As a growing field, the application of tolerogenic DCs remains mainly confined to nonclinical research. Non-human primates submitted to MHC-mismatched KT (under maintenance rapamycin) were infused with abatacept, a fusion protein of cytotoxic T lymphocyte antigen-4 (CTLA-4Ig), along with donor tolerogenic DCs, resulting in suppression of alloreactive $CD8^+$ memory T cells [32]. The expansion of tolerogenic $CD8^+$ memory T cells, along with down-regulation of Eomes gene and upregulation of programmed death 1 (PD-1) and CTLA-4, prolonged allograft survival [32]. Importantly, to minimize the risk for donor HLA sensitization, the infusion of autologous tolerogenic DCs is recommended [40]. Mice infused with autologous

t1.1 **Key Table**

t1.2 Table 1. Strategies and Immune Mechanisms Guiding Allograft Acceptance

Strategies	Core goal	Risk of GvHD	Preserve immune competence	Main mechanisms acting long-term	Acceptance in HLA-mismatched clinical trials	Refs
Full hematopoietic chimerism	Tolerance	+++	–	Central deletion	+	[14]
Mixed hematopoietic chimerism	Tolerance	+	–	Peripheral deletion	+	[25,27]
Tolerogenic leucocytes	Tolerance	–	+ (Hypothesized)	Peripheral suppression	+ (One study)	[31]
Apoptotic donor leucocytes	Tolerance	–	+ (Hypothesized)	Peripheral deletion	Not studied	[43]
Mesenchymal stem cells	Tolerance/trophic support	–	+ (Hypothesized)	Peripheral suppression	+ (One in four recipients; one study)	[50]
Co-stimulation blockade	Tolerance/immunosuppression	–	–	Peripheral deletion/suppression	–	[67,68]
Carriers of immunomodulatory agents	Localized tolerance/immunosuppression	–	+ (Hypothesized)	Regulatory T cell-mediated suppression at allograft site	Not studied	[72]
Immunoisolation	Physical barrier impairing direct immune recognition	–	+	Extend escape of direct immune recognition	+ (Varying results among studies)	[83,84]
Carriers of immunomodulatory cells	Localized immunosuppression	–	+ (Hypothesized)	Suppression at allograft site	Not studied	[8]

tolerogenic DCs achieved 300 days of IS-free survival after transplantation with MHC-mismatched livers. Graft-infiltrating host tolerogenic DCs were thought to induce alloreactive T cell suppression by cross-dressing donor antigens [41], probably from donor graft splenocyte-derived exosomes [42]. Also, simultaneous upregulation of PD-1 ligand (PD-L1) and IL-10 [41] occurred. Thus, tolerogenic DCs exert tolerogenic alloantigen presentation, inducing antigen-specific peripheral suppression.

With an alternative mechanism of action, regulatory macrophages were reported to provide non-antigen-specific peripheral suppression. The *in vivo* induction of graft-infiltrating host regulatory macrophages (Ly6-C^{lo} CD169^{+}), achieved through the intravenous injection of rapamycin-functionalized high-density lipoprotein nanoparticles, prolonged mice heart allograft survival for 100 days. Regulatory macrophages downregulated IL-6/TNF-α, promoting the expansion of graft-infiltrating Tregs and suppressing CD8^{+} T cells *in vitro* [33].

As in chimerism, most preclinical/clinical investigation on tolerogenic leucocytes remains restricted to KT/LT. Although autologous cell sources are preferred, their suppressive potential may vary among recipients [2,36]. Also, clinical research to prove that tolerogenic leucocytes induce sufficient suppression in humans is needed.

Apoptotic Donor Leucocytes

Injection of donor leucocytes treated *in vitro* with apoptosis-inducing agents comprises a peripheral tolerance-inducing strategy in which tolerogenic cells present donor antigens [43]. Non-human primates that received MHC-mismatched pancreatic islets achieved 1-year tolerance, following two

b0.2 Box 2. Immunological Mechanisms Driving Mixed Hematopoietic Chimerism

b2.3 **Sustained Mixed Chimerism**

b2.4 In sustained mixed chimerism, Treg-mediated suppression was important at initial stages. However, in the long-term,
b2.5 peripheral deletion of host alloreactive T cells was the main mechanism responsible for the achievement of tolerance. In
b2.6 a Treg-enriched context, host Tregs were able to inhibit both donor and host-derived DCs, rendering them tolerogenic
b2.7 through expression of PD-L1, which consequently impaired donor alloreactive T cells to trigger GvHD [100]. Moreover,
b2.8 in grafts enriched in resident donor alloreactive $CD4^+/CD8^+$ memory T cells [101], which includes lymphocyte-enriched or-
b2.9 gans such as the liver and intestine [102,103], a balance was initially established between GvH and host-versus-graft (HvG)
b2.10 responses [28]. Such delay in the replacement of donor by host T cells, along with expansion of GvH populations,
b2.11 prevented GvHD [101] and rejection [28]. Although the peripheral suppression of host alloreactive T cells may be initially
b2.12 triggered by Tregs and, eventually, by GvH reactivity seen in lymphocyte-enriched allografts, deletion of host alloreactive
b2.13 T cells will prevail in the long-term, as in transient chimeras (see later), through the intrinsic apoptotic pathway [21], rather
b2.14 than by the extrinsic Fas/FasL pathway [104].

b2.15 **Transient Mixed Chimerism**

b2.16 As in sustained chimerism, early tolerance induction in transient chimeras depends on Treg-mediated suppression. Initial
b2.17 post-transplant expansion of Tregs, as pre-existent host alloreactive Tregs, up to 6 months [105], induced tolerance in
b2.18 human recipients of HLA-mismatched CKBMT [106]. This was likely driven by *de novo* Treg production or lymphopenia-
b2.19 induced proliferation, where residual lymphocytes that remain after lymphocyte depletion conditioning undergo proliferation
b2.20 [106]. While Tregs are known to suppress host alloreactive T cells early post-transplantation, the achievement of long-term
b2.21 (>18 months) allograft tolerance seems not to be Treg-dependent [107].

b2.22 Early and transient Treg suppressive effects are thought to contribute to the formation of an initial tolerogenic microenvi-
b2.23 ronment [100], synergic with the peripheric persistent exposure of donor antigens (i.e., from kidney) in a tolerogenic context
b2.24 [27]. Tolerogenic donor antigen presentation, which probably lasted permanently in the absence of Treg expansion,
b2.25 gradually induced peripheral deletion of host alloreactive $CD4^+/CD8^+$ T cells overtime, mediating tolerance long-term, as
b2.26 seen in mixed chimerism-induced tolerant recipients [27]. Curiously, alloreactive clones were expanded in human recipients
b2.27 submitted to conventional IS, while anergy might have triggered graft rejection in one patient displaying donor-specific
b2.28 unresponsiveness *in vitro* [27] and sudden rejection of kidney allografts tolerated for 10 years in non-human primates [108].

181 injections of apoptotic donor leucocytes (ADLs) under a short course of IS [5]. Although dosage and
182 interval between infusions influenced the therapeutic efficacy in mice islet allografts [44], a human trial
183 found single-dose ADLs to be adjuvant in GvHD prophylaxis (NCT00524784[viii]) [45]. Preclinical re-
184 search established multiple mechanisms to occur following ADL infusion. First, defined subtypes of
185 host splenic APCs, $CD11c^+$ DCs, seem to phagocytize intravenously infused ADLs via specific recep-
186 tors for apoptotic cell uptake, further presenting their alloantigens. This mechanism guided >100 days
187 of mice islet allograft survival, in IS-free regimen [43]. ADLs also upregulated negative co-stimulatory
188 molecules (e.g., PD-L1/PD-L2) in APCs, initially promoting fast proliferation followed by significant
189 clonal deletion of $CD4^+$ T cells [43]. Conversely, the infusion of donor ADLs (along with IS) inhibited
190 memory, but not naïve, alloreactive T cells in murine islet allografts [46]. Transient nonphagocytized
191 ADLs also directly rendered $CD4^+$ T cells anergic in mice allografts, through alloantigen presentation
192 without upregulation of CD80/CD86 molecules (Signal 2) [43]. Lastly, ADLs triggered the TGF-β-
193 mediated proliferation of host Tregs, having an initial effect on mice allogeneic islet survival [44].

194 In summary, ADLs performed tolerogenic antigen presentation, inducing peripheral deletion/
195 anergy, in addition to the sole peripheral suppression seen in tolerogenic leucocytes *in vivo*.
196 Furthermore, since following ADL phagocytosis host APCs may perform antigen presentation
197 in an immunogenic [47] or tolerogenic fashion [43]; the influence of host microenvironment in
198 such differential behavior may be important.

199 *Mesenchymal Stem Cells*

200 Stem cells were reported both as trophic/immunosuppressive inducers and/or peripheral
201 tolerance-inducing agents. Studies in KT patients showed infused autologous/allogeneic mesen-
202 chymal stem cells (MSCs) to be safe [48,49]. Injection of autologous BM-MSCs also induced a

tolerogenic profile in HLA-mismatched KT recipients under low IS (NCT00752479[ix], NCT02012153[x]) [48], providing IS withdrawal in one of four subjects in a case report [50]. Additionally, donor-derived BM-MSCs reduced IS dosage and helped to prevent acute rejection in HLA-mismatched KT patients (NCT02563340[xi]) [51]. In contrast, while infusion of third party umbilical cord-derived MSCs via the renal artery proved safe in a clinical trial, they did not show adjuvant effect on kidney allograft survival (NCT02490020[xii]) [49]. The discrepant results between studies may be explained by the variable efficacy of MSCs, which depend on dosage, donor characteristics, retrieval location, priming, and expansion/isolation techniques [49,52].

Notably, multipotent MSCs are transiently **immune evasive** through a **'hit and run'** mechanism when infused [52,53]. The lasting IS of short-lived MSCs is explained through modulation of tolerogenic macrophages [54] and Tregs [6], besides anergy induction [55], either indirectly or directly. Through indirect contact, proinflammatory priming activated MSCs, upregulating the secretion of anti-inflammatory molecules in autoimmune diabetic mice [53]. Also, released trophic/proangiogenic elements may support engraftment [56,57]. Indeed, syngeneic BM-MSC-derived matrix metalloproteinases MMP-2 and MMP-9 impaired rejection of cotransplanted islet allografts (95 days) in IS-free mice [58]. *In vitro* coculture of BM-MSCs with peripheral blood mononuclear cells (PBMCs) led to the upregulation of PD-L1 and TGF-β in MSCs, generating stable functioning iTregs [59]. Besides secretion of soluble molecules, MSCs release exosomes and extracellular vesicles *in vitro* [60]. Efforts to prolong this immunosuppressive effect of MSCs have sought to increase their retention in mice models [52,53,61]. It is also known that MSCs directly contact immune cells, in a non-antigen-specific fashion. This has supported MSC-mediated division arrest anergy of activated $CD4^+$/$CD8^+$ T cells in mice [55]. Furthermore, in a study comprising 16 GvHD patients, host-activated cytotoxic populations ($CD56^+$ NK and $CD8^+$ T cells) induced perforin/Fas-mediated apoptosis of intraperitoneally (but not intravenously) infused MSCs. Patients harboring cytotoxic populations of MSCs above a defined threshold had predictive response to MSC infusion [6]. In a mice model of GvHD, host APCs phagocytized apoptotic MSCs and overexpressed indolamine-2,3-dioxygenase (IDO), improving the GvHD treatment [6]. The analysis of serum samples of eight steroid-resistant GvHD patients submitted to MSC infusion also showed that successful response to the therapy correlated with increased PGE2 levels [54]. One *in vitro* study that fine-tuned autologous MSCs to present an antigen of interest under concomitant suppressive signaling suggested an additional mechanism of action of MSCs, in which they may directly contact with immune cells in a tolerogenic antigen-specific fashion [62]. However, so far, MSCs have been mostly reported to perform peripheral suppression *in vivo*, in a non-antigen-specific fashion, although further mechanisms remain poorly studied.

Technologies based on tolerogenic cells have been effective in minimizing IS-related side effects, but clinical results still lack consistency depending on their method of administration, or targeted transplanted organ. Moreover, cell-based therapies are highly dependent on laborious *in vitro* manipulation. Therefore, cell-free suppression-inducing technologies have been raised as interesting alternatives.

Acellular Constructs

Co-stimulation Blockade

Co-stimulation blockade is an immunosuppressive and/or peripheral tolerance-inducing setup comprising drugs blocking costimulatory molecules (Signal 2) necessary for T cell activation, impairing alloreactive T cells.

Although IS by blockade of co-stimulatory molecules prevented renal rejection in MHC-mismatched non-human primates [63], its use as a standalone acceptance-inducing strategy

was contraindicated in humans for the case of anti-CD154 monoclonal antibody due to thromboembolism seen in monkeys [64], along with other severe toxicities [65]. However, the approach reduced IS-related morbidities in patients when combined with chimerism and other tolerance-inducing strategies [26,32]. Studies in mice skin allografts showed co-stimulatory blockade to induce anergy [66] and expansion of Tregs [67]. Murine models transplanted with allogeneic BM and treated with co-stimulation blockade exhibited deletion of alloreactive T cells through activation-induced cell death [68]. Interestingly, co-stimulation blockade triggered the expansion of residual host alloreactive $CD4^+$ T cells in mice skin allografts. Those clones remained functional after tolerance was established, but absent at the Treg-enriched allograft site. Thus, alloreactive $CD4^+$ T cells were kept in check by the immunosuppressive environment locally created by treatment (namely by host Tregs), which impaired their migration and/or expansion in murine skin allografts [67]. A major obstacle to tolerance comprises host alloreactive memory T cells, which are normally resistant to multiple co-stimulation blockade regimens [69] and suppression-mediated Tregs [70], giving rise to **heterologous immunity**. Administration of an anti-CD2 fusion protein, alefacept, resulted in the deletion of pre-existing alloreactive memory $CD8^+$ T cells in non-human primates submitted to MHC-mismatched CKBMT [65]. The search for technologies with reduced systemic toxicity has led to the development of setups that promote localized graft acceptance, which are gaining momentum (see later).

Carriers of Immunomodulatory Agents

Cotransplantation of allografts with biomaterials that locally provide (i) soluble immunosuppressive molecules, (ii) prolonged presentation of cell-surface negative co-stimulatory molecules, or (iii) presentation of antigens/MHC molecules, integrate localized immunosuppressive/tolerance strategies. Although these strategies remain at the preclinical stage of development, they have been considered highly promising to reduce systemic IS.

Delivery of Tolerogenic Material

Strategies based on the localized presentation of cell-surface negative co-stimulatory molecules have been mostly restricted to mice islet allograft models. Allogeneic islets were co-delivered with poly(ethylene glycol) (PEG) microgels decorated with chimeric streptavidin/programmed cell death-1 (SA-PD-L1) to the epididymal fat pad of mice, along with a 2-week rapamycin course. Most recipients maintained islet function for 100 days, and $FoxP3^+CD4^+$ T cells (Tregs) were expanded at the graft site [7]. Similar results were obtained in identical models by immobilizing streptavidin-Fas ligand (SA-FasL) in microgels [71] and scaffolds [72]. Since in the latter strategy host alloreactive T cells remained functional in the spleen, it was speculated that allograft-localized Tregs maintained tolerance in IS-free mice [72]. This phenomenon shares similarities with the one seen in co-stimulation blockade regimens, where localized Treg-mediated suppression kept peripheral alloreactive cells in check upon mouse skin allograft transplantation. Alternative approaches have relied on the local delivery of soluble immunomodulators. Incubation of PBMCs with a macroscale porous agarose cryogel scaffold loaded with microparticles enabled the sequential release of immunomodulators capable of inducing tolerogenic phenotypes of DCs, further impairing allogeneic T cell proliferation *in vitro* [73].

The surface of pancreatic islets has also been directly engineered to transiently present immunomodulators [74,75]. Immobilization of SA-Fas-L on the surface of allogeneic mouse islets induced long-term localized tolerance (<100 days), under an initial 2-week rapamycin course. This strategy also reduced, but did not abrogate, alloreactive T cell expansion in graft-draining lymph nodes. Such localized immunomodulation/tolerance was antigen-specific, requiring persistent alloantigen presentation and long-term (130 days) Treg expansion [74].

Clinician's Corner

Gold standard immunosuppression therapies administered systemically to prevent allograft rejection in allogeneic transplantation are known to induce severe morbidities in the long-term, compromising patients' safety and quality of life, as well as hampering the function of transplanted organs or tissues. Therefore, the development of safer strategies to induce allograft acceptance is required.

Protocols targeting immune tolerance based on the achievement of hematopoietic chimeric states in HLA-disparate recipients exhibit considerable risk for GvHD, apparently favoring a limited number of transplanted tissues, namely liver and kidney. Novel and less toxic therapies, including infusion of tolerogenic cells (e.g., Tregs) and stem cells, seem to have mainly minimized IS-related side effects and have shown promising ability to induce graft acceptance in non-human primate models, as well as in humans.

The use of biomaterials for allogeneic transplantation purposes has been explored since the 1980s for the shielding of pancreatic islets from immune recognition, however, it mainly translated into poor allograft function. In recent years, the use of biomaterials and other classical key players in regenerative medicine (including mesenchymal stem cells) have paved the way for the establishment of innovative strategies to achieve localized immunosuppression. To date, the efficacy of these strategies has only been proven in rodent models and further in-depth investigation to elucidate their pertinence in larger animal models is needed.

Future efforts targeting the establishment of (i) less toxic regimens/bioengineered strategies that benefit recipient response over standard immunosuppression, as well as (ii) identification of patient subgroups with predictive response to defined therapeutic schemes and administration routes, and (iii) effective technologies to follow up the recipient response post-transplantation may be key for taking full advantage of recent multidisciplinary efforts that bring together the know-how of the medical

community, immunologists, and engineers to design bioengineered technological approaches.

Strategies comprising local delivery of immunomodulators to allografts, either in a soluble form or as transient presenters of cell surface molecules, are intended to create a tolerogenic microenvironment at the micro-scale allograft site. The latter has been, so far, restricted to microtissue transplantation, with applications mostly focused on pancreatic islets. The induction of such tolerogenic microenvironment at the allograft site was hypothesized to induce the tolerogenic presentation of alloantigens, namely in typologies presenting cell surface immunomodulatory molecules [74]. Such phenomenon has been related to the local expansion of Tregs, seeking to restrain host alloreactive cell expansion in peripheral sites, while suppressing them at the allograft.

Delivery of Antigens

Albeit the intravenous delivery of antigens led to severe toxicity in murine and non-human primate models [76], technologies that redirect antigen exposure to sites of interest, promoting antigen presentation into a tolerogenic context, are reinvigorating this recent field. However, most approaches have been mainly restricted to preclinical studies targeting autoimmune diseases. For example, mice with autoimmune diabetes achieved tolerance following intravenous single-
Q7 infusion of antigen-decorated poly lactic-co-glycolic acid (PLGA) nanoparticles. Macrophages exhibiting scavenger receptors (e.g., MARCO) capable of binding to polyanionic surfaces were sought to internalize nanoparticles and therefore perform antigen presentation in a tolerogenic context, rendering mice tolerant [77]. In the same study, antigen-functionalized nanoparticles led to the expansion of pancreatic antigen-specific Tregs with upregulated PD-1 and CTLA-4, which were pivotal to sequestrate antigen-specific effector T cells in the spleen [77]. Another approach delivered antigen-laden hydrogels to autoimmune diabetic mice. Although *in situ* induced tolerogenic DCs were hypothesized to perform antigen presentation, and promoted antigen-specific Treg expansion, the system did not impair expansion of antigen-specific effector $CD4^+$ T cells in locations presenting the disease-associated antigen (islets and hydrogel), leading to disease progression [76].

Consistent with the delivery of localized immunosuppressants, technologies focused on the delivery of antigens in autoimmune diseases promote the local expansion of host Tregs at the site of antigen-reactive responses. The success of the acceptance strategy relied on preventing concomitant local expansion of antigen-reactive cells, entrapping them in peripheral regions, thereby promoting an adequate localized suppression.

Mimicking Allogeneic MHC-System

Although the delivery of MHC molecule mimics has achieved promising results, this technology is still poorly explored for allogeneic transplantation and studies in large-scale models are still in need. To target and specifically delete host alloreactive T cells in a mice skin allotransplant, polyethyleneimine (PEI)-coated PLGA nanoparticles covalently functionalized with five different molecules were developed. The first tagged molecule, a recombinant protein mimicking mouse allogeneic MHC-I, targeted T cells; chimeric CD47 protein, a mimicker of the 'don't eat me' signal, intended to avoid phagocyte-mediated destruction; and finally, different immunosuppressants as anti-Fas monoclonal antibody, a fusion protein of PD-L1 and TGF-β were also added to the nanoparticle system. Infusion of nanoparticles triggered apoptosis of host alloreactive $CD8^+$ T cells with specificity for allogeneic MHC-I and also led to the expansion of Tregs, permitting 45 days of skin allograft survival [78].

Additional applications for MHC-laden carriers and antigen-laden systems can be found in the literature [79,80].

Approaches Combining Biomaterials and Cells

Allograft Immunoisolation

Physical immunoisolation of avascular cell/microtissue allografts in semipermeable matrices, through encapsulation in semipermeable nonbiodegradable materials (e.g., alginate hydrogels) is believed to hamper direct immune recognition. The concept is based on the design of biomaterials that allow the diffusion of oxygen/vital molecules to cells, while impairing contact with external inflammatory cells and high molecular weight soluble components of the immune system [9,81]. For conceptually not enabling the reperfusion of allografts with the host's vascular system, preclinical and clinical applications have been limited to pancreatic islet transplantation. A recent *in vitro* study, however, questioned the efficacy of alginate-barium hydrogels on the shielding of islets, since indirect antigen recognition by $CD8^+$ T cells was observed [82].

Studies in non-human primates showed alginate macroencapsulated islet allografts remain functional without IS [81], but translational clinical outcomes using alginate-based microencapsulation have substantially varied [83,84]. Promising results from a case report showed that some patients that received alginate microencapsulated islets achieved IS-free graft function for 3 years [83]. Conversely, in a human trial, a subcutaneous allogeneic islet macroencapsulation device comprising an internal oxygen module of gas-permeable silicon-rubber membranes further linking two oxygen tanks (~30 hours autonomy) was accepted without IS (6 months). However, allograft function lasted 1 day, forming pericapsular fibrosis in most recipients (NCT02064309[xiii]) [9]. Several *in vitro* and preclinical studies aimed to improve encapsulated islets' survival and function over time. Mice models received islet-containing constructs comprising the release of trophic/proangiogenic molecules from encapsulating biomaterials [85], the incorporation of immunomodulatory molecules in materials [86], as well as the cotransplantation of islets with other cell types [87]. Tailoring the device topography [88] and conformational design (e.g., donut-shaped [89], fiber-shaped [90], or 3D macroporous structures [91]), biomaterial chemistry, and mechanical properties, as well as the inclusion of extracellular matrix proteins in the encapsulating matrix [85,88,92] have been explored as roadways to improve/extend transplanted islet function.

Carriers of Immunomodulatory Cells

Few reports in mice models comprise the use of biomaterial-assisting immunomodulatory cells as localized IS/tolerance towards an allogeneic transplant [8,87]. Intraperitoneal delivery of MSCs primed with proinflammatory cytokines provided 50-day survival of co-encapsulated mice islet allografts, without IS [87]. Additionally, a recent study in a rat model described the fabrication of a 3D printed compartmentalized system targeted at the localized protection of allogeneic endocrine Leydig cells. The biofabricated device comprised a U-shaped immunosuppressive drug reservoir to elute CTLA-4Ig to a central cell reservoir, in which BM-MSCs were encapsulated in a pluronic G-127 macrogel. The reservoir had 100–300-μm external openings that allowed host vascularization, achieved through 6-week prevascularization. As a result, although fibrotic tissue covered the implant, the local delivery of CTLA-4Ig locally impaired T cell activation, allowing allogeneic cell survival for 31 days [8]. Additional approaches to implement carriers of immunomodulatory cells may be found in reference [93].

Concluding Remarks

The goal of achieving allograft acceptance has inspired the medical and bioengineering fields to design multidisciplinary technologies (see Clinician's Corner). However, the exact mechanisms that orchestrate allogeneic acceptance are frequently difficult to evaluate during the clinical follow-up. The biological mechanisms of self-tolerance have driven the development of

Outstanding Questions

How can we learn from biological mechanisms of immunological tolerance and effectively apply this know-how to the development of bioengineered safe and affordable therapies to manage allogeneic transplantation?

Given the difficult extrapolation of the beneficial outcomes seen in animal models to reliable human therapies, how can we develop proper preclinical models to study novel trends, easily translatable into humans?

Considering the scarcity of biomarkers to infer on patients' predictive response to treatments over time, how can we design diagnostic tools to stratify patients that may benefit from new therapies?

Since chronic rejection is recurrent and minor histocompatibility antigens are still unable to be matched in humans, how can we perform allogeneic transplantation free of expiry date?

Bioengineered approaches that orchestrate tolerogenic alloantigen presentation mechanisms already achieved localized tolerance in murine models. How can we translate the localized tolerance observed in mice to trigger operational tolerance in humans?

different therapies, mostly based on cell and/or protein delivery. Emerging fields comprising biomaterials and engineered bioreactors will benefit from continuing to take lessons from human biology, offering the hope to deliver affordable and reproducible technologies to the marketplace. Ideally noninvasive approaches to monitor recipient response post-transplantation (e.g., sequencing of a TCR region of alloreactive T cells [28]) may work as complementary tools to stratify recipients with predictive response to treatment and anticipate rescue therapy.

A frequently overlooked limitation in the development of acceptance-inducing strategies is the difficult extrapolation between animal models and humans (see Outstanding Questions). Most therapeutic studies are performed in murine models, poor in alloreactive memory T cells. Moreover, some studies use animals matched for minor histocompatibility antigens, reported to drive rejection in humans [2]. Preclinical humanized models are well accepted to facilitate the extrapolation to clinical practice.

New trends in allograft acceptance technology may find inspiration in cancer therapeutics, including on the subcutaneous placement of hydrogels capable of recruiting DCs and modifying their cell surface, enabling posterior-targeted DC modulation/therapy *in vivo* [94]. Beyond chemically engineered surfaces, the exploitation of physical properties of biomaterials also offer promising cues to control immune response [95].

Collectively, multidisciplinary approaches based on the engineering of proteins, cells, and biomaterials may pave the way to reduce immunosuppressant use, monitor recipient response, and improve quality of life of transplanted individuals.

Acknowledgments

This work was supported by the Programa Operacional Competitividade e Internacionalização, in the component FEDER, and by national funds (OE) through FCT/MCTES, in the scope of the projects 'TranSphera' (PTDC/BTM-ORG/30770/2017). This work was also developed within the scope of the project CICECO-Aveiro Institute of Materials, UIDB/50011/2020 and UIDP/50011/2020, financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. M.B.O. acknowledges the individual contract CEECIND/03605/2017. A.R.S. acknowledges the PhD grant SFRH/BD/145765/2019.

Declaration of Interests

The authors declare no conflict of interest.

Resources

[i] www.transplant-observatory.org/global-report-2018/
[ii] https://optn.transplant.hrsa.gov/data/
[iii] www.organdonor.gov/statistics-stories/statistics.html
[iv] https://clinicaltrials.gov/ct2/show/NCT00497926
[v] https://clinicaltrials.gov/ct2/show/NCT03292445
[vi] https://clinicaltrials.gov/ct2/show/NCT00801632
[vii] https://clinicaltrials.gov/ct2/show/NCT02145325
[viii] https://clinicaltrials.gov/ct2/show/NCT00524784
[ix] https://clinicaltrials.gov/ct2/show/NCT00752479
[x] https://clinicaltrials.gov/ct2/show/NCT02012153
[xi] https://clinicaltrials.gov/ct2/show/NCT02563340
[xii] https://clinicaltrials.gov/ct2/show/NCT02490020
[xiii] https://clinicaltrials.gov/ct2/show/NCT02064309

References

1. Wekerle, T. *et al.* (2017) Strategies for long-term preservation of kidney graft function. *Lancet* 389, 2152–2162
2. Pilat, N. *et al.* (2017) Combining adoptive Treg transfer with bone marrow transplantation for transplantation tolerance. *Curr. Transplant. Rep.* 4, 253–261
3. Sellares, J. *et al.* (2012) Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am. J. Transplant.* 12, 388–399
4. Mikami, N. *et al.* (2020) Epigenetic conversion of conventional T cells into regulatory T cells by CD28 signal deprivation. *Proc. Natl. Acad. Sci. U. S. A.* 117, 12258–12268
5. Singh, A. *et al.* (2019) Long-term tolerance of islet allografts in nonhuman primates induced by apoptotic donor leukocytes. *Nat. Commun.* 10, 3495
6. Galleu, A. *et al.* (2017) Apoptosis in mesenchymal stromal cells induces in vivo recipient-mediated immunomodulation. *Sci. Transl. Med.* 9, eaam7828
7. Coronel, M.M. *et al.* (2020) Immunotherapy via PD-L1–presenting biomaterials leads to long-term islet graft survival. *Sci. Adv.* 6, eaba5573
8. Paez-Mayorga, J. *et al.* (2020) Neovascularized implantable cell homing encapsulation platform with tunable local immunosuppressant delivery for allogeneic cell transplantation. *Biomaterials* 257, 120232
9. Carlsson, P.O. *et al.* (2018) Transplantation of macroencapsulated human islets within the bioartificial pancreas betaAir to patients with type 1 diabetes mellitus. *Am. J. Transplant.* 18, 1735–1744
10. Zuber, J. and Sykes, M. (2017) Mechanisms of mixed chimerism-based transplant tolerance. *Trends Immunol.* 38, 829–843
11. Mahr, B. *et al.* (2017) Transplantation tolerance through hematopoietic chimerism: progress and challenges for clinical translation. *Front. Immunol.* 8, 1762
12. Anderson, D. *et al.* (1951) The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. *Heredity* 5, 379–397
13. Oura, T. *et al.* (2015) Transient mixed chimerism for allograft tolerance. *Chimerism* 6, 21–26
14. Leventhal, J. *et al.* (2013) Tolerance induction in HLA disparate living donor kidney transplantation by donor stem cell infusion: durable chimerism predicts outcome. *Transplantation* 95, 169–176
15. Balner, H. (1964) Persistence of tolerance towards donor-type antigens after temporary chimerism in rats. *Transplantation* 2, 464–474
16. Kawai, T. *et al.* (1995) Mixed allogeneic chimerism and renal allograft tolerance in cynomolgus monkeys. *Transplantation* 59, 256–262
17. Orlando, G. *et al.* (2011) How regenerative medicine may contribute to the achievement of an immunosuppression-free state. *Transplantation* 92, 36–38
18. Duran-Struuck, R. *et al.* (2017) Effect of ex vivo-expanded recipient regulatory T cells on hematopoietic chimerism and kidney allograft tolerance across MHC barriers in cynomolgus macaques. *Transplantation* 101, 274–283
19. Sykes, M. (2015) Immune tolerance in recipients of combined haploidentical bone marrow and kidney transplantation. *Bone Marrow Transplant.* 50, S82–S86
20. Perry, J.S.A. *et al.* (2018) Transfer of cell-surface antigens by scavenger receptor CD36 promotes thymic regulatory T cell receptor repertoire development and allo-tolerance. *Immunity* 48, 923–936
21. Cippa, P.E. *et al.* (2013) Targeting apoptosis to induce stable mixed hematopoietic chimerism and long-term allograft survival without myelosuppressive conditioning in mice. *Blood* 122, 1669–1677
22. Speiser, D. *et al.* (1992) T cell immunoincompetence in allogeneic chimerism. *N. Engl. J. Med.* 326, 1028–1029
23. Ildstad, S. *et al.* (2015) Facilitating cells: translation of hematopoietic chimerism to achieve clinical tolerance. *Chimerism* 6, 33–39
24. Leventhal, J. *et al.* (2017) *Eight Year Follow-Up of a Phase 2 Clinical Trial to Induce Tolerance in Living Donor Renal Transplant Recipients*. 2017 American Transplant Congress
25. Scandling, J.D. *et al.* (2015) Chimerism, graft survival, and withdrawal of immunosuppressive drugs in HLA matched and mismatched patients after living donor kidney and hematopoietic cell transplantation. *Am. J. Transplant.* 15, 695–704
26. Kawai, T. *et al.* (2014) Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. *Am. J. Transplant.* 14, 1599–1611
27. Morris, H. *et al.* (2015) Tracking donor-reactive T cells: evidence for clonal deletion in tolerant kidney transplant patients. *Sci. Transl. Med.* 7, 1–11
28. Zuber, J. *et al.* (2016) Bidirectional intragraft alloreactivity drives the repopulation of human intestinal allografts and correlates with clinical outcome. *Sci. Immunol.* 1, eaah3732
29. Sawitzki, B. *et al.* (2020) Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. *Lancet* 395, 1627–1639
30. Mathew, J.M. *et al.* (2018) A phase I clinical trial with ex vivo expanded recipient regulatory T cells in living donor kidney transplants. *Sci. Rep.* 8, 7428
31. Todo, S. *et al.* (2016) A pilot study of operational tolerance with a regulatory T-cell-based cell therapy in living donor liver transplantation. *Hepatology* 64, 632–643
32. Ezzelarab, M.B. *et al.* (2016) Eomesodermin(lo) CTLA4(hi) alloreactive CD8+ memory T cells are associated with prolonged renal transplant survival induced by regulatory dendritic cell infusion in CTLA4 immunoglobulin-treated nonhuman primates. *Transplantation* 100, 91–102
33. Braza, M.S. *et al.* (2018) Inhibiting inflammation with myeloid cell-specific nanobiologics promotes organ transplant acceptance. *Immunity* 49, 819–828
34. Lee, K. *et al.* (2014) Attenuation of donor-reactive T cells allows effective control of allograft rejection using regulatory T cell therapy. *Am. J. Transplant.* 14, 27–38
35. Nikolouli, E. *et al.* (2017) Alloantigen-induced regulatory T cells generated in presence of vitamin C display enhanced stability of Foxp3 expression and promote skin allograft acceptance. *Front. Immunol.* 8, 748
36. Pilat, N. *et al.* (2019) Treg-mediated prolonged survival of skin allografts without immunosuppression. *Proc. Natl. Acad. Sci. U. S. A.* 116, 13508–13516
37. Sicard, A. *et al.* (2020) Donor-specific chimeric antigen receptor Tregs limit rejection in naive but not sensitized allograft recipients. *Am. J. Transplant.* 20, 1562–1573
38. Lee, J.H. *et al.* (2017) Tolerogenic dendritic cells are efficiently generated using minocycline and dexamethasone. *Sci. Rep.* 7, 15087
39. Oh, N.A. *et al.* (2020) Plasmacytoid dendritic cell-driven induction of Treg is strain specific and correlates with spontaneous acceptance of kidney allografts. *Transplantation* 104, 39–53
40. Ezzelarab, M.B. *et al.* (2017) Renal allograft survival in nonhuman primates infused with donor antigen-pulsed autologous regulatory dendritic cells. *Am. J. Transplant.* 17, 1476–1489
41. Ono, Y. *et al.* (2018) Graft-infiltrating PD-L1(hi) cross-dressed dendritic cells regulate antidonor T cell responses in mouse liver transplant tolerance. *Hepatology* 67, 1499–1515
42. Marino, J. *et al.* (2016) Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation. *Sci. Immunol.* 1, aaf8759
43. Kheradmand, T. *et al.* (2012) Ethylenecarbodiimide-fixed donor splenocyte infusions differentially target direct and indirect pathways of allorecognition for induction of transplant tolerance. *J. Immunol.* 189, 804–812
44. Luo, X. *et al.* (2008) ECDI-fixed allogeneic splenocytes induce donor-specific tolerance for long-term survival of islet transplants via two distinct mechanisms. *Proc. Natl. Acad. Sci. U. S. A.* 105, 14527–14532
45. Mevorach, D. *et al.* (2014) Single infusion of donor mononuclear early apoptotic cells as prophylaxis for graft-versus-host disease in myeloablative HLA-matched allogeneic bone marrow transplantation: a phase I/IIa clinical trial. *Biol. Blood Marrow Transplant.* 20, 58–65

46. Dangi, A. *et al.* (2020) Donor apoptotic cell-based therapy for effective inhibition of donor-specific memory T and B cells to promote long-term allograft survival in allosensitized recipients. *Am. J. Transplant.* 20, 2728–2739
47. Larson, S.R. *et al.* (2016) Ly6C(+) monocyte efferocytosis and cross-presentation of cell-associated antigens. *Cell Death Differ.* 23, 997–1003
48. Perico, N. *et al.* (2018) Long-term clinical and immunological profile of kidney transplant patients given mesenchymal stromal cell immunotherapy. *Front. Immunol.* 9, 1359
49. Sun, Q. *et al.* (2018) Allogeneic mesenchymal stem cells as induction therapy are safe and feasible in renal allografts: pilot results of a multicenter randomized controlled trial. *J. Transl. Med.* 16, 52
50. Casiraghi, F. *et al.* (2020) Kidney transplant tolerance associated with remote autologous mesenchymal stromal cell administration. *Stem Cells Transl. Med.* 9, 427–432
51. Peng, Y. *et al.* (2013) Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: a clinical pilot study. *Transplantation* 95, 161–168
52. Levy, O. *et al.* (2020) Shattering barriers toward clinically meaningful MSC therapies. *Sci. Adv.* 6, eaba6884
53. Oliveira, R.L. *et al.* (2017) In vivo immunogenic response to allogeneic mesenchymal stem cells and the role of preactivated mesenchymal stem cells cotransplanted with allogeneic islets. *Stem Cells Int.* 2017, 9824698
54. Cheung, T.S. *et al.* (2019) Apoptotic mesenchymal stromal cells induce prostaglandin E2 in monocytes: implications for the monitoring of mesenchymal stromal cell activity. *Haematologica* 104, e438
55. Glennie, S. *et al.* (2005) Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. *Blood* 105, 2821–2827
56. Takahashi, Y. *et al.* (2018) Self-condensation culture enables vascularization of tissue fragments for efficient therapeutic transplantation. *Cell Rep.* 23, 1620–1629
57. Gamble, A. *et al.* (2018) Improved islet recovery and efficacy through co-culture and co-transplantation of islets with human adipose-derived mesenchymal stem cells. *PLoS One* 13, e0206449
58. Ding, Y. *et al.* (2009) Mesenchymal stem cells prevent the rejection of fully allogenic islet grafts by the immunosuppressive activity of matrix metalloproteinase-2 and -9. *Diabetes* 58, 1797–1806
59. Azevedo, R.I. *et al.* (2020) Mesenchymal stromal cells induce regulatory T cells via epigenetic conversion of human conventional CD4 T cells in vitro. *Stem Cells* 38, 1007–1019
60. Wang, B. *et al.* (2016) Mesenchymal stem cells deliver exogenous microRNA-let7c via exosomes to attenuate renal fibrosis. *Mol. Ther.* 24, 1290–1301
61. Mao, A.S. *et al.* (2019) Programmable microencapsulation for enhanced mesenchymal stem cell persistence and immunomodulation. *Proc. Natl. Acad. Sci. U. S. A.* 116, 15392–15397
62. van Megen, K.M. *et al.* (2019) Activated mesenchymal stromal cells process and present antigens regulating adaptive immunity. *Front. Immunol.* 10, 694
63. Kirk, A.D. *et al.* (1999) Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nat. Med.* 5, 686–693
64. Kawai, T. *et al.* (2000) Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat. Med.* 6, 114
65. Lee, S. *et al.* (2013) Alefacept promotes immunosuppression-free renal allograft survival in nonhuman primates via depletion of recipient memory T cells. *Am. J. Transplant.* 13, 3223–3229
66. Quezada, S.A. *et al.* (2005) Analysis of the underlying cellular mechanisms of anti-CD154-induced graft tolerance: the interplay of clonal anergy and immune regulation. *J. Immunol.* 175, 771–779
67. Chai, J.G. *et al.* (2015) Allospecific CD4(+) T cells retain effector function and are actively regulated by Treg cells in the context of transplantation tolerance. *Eur. J. Immunol.* 45, 2017–2027
68. Wekerle, T. *et al.* (2001) Peripheral deletion after bone marrow transplantation with costimulatory blockade has features of both activation-induced cell death and passive cell death. *J. Immunol.* 166, 2311–2316
69. Burwitz, B.J. *et al.* (2017) Allogeneic stem cell transplantation in fully MHC-matched Mauritian cynomolgus macaques recapitulates diverse human clinical outcomes. *Nat. Commun.* 8, 1418
70. Mcintosh, C. *et al.* (2020) Giving effector/ memory T cells a new (suppressive) purpose. *Am. J. Transplant.* Published online February 24, 2020. https://doi.org/10.1111/ajt.15804
71. Headen, D.M. *et al.* (2018) Local immunomodulation with Fas ligand-engineered biomaterials achieves allogeneic islet graft acceptance. *Nat. Mater.* 17, 732–739
72. Skoumal, M. *et al.* (2019) Localized immune tolerance from FasL-functionalized PLG scaffolds. *Biomaterials* 192, 271–281
73. Srinivasan, S. and Babensee, J.E. (2020) Controlled delivery of immunomodulators from a biomaterial scaffold niche to induce a tolerogenic phenotype in human dendritic cells. *ACS Biomater. Sci. Eng.* 6, 4062–4076
74. Woodward, K.B. *et al.* (2020) Pancreatic islets engineered with a FasL protein induce systemic tolerance at the induction phase that evolves into long-term graft-localized immune privilege. *Am. J. Transplant.* 20, 1285–1295
75. Batra, L. *et al.* (2020) Localized immunomodulation with PD-L1 results in sustained survival and function of allogeneic islets without chronic immunosuppression. *J. Immunol.* 204, 2840–2851
76. Verbeke, C.S. *et al.* (2017) Multicomponent injectable hydrogels for antigen-specific tolerogenic immune modulation. *Adv. Healthc. Mater.* 6, 28116870
77. Prasad, S. *et al.* (2018) Tolerogenic Ag-PLG nanoparticles induce Tregs to suppress activated diabetogenic CD4 and CD8 T cells. *J. Autoimmun.* 89, 112–124
78. Shahzad, K.A. *et al.* (2018) On-target and direct modulation of alloreactive T cells by a nanoparticle carrying MHC alloantigen, regulatory molecules and CD47 in a murine model of alloskin transplantation. *Drug Deliv.* 25, 703–715
79. Gammon, J.M. and Jewell, C.M. (2019) Engineering immune tolerance with biomaterials. *Adv. Healthc. Mater.* 8, e1801419
80. Stabler, C.L. *et al.* (2019) Engineering immunomodulatory biomaterials for type 1 diabetes. *Nat. Rev. Mater.* 4, 429–450
81. Bochenek, M.A. *et al.* (2018) Alginate encapsulation as long-term immune protection of allogeneic pancreatic islet cells transplanted into the omental bursa of macaques. *Nat. Biomed. Eng.* 2, 810–821
82. Li, Y. *et al.* (2020) In vitro platform establishes antigen-specific CD8(+) T cell cytotoxicity to encapsulated cells via indirect antigen recognition. *Biomaterials* 256, 120182
83. Basta, G. *et al.* (2011) Long-term metabolic and immunological follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. *Diabetes Care* 34, 2406–2409
84. Tuch, B.E. *et al.* (2009) Safety and viability of microencapsulated human islets transplanted into diabetic humans. *Diabetes Care* 32, 1887–1889
85. Weaver, J.D. *et al.* (2017) Vasculogenic hydrogel enhances islet survival, engraftment, and function in leading extrahepatic sites. *Sci. Adv.* 3, e1700184
86. Hu, S. *et al.* (2021) Toll-like receptor 2-modulating pectin-polymers in alginate-based microcapsules attenuate immune responses and support islet-xenograft survival. *Biomaterials* 266, 120460
87. Vaithilingam, V. *et al.* (2017) Co-encapsulation and co-transplantation of mesenchymal stem cells reduces pericapsular fibrosis and improves encapsulated islet survival and function when allografted. *Sci. Rep.* 7, 10059
88. Groot Nibbelink, M. *et al.* (2018) An important step towards a prevascularized islet microencapsulation device: in vivo prevascularization by combination of mesenchymal stem cells on micropatterned membranes. *J. Mater. Sci. Mater. Med.* 29, 174
89. Ernst, A.U. *et al.* (2019) Interconnected toroidal hydrogels for islet encapsulation. *Adv. Healthc. Mater.* 8, e1900423
90. An, D. *et al.* (2018) Designing a retrievable and scalable cell encapsulation device for potential treatment of type 1 diabetes. *Proc. Natl. Acad. Sci. U. S. A.* 115, E263–E272

91. Liu, X. *et al.* (2019) Development of a coaxial 3D printing platform for biofabrication of implantable islet-containing constructs. *Adv. Healthc. Mater.* 8, e1801181
92. Jiang, K. *et al.* (2018) 3-D physiomimetic extracellular matrix hydrogels provide a supportive microenvironment for rodent and human islet culture. *Biomaterials* 198, 37–48
93. Dellacherie, M.O. *et al.* (2019) Macroscale biomaterials strategies for local immunomodulation. *Nat. Rev. Mater.* 4, 379–397
94. Wang, H. *et al.* (2020) Metabolic labeling and targeted modulation of dendritic cells. *Nat. Mater.* 19, 1244–1252
95. Zhang, J. *et al.* (2020) Micropatterned soft hydrogels to study the interplay of receptors and forces in T cell activation. *Acta Biomater.* 119, 234–246
96. Lechler, R.I. *et al.* (2003) The complementary roles of deletion and regulation in transplantation tolerance. *Nat. Rev. Immunol.* 3, 147–158
97. Abbas, A.K. and Lichtman, A.H. (2011) *Basic Immunology: Functions and Disorders of the Immune System* (3rd edn), Saunders/Elsevier
98. Siu, J.H.Y. *et al.* (2018) T cell allorecognition pathways in solid organ transplantation. *Front. Immunol.* 9, 2548
99. Owen, J. *et al.* (2013) *Kuby Immunology* (7th edn), W.H. Freeman and Company
100. Kinsella, F.A.M. *et al.* (2019) Mixed chimerism established by hematopoietic stem cell transplantation is maintained by host and donor T regulatory cells. *Blood Adv.* 3, 734–743
101. Li, H.W. *et al.* (2015) Rapid functional decline of activated and memory graft-versus-host-reactive T cells encountering host antigens in the absence of inflammation. *J. Immunol.* 195, 1282–1292
102. Wang, X.Q. *et al.* (2012) Hematopoietic chimerism in liver transplantation patients and hematopoietic stem/progenitor cells in adult human liver. *Hepatology* 56, 1557–1566
103. Fu, J. *et al.* (2019) Human intestinal allografts contain functional hematopoietic stem and progenitor cells that are maintained by a circulating pool. *Cell Stem Cell* 24, 227–239
104. Kurtza, J. *et al.* (2003) Lack of role for CsA-sensitive or Fas pathways in the tolerization of CD4 T cells via BMT and anti-CD40L. *Am. J. Transplant.* 3, 804–816
105. Savage, T.M. *et al.* (2018) Early expansion of donor-specific Tregs in tolerant kidney transplant recipients. *JCI Insight* 3, e124086
106. Sprangers, B. *et al.* (2017) Origin of enriched regulatory T cells in patients receiving combined kidney-bone marrow transplantation to induce transplantation tolerance. *Am. J. Transplant.* 17, 2020–2032
107. Andreola, G. *et al.* (2011) Mechanisms of donor-specific tolerance in recipients of haploidentical combined bone marrow/kidney transplantation. *Am. J. Transplant.* 11, 1236–1247
108. Yamada, Y. *et al.* (2015) Repeated injections of IL-2 break renal allograft tolerance induced via mixed hematopoietic chimerism in monkeys. *Am. J. Transplant.* 15, 3055–3066