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REVIEW

Current Status of Allograft Tolerance in Intestinal Transplantation

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Solid organ transplantation has become a clinical practice after the development of different immunosuppressive drugs that allowed controlling rejection. The price to be paid for that is the permanent risk of infections and malignancies and a significant drug-associated toxicity. The establishment of transplant tolerance has been the "holy grail" for transplantation medicine since its beginnings. Different experimental approaches and clinical trials resulted in the accumulation of knowledge on mechanisms and strategies that favor the establishment of tolerance without achieving the objective of autonomous allograft tolerance in the clinical field. Development of tolerance in intestinal transplantation constitutes a challenging situation due to several particular features that contribute to the generation of a strong allogeneic response. In the present review, we summarize the different immune mechanisms that may contribute to allograft tolerance. The different barriers that should be bypassed in intestinal transplantation to tolerate the graft are discussed. Finally, we revise the strategies that were applied with different degrees of success in the clinical field including the most promising recent approaches and the forthcoming candidates in the field that might be translated into clinical trials in the near future.

Keywords: adoptive cellular therapy, chimerism, operational tolerance, rejection, small bowel

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INTRODUCTION

The improvement of surgical techniques, the experience gained in patient management, and the development of new immunosuppressive strategies have supported the refinement of intestinal transplantation (ITx) to become a therapeutic option for patients with permanent intestinal failure [1–3]. Most of the surgical challenges associated with ITx and multivisceral transplantation (MVTx) have been described and standardized, but the procedure remains challenging since immune-related adverse events are frequent. Over the last decades, physicians have successfully reduced the incidence of intestinal graft loss pertaining to acute cellular rejection (ACR) by performing a protocolized endoscopic follow-up of the graft and by using potent immunosuppressive medications, which in average tend to be more aggressive than for other solid organ transplantations (SOTx) [1, 4, 5].

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The intestine harbors the highest load of lymphoid cell populations and lymphoid structures among the different organs that can be transplanted. Besides, the gut lumen is colonized by a complex microbiota containing a high number of immunostimulatory molecules. This high immunogenicity of the intestinal allograft constitutes the main reason for the unique immune challenges observed in the ITx recipients, probably only comparable to lung transplantation. An adequate immune balance would result in reduction of ACR and chronic rejection, the current most frequent indications for intestinal retransplantation [5–7]. Therefore, the ultimate solution might be to induce tolerance to the donor tissue in the recipient. A tolerant intestinal recipient would no longer require administration of immunosuppressive drugs with the consequent benefit of a reduction in the long-term drug-related morbidities (renal toxicity, hypertension, diabetes, among others). However, the possibility of inducing a reproducible state of tolerance remains an infrequent event in clinical transplantation, and even more uncommon to be achieved in ITx [7, 8].

In the present review, we summarize the intestinal immunological features that are critical for the establishment of intestinal graft tolerance, discuss the rationale and the results obtained with tolerogenic strategies used in experimental and clinical studies so far, and we revise promising approaches that may be translated into clinical practice in the future.

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IMMUNE TOLERANCE MECHANISMS AND HOW THEY CONTRIBUTE TO ALLOGRAFT TOLERANCE

The general term immune tolerance defines the state in which the immune system does not respond or fails to react to a foreign or a self-antigen. Immune tolerance includes several forms: gestational tolerance, central tolerance, and peripheral tolerance comprising oral tolerance. In transplantation biology, the term "transplantation tolerance" was proposed more than 50 years ago when Medawar and colleagues provided the basic knowledge for acquired tolerance to alloantigens [9]. Transplantation tolerance is not a physiological mechanism, because immune pathways were not evolutionary designed to confront allograft implantation. To achieve transplantation tolerance, different tolerogenic mechanisms have to be manipulated in order to induce long-lasting acceptance of the allograft.

Gestational Tolerance

The phenomenon of tolerating paternal antigens from fetal tissues during pregnancy, which is referred as gestational or fetomaternal tolerance, includes several mechanisms: (1) separation of blood circulation between fetus and mother; (2) the pregnant uterus serves as an immune privileged site; and (3) fetal factors regulate maternal immune responses [10]. Without going into further details that are beyond the scope of this review, but worthy to mention is the striking evidence that trophoblasts, specialized cells of the placenta, have the capacity to target immune cells by the human leukocyte antigen-G (HLA-G), a non-classical HLA class I molecule, resulting in tolerance induction [11, 12]. Beyond trophoblast cells, diverse other cell types such as endothelial, hematopoietic, and epithelial cells can express membrane bound or soluble HLA-G under different conditions contributing to tolerogenic circuits [13]. Several studies have proven a correlation between increased level of soluble HLA-G in serum or biopsies from heart [14], kidney [15], liver-kidney [16], and liver [17] allografts and low frequency of ACR. Furthermore, a recent study has reported that liver transplant (LTx) recipients with operational tolerance have a higher expression level of HLA-G on monocytoid DC than healthy controls or patients with maintenance

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immunosuppression [18]. Unfortunately, no data are currently available on the role of soluble or cell surface-expressed HLA-G molecule in ITx patients.

Central Tolerance

Central tolerance is the term used for thymocyte selection during T-cell maturation phase in the thymus [19]. Low affinity T-cell receptor (TCR) binding of double-positive thymocytes leads to positive selection by inducing an anti-apoptotic signal mechanism. Self-reactive T cells are depleted during T-cell maturation in the thymus by the mechanism of clonal deletion, also referred to as negative selection, which means apoptotic cell death of T cells bearing TCR with high avidity to self-peptide/major histocompatibility complex (MHC) [19, 20]. The rationale of using central tolerance mechanisms to favor allograft tolerance has been tested in different experimental settings by direct intrathymic inoculation of alloantigen showing improved graft survival [21]. However, intrathymic antigen delivery has not reached routine clinical applications yet. Some strategies applied to the ITx clinical practice may be partially relying on central tolerance generation in favor of establishing allograft tolerance. Starzl and coworkers at Pittsburgh have used a protocol of combining donor bone marrow transfusion (so-called bone marrow augmentation) with ITx or MVTx from the same donor in order to achieve central engraftment of hematopoietic cells [5, 6]. These experiences are commented in more details in subsequent sections.

Peripheral Tolerance

The term peripheral tolerance or postthymic tolerance is used to explain the phenomenon of peripheral abrogation of activation of auto-reactive T cells, which escaped clonal deletion in the thymus [22, 23]. Several mechanisms may contribute to peripheral tolerance, among them: (1) T-cell activation by TCR–MHC interaction with an antigen presenting cell (APC) that is not displaying co-activatory signals leading to T-cell anergy [24]; (2) suppression through specialized regulatory dendritic cells (DC) leading to clonal deletion or generation of regulatory T cells (Treg); or (3) bystander suppression through Treg cells [25]. Furthermore, several cell types and mechanisms may contribute to shape T-cell response to generate Treg cells including, and not restricted to, particular subsets of DC, myeloid-derived suppressor cells, mesenchymal stem cells (MSC), or regulatory B cells [26–30]. Although a detailed discussion of these mechanisms is beyond the scope of the present review, a brief update is included in the following section. Several approaches tested in the clinic to favor graft acceptance have a rationale related to peripheral tolerance, such as the costimulatory blockade and Treg cell therapy. Various therapeutics (recombinant antibodies or proteins) have been developed to target costimulatory interactions between CD28-B7 or CD40-CD154 with promising results in different experimental models [31]. In the ITx setting, the blockade of either the CD28-B7 costimulatory pathway with the fusion protein CTLA4Ig or the CD40-CD154 pathway with the monoclonal antibody to CD154 (MR1) failed to hinder ACR in murine models [32, 33]. Concerning Treg cell therapy, there is evidence that promoting Treg induction through different strategies improves survival of heart [34], liver [35], and kidney allografts [36], whereas no attempts have been made in this direction for intestinal allografts.

Oral Tolerance, a Special Case of Peripheral Tolerance with Potential to Induce Intestinal Allograft Tolerance

Thymus-derived tolerance mechanisms are not suited for harmless foreign antigens from the intestine. Therefore, oral tolerance has evolved as an additional peripheral tolerance with a set of mechanisms for nonresponsiveness against food and commensal antigens administered by the oral route [37, 38]. Different doses of antigen

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administered orally subsequently induce different tolerogenic mechanisms from the induction of Treg cells to anergy and clonal deletion [39–42]. The main players of oral tolerance mechanisms are Treg cells expressing transforming growth factor-beta (TGF-β) and IL-10 typically but not exclusively expressing the transcription factor forkhead box P3 (FoxP3). Treg cells were named differently during their history of discovery such as Th3 [43] or Tr1 [44]. Treg cells are further subdivided into natural FoxP3$^+$ Treg (nTreg) and induced FoxP3$^+$ Treg (iTreg), whereas nTreg cells undergo thymic selection and iTreg are generated from naïve CD4 T cells in the peripheral immune system [45]. Another important regulatory cell type are CD103$^+$ (integrin chain alphaE) dendritic cells (CD103$^+$ DC), which are migratory DC located in the lamina propria and mesenteric lymph nodes (MLN), but are not found in blood stream or other lymphatic organs [46].

Oral tolerance induction with alloantigens of MHC class molecules has been tested as a tolerogenic therapy for SOTx. Womer and colleagues [47] performed a pilot study with four renal allograft patients feeding donor HLA-DR2 peptides and subsequently analyzing *in vitro* T-cell proliferative response. They found that a low dose (0.5 mg/d) administration suppressed alloreactivity, but not higher doses. Although promising, this type of studies has to be extended to a larger group of patients for a longer period of time and to other MHC peptides. It is tempting to consider that the small bowel with its oral tolerance machinery might be highly receptive for such type of treatment, since T cells that are activated in the context of the intestinal mucosa tend to recirculate to mucosal sites [48] generating a circle of activation and relocalization of Treg cells that may be exploited to induce intestinal allograft tolerance. To our knowledge, no animal studies have been conducted so far attempting such a protocol for intestinal allografts, nor any clinical trials in other SOTx.

DEFINING TRANSPLANTATION TOLERANCE IN CLINICAL PRACTICE

The accepted definition for transplantation tolerance in animal models includes three major components: (1) prolonged allograft survival with normal graft function in the absence of all immunosuppressive drugs; (2) evidence of suppressed or absent donor-specific response using *in vitro* assays; (3) appearance of a histologically normal graft at the end of the experiment. How to apply this definition in the clinical practice, where transplantation tolerance is also called operational tolerance, has become challenging because there is no clear consensus on how to define normal or stable graft function. It is well recognized that the clinical variables routinely used for monitoring graft function are not highly sensitive to detect early graft failure. In the case of ITx, there is a lack of biochemical markers to assess graft function. Considering these limitations, to categorize a patient as tolerant in a clinical study only the first component of the definition is usually applied [7].

Spontaneous Graft Acceptance

A small number of transplanted patients, particularly with renal or liver allografts, become spontaneously tolerant after withdrawing of the immunosuppressive regimen. This phenomenon is a rare event, and still unpredictable (between 5% and 20% of liver, and arguably less than 5% of renal allografts become spontaneously tolerant) [7, 8, 49–51]. To the best of our knowledge, no intestinal allograft recipient has been reported as spontaneously tolerant except the case of ITx among identical twins with living donation [52]. Whether this phenomenon is natural and which of the tolerance mechanisms is responsible for obtaining it is not completely understood. Important efforts are being made in order to identify particular features of the group of patients that develop spontaneous tolerance. A European consortium, dedicated to study

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spontaneous operational tolerance in kidney transplant recipients, has identified several features on these patients that may provide a hint on the mechanisms ongoing in spontaneous tolerance. Among these mechanisms, an increase of peripheral B cells with regulatory phenotype [53], upregulation of particular set of genes [54], and regulatory miRNAs [55] in peripheral blood mononuclear cells has been described. A similar approach was developed by several groups on operational tolerant LTx patients, finding as common features an increase of plasmacytoid DC populations in peripheral blood [56], an increase of FoxP3$^+$ T cells with suppressive capacity [57], and an increase of specific subsets of regulatory gamma delta T cells [58]. In spite of some common aspects shared by most of these studies, it is becoming clear that organ-specific tolerogenic circuits may be operating in each case.

BARRIERS TO ACHIEVING TOLERANCE IN INTESTINAL TRANSPLANTATION

Experimental studies proved the difficulties to induce tolerance in mice after performing ITx, whereas mice became tolerant to liver engraftments [59, 60]. It has been demonstrated that a certain hierarchy of immunogenicity exists among the different organs to be transplanted. In coincidence, in the clinical setting, risk of rejection follows this pattern: from lower to higher risk for liver, kidney/heart/pancreas, and lung/intestine. The same order has been observed for the occurrence of acquisition of spontaneous allograft tolerance [7]. We will discuss in this section some of the current obstacles, and how they might be overcome using the actual knowledge of tolerance mechanisms and the available therapeutic options.

Innate Immunity

Innate immunity is the first line of defense against harmful pathogens and an important link for initiating adaptive immunity. The molecular players, pattern recognition receptors capable of interacting directly with pathogen-associated molecular patterns and the cellular players, such as epithelial cells, myeloid cells, and innate lymphocytes, are heavily represented in the intestinal mucosa. Several excellent recent reviews cover studies on the interaction among these players [61–63]. Furthermore, self-derived molecules released upon tissue injury can also activate innate immunity [64], becoming an increasingly considered harmful component in SOTx [10, 65, 66].

Any SOTx is subjected to ischemia–reperfusion injury (IRI) as part of the transplant procedure. The activation of the innate immune response is a component of the tissue damage caused by this process [67]. In the case of ITx, the complexity of the surgical procedure implies ischemia during operation and the intestinal barrier itself is highly labile to IRI [68–70]. This was pointed out in a rodent study comparing ITx and heart Tx with tolerance donor-specific blood transfusion model. The authors "mimicked" ITx features by remote intestinal IRI (clamping of the superior mesenteric artery for 30 min) or by the administration of LPS through the penile vein in the tolerogenic heart Tx model [71]. Their results showed that the beneficial tolerance induction through the blood transfusion in the heart Tx is completely abandoned by the addition of IRI or LPS, two major "danger" signals that induce innate immunity. It has to be kept in mind that the intestinal microbiota itself constitutes a potent inducer of innate activation in the case of barrier damage making the situation of ITx delicate [72]. Consequently, attenuating the effects of IRI and the subsequent activation of the innate response has been considered as a central part of any strategy tending to minimize immunogenicity of intestinal allograft and shifting it to a tolerogenic status. Several strategies have been proposed to prevent or reduce IRI in ITx such as donor immunosuppressive preconditioning [73–75], cytokine administration [76], ischemic preconditioning [77, 78], and

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antioxidant administration [77]. However, none of these strategies has become part of a routine clinical practice yet. It is worth to note that some ITx programs include an intestinal decontamination phase for donor and recipient, in order to minimize translocation of microbial products in the early posttransplant period [72]. However, no comparative analysis has been performed between groups of patients that underwent ITx using the decontamination procedure and those not using it.

T-Cell-Mediated Rejection Process

T-cell-mediated immune response with its main players, $CD4^+$ and $CD8^+$ T cells, leading to alloreactivity and ACR is certainly one of the main barriers before tolerance might be induced. The process is well documented and the main molecular and cellular players have been identified. Newell and colleagues elegantly evaluated the particularity of $CD4^+$ and $CD8^+$ T cells in the ITx setting. By treating mice with depleting antibodies for either CD4 or CD8 T cells before ITx, they showed that both cell types are involved in the ACR process [79]. This is in contrast to studies in other SOTx. For example in a study of heart Tx, depletion of $CD4^+$ T cells was sufficient to abrogate rejection [80]. In a subsequent study, Newell and colleagues used knockout mice, either for CD4 or for CD8, to test the role of these cells in the ACR process [81]. Surprisingly, the CD4 and CD8 knockout models did not abrogate the ACR process in contrast to the specific-monoclonal antibodies depleting experiments. The authors argue that probably the depleting monoclonal antibodies mediate their protective effect on allograft rejection through a different mechanism such as the induction of a regulatory cell population. However, these experiments emphasize an important particularity of the ITx compared to other SOTx.

Secondary Lymphoid Structures

The intestinal allograft carries secondary lymphoid structures such as MLN, Peyer's patches (PP), and isolated lymphoid follicles (ILF), and it is the allograft among SOTx with the highest load of lymphoid tissue [82, 83]. Newell's group has shown that donor lymphoid structures participate in the generation of allograft rejection mechanisms [84]. By using splenectomized lymphotoxin alpha deficient mice, which have no MLN, PP, and peripheral LN, they showed that donor organs contribute to this process. Furthermore, extensive proliferation and increased frequency of interferon-gamma (IFN-γ)-producing alloreactive recipient T cells occurred primarily in the allograft MLN relative to the recipient LN and spleen, concluding that the allograft secondary lymphoid organs play a primary role in initiating recipient anti-donor immune response.

On the other hand, allograft lymphoid organs are fundamental for various processes that may contribute to graft homeostasis such as production of IgA against commensals and mounting an adaptive immune response against invasive pathogens entering the gut lumen [85, 86]. IgA synthesis contributes to overall intestinal immune homeostasis, which should be sustained to avoid local inflammatory reactions [87, 88] that may lead to ACR. In the context of tolerance, as it was shown in the oral tolerance section, MLN are the essential site for tolerance induction. Until now, no strategies were tested in order to manipulate this capacity to favor induction of Treg against alloreactive harmful T cells.

Another aspect to be considered after the ITx operation is the fact that efferent lymphatic vessels coming from the MLN are not reconstructed. Whether this mechanical disruption has an impact on leukocyte trafficking and subsequently influencing alloimmune reactivity or suppressor function has not been definitively clarified. It is thought that lymphatics are regenerated naturally by lymphangiogenesis after the first few weeks posttransplant as it was shown in animal models [89–91]. However, Kellersman and colleagues demonstrated in a rat model that immediate reconstruction of

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the intestinal lymphatic vessels improves the survival rates of ITx transplanted animals [92]. During the first two to three postoperative weeks, lymph containing immune cells from the mesentery is routinely collected by a drainage system in human transplant. We analyzed cellular content and found that the turnover from donor CD8 T cells to recipient T cells is completed during the second week posttransplant [93]. This analysis of gut draining cells might be interesting for studies of cellular subtypes with memory or regulatory functions and might provide a hint for future outcome of allograft survival.

Alloreactive Memory T Cells

The capacity of the adaptive immunity to form memory T and B cells is a benefit against invading pathogens and also a main focus to achieve in vaccinology, whereas memory cells are a major hurdle for tolerance induction in transplant biology [94, 95]. As part of its highly immunogenic properties, the intestinal allograft has a high number of memory cells situated in the lamina propria.

Memory cells and SOTx is a complex field with many faces. Here, we will discuss shortly some major aspects on memory T-cell biology, such as costimulatory blockade avoidance, lymphopenia-induced proliferation, cross-reactivity, and pretransplant alloreactive memory cells, which are certainly all influencing survival rate of allografts. Memory T cells do not need costimulatory signals for activation, which means that immunosuppressive strategies focused in blocking costimulatory signals and subsequently induce tolerance through the mechanism of anergy are not effective on memory T cells [95].

Lymphopenia-induced expansion of the memory T-cell pool may arise upon different immunosuppressive treatment such as sirolimus, T-cell depleting antibodies, and costimulation blockade [96, 97]. This is due to the fact that memory T cells expand quicker than naïve T cells, when the pool of T cells have to be refilled [98].

Cross-reactivity is a further threat to allografts. This was elegantly evaluated in two different murine infection models one with *Leishmania major* [99] and the other with lymphocytic choriomeningitis virus [100] both combined with skin grafts. Although experimentally or clinically not yet evaluated in ITx, the high incidence of environmental exposure makes the intestinal allograft susceptible for cross-reactivity. Even more, each individual has a different and heterologous set of memory T cells due to its infectious history [101]. This may partly explain the higher allograft acceptance rate in children compared to adults. Furthermore, the memory T-cell load is more heterologous in humans compared to mouse models, which are exposed to a germ-free environment making the evaluation of experimental protocols for clinical settings difficult.

The presence of alloreactive memory T cells before transplantation, due to retransplantation, blood transfusion, or pregnancies, has been evaluated in various human studies and it became clear that their presence causes higher risk for ACR [102, 103]. Unfortunately, the field of alloreactive memory cells was not a focus of the ITx community so far. Therefore, studies of the impact of alloreactive memory T cells are certainly missing in the ITx field.

OVERCOMING BARRIERS TO OBTAINING TOLERANCE IN INTESTINAL TRANSPLANTATION

So far, we have shown the various tolerance mechanisms and the particular barriers that hinder tolerance induction in ITx. In this section, we discuss the different clinical protocols attempting to overcome these barriers and finding a way to induce tolerance in ITx patients. We discuss the first attempts from the Pittsburgh group, then the different protocol of cellular immunotherapy, and finally the latest attempt, a promising

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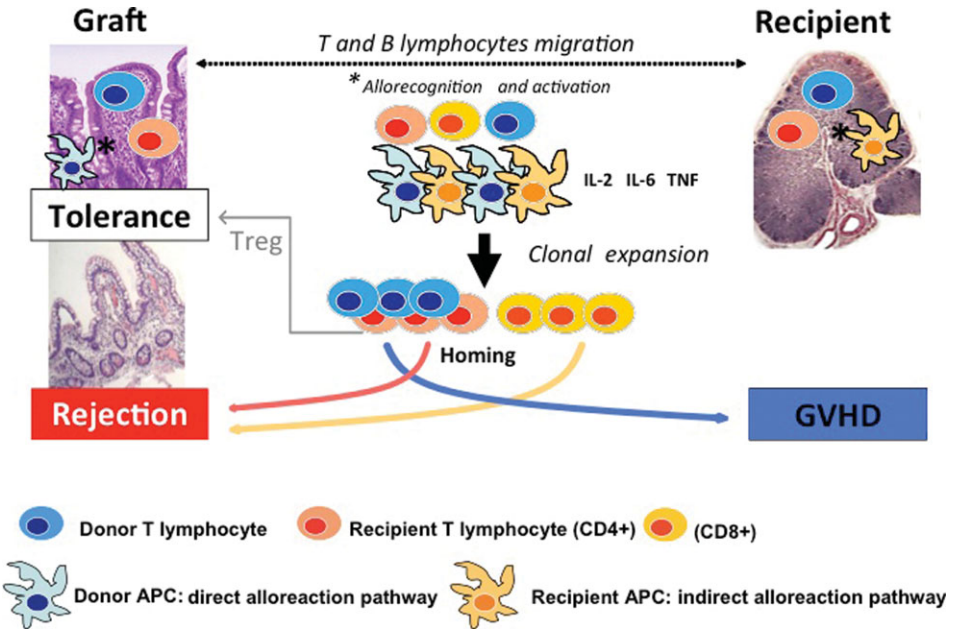

FIGURE 1. Immunological basis of intestinal allorecognition, rejection, graft versus host disease, and tolerance. Multiple immune interactions either in donor or recipient tissues result in triggering rejection in the graft tissue if not properly controlled. APC, antigen presenting cells; GVHD, graft versus host disease; IL, interleukin; TNF, tumor necrosis factor.

multifactorial immunomodulatory approach from the Leuven group headed by Prof. Pirenne.

Strategies on the Road Toward Induced Tolerance in Intestinal Transplantation

In contrast with other organs, the profound bidirectional immune interaction between the intestinal allograft and the host immune system have challenged the understanding and practical achievement of intestinal graft tolerance (Figure 1) [5, 72, 104]. Based on the evolving comprehension of different mechanisms that might promote and maintain tolerance, numerous protolerogenic regimens have been proposed. The translation from research to clinical use has not been performed as successful as expected, but a number of approaches have become part of the current clinical regimes.

Among different strategies to induce transplant tolerance, the concept of reducing the T-cell load in the recipient was first established by the Pittsburgh group in 1995 [5]. Cyclophosphamide was the first drug introduced for induction immunosuppression as lymphocyte depleting therapy in ITx, which was later replaced by anti-IL-2R antibodies (Ab) (daclixumab and basiliximab) [105], then by anti-thymocyte globulin (ATG), and by the anti-CD52 Ab (alemtuzumab) [5]. The introduction of a T-cell depleting protocol led to a significant (10%–20%) reduction of the early posttransplant rejection, whereas only a marginal improvement for the long-term and no operational tolerance were achieved [5]. In addition to the initial protocol, a single infusion of unmodified donor bone marrow cells and *ex vivo* graft irradiation was added in order to favor the development of tolerance through central and peripheral mechanisms. The rationale of this strategy is commented in the next section.

Another strategy for achieving tolerance was the development of interventional strategies to lower the antigenicity and immunoreactive cell populations in the graft. In the year 2000, the Pittsburgh group included in their protocol a 7.5 Gy *ex vivo* irradiation of the graft to deplete proliferating activated T cells. However, the efficacy of this

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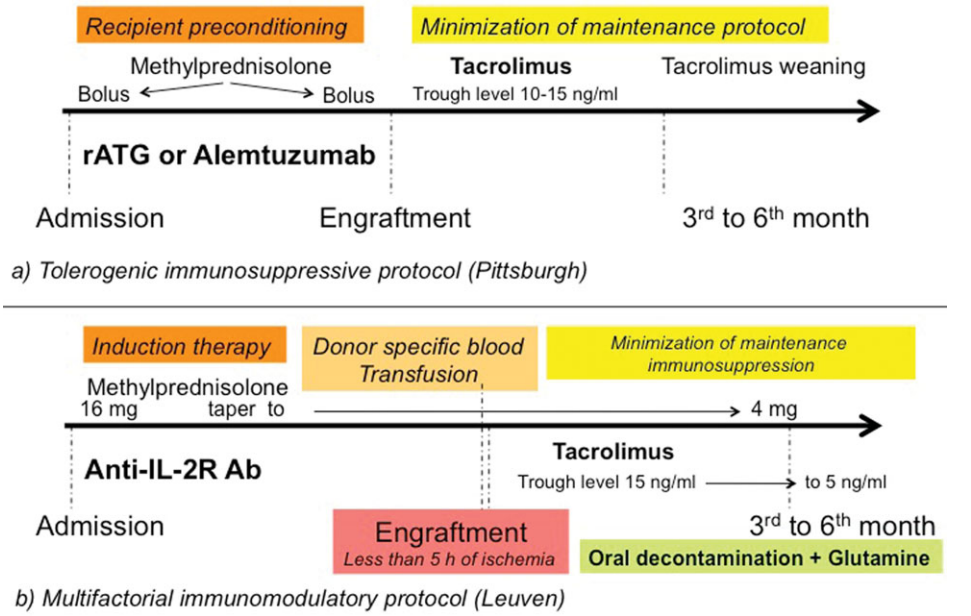

FIGURE 2. Comparison between the tolerogenic immunosuppressive protocol proposed by the Pittsburgh group and the multifactorial immunomodulatory protocol introduced by the Leuven group.

procedure was not satisfactory and it was abandoned [5]. With similar rationale, different programs started to treat the donor with T-cell depleting drugs (thymoglobulin, OKT3 or combined therapy) before the organ retrieval [103]. The main aim of this intervention was to reduce graft versus host disease (GVHD). The extra benefit of donor pretreatment to deplete T cells is the decrease of IRI as shown in a rat model for kidney Tx [75].

In 2001, the Pittsburgh group and others started to use the tolerogenic immunosuppressive protocol based on two major principles: (1) recipient preconditioning with a single high dose of an anti-lymphocyte treatment (rATG or alemtuzumab) in combination with minimization of posttransplant maintenance immunosuppression; and (2) elimination of corticosteroids that were part of the maintenance immunosuppression (Figure 2a). High doses of cortocosteroids and calcineurin inhibitors can negatively influence the development of Treg cells affecting the balance toward tolerance [106, 107]. In spite of improvement of patients and graft survival with these protocols, there is still lack of evidence of full clinical allograft tolerance.

Cellular Immunotherapy: Infusion of Donor Cells, Concepts and Clinical Applications

The infusion of donor hematopoietic cells at the time of transplantation has been used as a tolerogenic approach in clinical transplantation based on evidence from animal models [108, 109]. The evolution of this finding took different approaches; one was based on using myeloablative therapy followed by BMTx and SOTx, whereas other groups have used nonmyeloablative donor bone marrow cells infusion so called bone marrow augmentation.

In different clinical SOTx, the Pittsburgh group used the strategy of bone marrow augmentation with partial success depending on the type of graft [6]. The persistence of donor-derived hematopoietic cells has been documented in stable long-term transplant recipients, and this "micro-chimerism" was considered necessary to achieve inducible graft tolerance presumably by central and peripheral tolerance mechanisms

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[6]. In ITx, this protocol was tested on 37 transplants performed with a single infusion of 3 to 5 $\times$ 10^8 unmodified donor bone marrow cells at the time of the engraftment. In 19 transplants, a low dose of *ex vivo* irradiation of the graft was added to the protocol [5]. The protocol of bone marrow augmentation was abandoned due to long-term disappointing outcomes [110], and protocols were adapted to the "tolerogenic immunosuppression protocol" described before.

So far, there have been some successful phase II clinical trials that have achieved operational tolerance in kidney transplant patients using living donors and a modified BMTx procedure at the time of SOTx [111–113]. These encouraging results warrant the expansion of these strategies to other SOTx.

Multifactorial Immunomodulatory Approach: The Closest Innovation to Intestinal Transplant Operational Tolerance

In 2006, Pirenne and Kawai [114] published the first series of four patients under a novel immunomodulatory protocol (Figure 2b) using: (1) donor-specific blood transfusion (300–600 mL of whole blood collected at the time of the procurement given to the recipient during the transplant through the portal vein) [115, 116]; (2) a standard dose of induction therapy with anti-IL-2R antibodies (basiliximab 20 mg on day 1 and 5 postoperation); (3) maintenance immunosuppression avoiding high steroid doses (16 mg of glucocorticoid was given in the first doses and reduced to 4 mg over 3–6 months) and low doses of calcineurin inhibitor (initial attended target levels of tacrolimus 15 ng/ml tapered to 10 ng/ml and to 5 ng/ml over the same 3–6 months period); and (4) measures to limit the inflammatory reaction within the intestinal graft (ideal donors were selected, ischemia time was minimized to less than 5 h, small bowel decontamination was started in the donor and given for 3 months, and glutamine was administered parenterally and enterally posttransplantation). Following this protocol, no single rejection episode was documented in 250 biopsies, no GVHD was diagnosed. The presence of chimerism was monitored once a week during the first month and once a month thereafter using HLA serotyping of peripheral blood and genetic techniques finding only one recipient presented transient chimerism.

The preliminary results of this multifactorial approach were confirmed in a follow-up report published in 2009 [72] including 7 consecutive patients (3 isolated –one with kidney, and 4 combined liver-intestine). With a follow-up period between 8 months and 8 years, only one over seven patients developed a single episode of ACR 4 months after transplant and died due to a fungal infection. Some of the immune mechanisms that may contribute to this positive outcome are higher number of $CD4^+$ $CD25^{high}$ T cells in peripheral blood, and $FoxP3^+$ T cells detected by RT-PCR and immunostaining in the intestinal mucosa [72]. Those preliminary findings are consistent with the ones observed for spontaneous tolerance in kidney [117] and LTx [118].

Although true tolerance has not been achieved yet in ITx, and in spite of the reduced number of cases, this new strategy presents the best long-term results in terms of rejection-free and patient survival reported in the field being close to the dreamed "operational tolerance." The prospective application of the described protocol is needed in other centers around the world in order to validate the reported findings facilitating graft acceptance and reducing the doses for long-term immunosuppression and the expected side effects.

FUTURE PERSPECTIVES

There are several strategies aiming to generate allograft tolerance and immunomodulation, currently under development for other SOTx or for immune- mediated pathologies that appear promising and that could possibly constitute a new step toward the

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long sought tolerance in ITx. Among them, there are several approaches based on adoptive cellular therapies oriented to generate tolerance by promoting the differentiation to Tregs, mainly by peripheral tolerance mechanisms. The infusion of tolerogenic DC has been successfully proven in experimental models and there are currently clinical trials under way for kidney transplant patients that have shown promising results so far [119]. Furthermore, there are several studies that have shown that the infusion of MSC promotes generation of Treg-based tolerance with positive results in experimental models. This strategy has turned out to be successful in ongoing clinical trials for kidney transplantation [120].

Among other strategies aimed to skew the immune response in favor to the graft, some molecules derived from parasites with potent immunomodulatory properties appear as attractive candidates to be used in SOTx. Sj16, a peptide derived from *Schistosoma japonicum* was shown to have a potent capacity to induce $CD4^+$ $CD25^+$ Treg cells *in vitro* and *in vivo* via IFN-γ and IL-10 [121], is an interesting candidate to be added to the immunomodulatory armamentarium used in the SOTx field.

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CONCLUSION

Over the last decades, tolerance has evolved from a laboratory research topic to a possible available therapeutic approach. Up to now, more knowledge than favorable clinical results has been obtained. However, operational tolerance has been successfully achieved and promoted in kidney and liver. The complexity of the small bowel allograft makes it difficult to rationalize strategies toward this end. Along the history, problems associated to the relatively low number of transplanted patients, the difficulties to perform prospective randomized trials, and the inherent risk of weaning immunosuppression in a highly reactive graft have impaired the progression to reach clinical intestinal transplant tolerance. Nevertheless, the multifactorial treatment approach seems to have overcome some of the hurdles to skew immune homeostasis to the regulatory side. It should be kept in mind that each transplanted solid organ has its particularity that has to be wisely considered and evaluated in the construction of future strategies for graft acceptance.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Fishbein TM. Intestinal transplantation. N Engl J Med 2009;361(10):998–1008.
[2] Selvaggi G, Tzakis AG. Small bowel transplantation: technical advances/updates. Curr Opin Organ Transplant 2009;14(3):262–266.
[3] Ueno T, Fukuzawa M. Current status of intestinal transplantation. Surg Today 2010;40(12): 1112–1122.
[4] Fishbein TM, Gondolesi GE, Kaufman SS. Intestinal transplantation for gut failure. Gastroenterology 2003;124(6):1615–1628.

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[5] Abu-Elmagd KM, Costa G, Bond GJ, et al. Evolution of the immunosuppressive strategies for the intestinal and multivisceral recipients with special reference to allograft immunity and achievement of partial tolerance. Transpl Int 2009;22(1):96–109.
[6] Starzl TE, Murase N, Abu-Elmagd K, et al. Tolerogenic immunosuppression for organ transplantation. Lancet 2003;361(9368):1502–1510.
[7] Newell KA. Clinical transplantation tolerance. Semin Immunopathol 2011;33(2):91–104.
[8] Owens ML, Maxwell JG, Goodnight J, Wolcott MW. Discontinuance of immunosuprression in renal transplant patients. Arch Surg 1975;110(12):1450–1451.
[9] Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature 1953;172(4379):603–606.
[10] Erlebacher A. Mechanisms of T cell tolerance towards the allogeneic fetus. Nat Rev Immunol 2013;13(1):23–33.
[11] Hunt JS, Petroff MG, McIntire RH, Ober C. HLA-G and immune tolerance in pregnancy. FASEB J 2005;19(7):681–693.
[12] Carosella ED, Moreau P, Lemaoult J, Rouas-Freiss N. HLA-G: from biology to clinical benefits. Trends Immunol 2008;29(3):125–132.
[13] Carosella ED. The tolerogenic molecule HLA-G. Immunol Lett 2011;138(1):22–24.
[14] Lila N, Amrein C, Guillemain R, et al. Human leukocyte antigen-G expression after heart transplantation is associated with a reduced incidence of rejection. Circulation 2002;105(16):1949–1954.
[15] Crispim JC, Duarte RA, Soares CP, et al. Human leukocyte antigen-G expression after kidney transplantation is associated with a reduced incidence of rejection. Transpl Immunol 2008;18(4):361–367.
[16] Creput C, Le Friec G, Bahri R, et al. Detection of HLA-G in serum and graft biopsy associated with fewer acute rejections following combined liver-kidney transplantation: possible implications for monitoring patients. Hum Immunol 2003;64(11):1033–1038.
[17] Zarkhin V, Talisetti A, Li L, et al. Expression of soluble HLA-G identifies favorable outcomes in liver transplant recipients. Transplantation 2010;90(9):1000–1005.
[18] Castellaneta A, Mazariegos GV, Nayyar N, et al. HLA-G level on monocytoid dendritic cells correlates with regulatory T-cell Foxp3 expression in liver transplant tolerance. Transplantation 2011;91(10):1132–1140.
[19] Griesemer AD, Sorenson EC, Hardy MA. The role of the thymus in tolerance. Transplantation 2010;90(5):465–474.
[20] Ramsdell F, Fowlkes BJ. Clonal deletion versus clonal anergy: the role of the thymus in inducing self tolerance. Science 1990;248(4961):1342–1348.
[21] Naji A. Induction of tolerance by intrathymic inoculation of alloantigen. Curr Opin Immunol 1996;8(5):704–709.
[22] Miller JF, Morahan G. Peripheral T cell tolerance. Annu Rev Immunol 1992;10:51–69.
[23] Xing Y, Hogquist KA. T-cell tolerance: central and peripheral. Cold Spring Harb Perspect Biol 2012;4(6):a006957.
[24] Chappert P, Schwartz RH. Induction of T cell anergy: integration of environmental cues and infectious tolerance. Curr Opin Immunol 2010;22(5):552–559.
[25] Bilate AM, Lafaille JJ. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. Annu Rev Immunol 2012;30:733–758.
[26] Ezzelarab M, Thomson AW. Tolerogenic dendritic cells and their role in transplantation. Semin Immunol 2011;23(4):252–263.
[27] Sordi V, Piemonti L. Therapeutic plasticity of stem cells and allograft tolerance. Cytotherapy 2011;13(6):647–660.
[28] Shalev I, Selzner N, Shyu W, et al. Role of regulatory T cells in the promotion of transplant tolerance. Liver Transpl 2012;18(7):761–770.
[29] Wood KJ, Bushell A, Hester J. Regulatory immune cells in transplantation. Nat Rev Immunol 2012;12(6):417–430.
[30] Coelho V, Saitovitch D, Kalil J, Silva HM. Rethinking the multiple roles of B cells in organ transplantation. Curr Opin Organ Transplant 2013;18(1):13–21.
[31] Priyadharshini B, Greiner DL, Brehm MA. T-cell activation and transplantation tolerance. Transplant Rev (Orlando) 2012;26(3):212–222.
[32] Newell KA, He G, Guo Z, et al. Cutting edge: blockade of the CD28/B7 costimulatory pathway inhibits intestinal allograft rejection mediated by CD4+ but not CD8+ T cells. J Immunol 1999;163(5):2358–2362.
[33] Meng L, Guo Z, Kim O, et al. Blockade of the CD40 pathway fails to prevent CD8 T cell-mediated intestinal allograft rejection. Transplant Proc 2001;33(1–2):418–420.
[34] Wolf D, Schreiber TH, Tryphonopoulos P, et al. Tregs expanded *in vivo* by TNFRSF25 agonists promote cardiac allograft survival. Transplantation 2012;94(6):569–574.

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[35] Abe Y, Urakami H, Ostanin D, et al. Induction of Foxp3-expressing regulatory T-cells by donor blood transfusion is required for tolerance to rat liver allografts. PLoS One 2009;4(11):e7840.
[36] Bestard O, Cassis L, Cruzado JM, et al. Costimulatory blockade with mTor inhibition abrogates effector T-cell responses allowing regulatory T-cell survival in renal transplantation. Transpl Int 2011;24(5):451–460.
[37] Weiner HL, da Cunha AP, Quintana F, Wu H. Oral tolerance. Immunol Rev 2011;241(1):241–259.
[38] Pabst O, Mowat AM. Oral tolerance to food protein. Mucosal Immunol 2012;5(3):232–239.
[39] Friedman A, Weiner HL. Induction of anergy or active suppression following oral tolerance is determined by antigen dosage. Proc Natl Acad Sci USA 1994;91(14):6688–6692.
[40] Chen Y, Inobe J, Marks R, et al. Peripheral deletion of antigen-reactive T cells in oral tolerance. Nature 1995;376(6536):177–180.
[41] Chen Y, Inobe J, Kuchroo VK, et al. Oral tolerance in myelin basic protein T-cell receptor transgenic mice: suppression of autoimmune encephalomyelitis and dose-dependent induction of regulatory cells. Proc Natl Acad Sci USA 1996;93(1):388–391.
[42] Siewert C, Lauer U, Cording S, et al. Experience-driven development: effector/memory-like alphaE+Foxp3+ regulatory T cells originate from both naive T cells and naturally occurring naive-like regulatory T cells. J Immunol 2008;180(1):146–155.
[43] Chen Y, Kuchroo VK, Inobe J, et al. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science 1994;265(5176):1237–1240.
[44] Groux H, O'Garra A, Bigler M, et al. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997;389(6652):737–742.
[45] Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity 2009;30(5):626–635.
[46] Milling S, Yrlid U, Cerovic V, MacPherson G. Subsets of migrating intestinal dendritic cells. Immunol Rev 2010;234(1):259–267.
[47] Womer KL, Magee CC, Najafian N, et al. A pilot study on the immunological effects of oral administration of donor major histocompatibility complex class II peptides in renal transplant recipients. Clin Transplant 2008;22(6):754–759.
[48] Mowat AM. Anatomical basis of tolerance and immunity to intestinal antigens. Nat Rev Immunol 2003;3(4):331–341.
[49] Zoller KM, Cho SI, Cohen JJ, Harrington JT. Cessation of immunosuppressive therapy after successful transplantation: a national survey. Kidney Int 1980;18(1):110–114.
[50] Lerut J, Sanchez-Fueyo A. An appraisal of tolerance in liver transplantation. Am J Transplant 2006;6(8):1774–1780.
[51] Ashton-Chess J, Giral M, Brouard S, et al. Spontaneous operational tolerance after immunosuppressive drug withdrawal in clinical renal allotransplantation. Transplantation 2007;84(10):1215–1219.
[52] Berney T, Genton L, Buhler LH, et al. Five-year follow-up after pediatric living related small bowel transplantation between two monozygotic twins. Transplant Proc 2004;36(2):316–318.
[53] Pallier A, Hillion S, Danger R, et al. Patients with drug-free long-term graft function display increased numbers of peripheral B cells with a memory and inhibitory phenotype. Kidney Int 2010;78(5):503–513.
[54] Braza F, Soulillou JP, Brouard S. Gene expression signature in transplantation tolerance. Clin Chim Acta 2012;413(17–18):1414–1418.
[55] Danger R, Pallier A, Giral M, et al. Upregulation of miR-142–3p in peripheral blood mononuclear cells of operationally tolerant patients with a renal transplant. J Am Soc Nephrol 2012;23(4):597–606.
[56] Mazariegos GV, Zahorchak AF, Reyes J, et al. Dendritic cell subset ratio in peripheral blood correlates with successful withdrawal of immunosuppression in liver transplant patients. Am J Transplant 2003;3(6):689–696.
[57] Pons JA, Revilla-Nuin B, Baroja-Mazo A, et al. FoxP3 in peripheral blood is associated with operational tolerance in liver transplant patients during immunosuppression withdrawal. Transplantation. 2008;86(10):1370–1378.
[58] Martinez-Llordella M, Puig-Pey I, Orlando G, et al. Multiparameter immune profiling of operational tolerance in liver transplantation. Am J Transplant 2007;7(2):309–319.
[59] Zhang Z, Zhu L, Quan D, et al. Pattern of liver, kidney, heart, and intestine allograft rejection in different mouse strain combinations. Transplantation 1996;62(9):1267–12672.
[60] Murase N, Ye Q, Nalesnik MA, et al. Immunomodulation for intestinal transplantation by allograft irradiation, adjunct donor bone marrow infusion, or both. Transplantation 2000;70(11):1632–1641.
[61] Medzhitov R. Origin and physiological roles of inflammation. Nature 2008;454(7203):428–435.
[62] Schenten D, Medzhitov R. The control of adaptive immune responses by the innate immune system. Adv Immunol 2011;109:87–124.
[63] Sonnenberg GF, Artis D. Innate lymphoid cell interactions with microbiota: implications for intestinal health and disease. Immunity 2012;37(4):601–610.

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[64] Hirsiger S, Simmen HP, Werner CM, et al. Danger signals activating the immune response after trauma. Mediators Inflamm 2012;2012:315941.
[65] Liu W, Li XC. An overview on non-T cell pathways in transplant rejection and tolerance. Curr Opin Organ Transplant 2010;15(4):422–426.
[66] Murphy SP, Porrett PM, Turka LA. Innate immunity in transplant tolerance and rejection. Immunol Rev 2011;241(1):39–48.
[67] Eltzschig HK, Eckle T. Ischemia and reperfusion—from mechanism to translation. Nat Med 2011;17(11):1391–1401.
[68] Mallick IH, Yang W, Winslet MC, Seifalian AM. Ischemia–reperfusion injury of the intestine and protective strategies against injury. Dig Dis Sci 2004;49(9):1359–1377.
[69] Lausada N, Stringa P, Cabanne A, et al. [Impact of ischemia–reperfusion injury on long survival rate in intestinal transplantation in rats]. Acta Gastroenterol Latinoam 2011;41(2):129–136.
[70] Stringa P, Lausada N, Romanin D, et al. Defining the nonreturn time for intestinal ischemia reperfusion injury in mice. Transplant Proc 2012;44(5):1214–1217.
[71] Kawai M, Kitade H, Koshiba T, et al. Intestinal ischemia reperfusion and lipopolysaccharide transform a tolerogenic signal into a sensitizing signal and trigger rejection. Transplantation 2009;87(10):1464–1467.
[72] Pirenne J, Kawai M. Intestinal transplantation: evolution in immunosuppression protocols. Curr Opin Organ Transplant 2009;14(3):250–255.
[73] Watson MJ, Ke B, Shen XD, et al. Treatment with antithymocyte globulin ameliorates intestinal ischemia and reperfusion injury in mice. Surgery 2012;152(5):843–850.
[74] Oltean M, Pullerits R, Zhu C, et al. Donor pretreatment with FK506 reduces reperfusion injury and accelerates intestinal graft recovery in rats. Surgery 2007;141(5):667–677.
[75] Cicora F, Roberti J, Vasquez D, et al. Preconditioning donor with a combination of tacrolimus and rapamacyn to decrease ischaemia–reperfusion injury in a rat syngenic kidney transplantation model. Clin Exp Immunol 2012;167(1):169–177.
[76] Farmer DG, Ke B, Shen XD, et al. Interleukin-13 protects mouse intestine from ischemia and reperfusion injury through regulation of innate and adaptive immunity. Transplantation 2011;91(7):737–743.
[77] Saeki I, Matsuura T, Hayashida M, Taguchi T. Ischemic preconditioning and remote ischemic preconditioning have protective effect against cold ischemia–reperfusion injury of rat small intestine. Pediatr Surg Int 2011;27(8):857–862.
[78] Stringa P, Romanin D, Lausada N, et al. Ischemic preconditioning and tacrolimus pre-treatment as strategies to attenuate intestinal ischemia–reperfusion injury in mice. Transplant Proc in press.
[79] Newell KA, He G, Hart J, Thistlethwaite JR, Jr. Treatment with either anti-CD4 or anti-CD8 monoclonal antibodies blocks alphabeta T cell-mediated rejection of intestinal allografts in mice. Transplantation 1997;64(7):959–965.
[80] Bishop DK, Chan S, Li W, et al. CD4-positive helper T lymphocytes mediate mouse cardiac allograft rejection independent of donor alloantigen specific cytotoxic T lymphocytes. Transplantation 1993;56(4):892–897.
[81] He G, Hart J, Kim OS, et al. The role of CD8 and CD4 T cells in intestinal allograft rejection: a comparison of monoclonal antibody-treated and knockout mice. Transplantation 1999;67(1):131–137.
[82] Brandtzaeg P, Pabst R. Let's go mucosal: communication on slippery ground. Trends Immunol 2004;25(11):570–577.
[83] Brandtzaeg P, Kiyono H, Pabst R, Russell MW. Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol 2008;1(1):31–37.
[84] Wang J, Dong Y, Sun JZ, et al. Donor lymphoid organs are a major site of alloreactive T-cell priming following intestinal transplantation. Am J Transplant 2006;6(11):2563–2571.
[85] Cerutti A, Rescigno M. The biology of intestinal immunoglobulin A responses. Immunity 2008;28(6):740–750.
[86] Newberry RD. Intestinal lymphoid tissues: is variety an asset or a liability? Curr Opin Gastroenterol 2008;24(2):121–128.
[87] Macdonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. Science. 2005;307(5717):1920–1925.
[88] MacDonald TT, Monteleone I, Fantini MC, Monteleone G. Regulation of homeostasis and inflammation in the intestine. Gastroenterology 2011;140(6):1768–1775.
[89] Schmid T, Koeroezsi G, Oberhuber G, et al. Lymphatic regeneration after small bowel transplantation. Transplant Proc 1990;22(4):2060–2061.
[90] Schier F, Uner A, Waldschmidt J. Microlymphography of spontaneous lymph vessel anastomosis in small bowel transplantation in the rat. J Pediatr Surg 1991;26(10):1239–1242.

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[91] Uner A, Weinberg AM, Nautrup CP, et al. Spontaneous reanastomosis between lymphatic vessels following syngeneic transplantation of the small intestine in the rat. Surg Radiol Anat 2001;23(6):383–387.
[92] Kellersman R, Zhong R, Kiyochi H, et al. Reconstruction of the intestinal lymphatic drainage after small bowel transplantation. Transplantation 2000;69(1):10–16.
[93] Meier D, Cagnola H, Ramisch D, et al. Analysis of immune cells draining from the abdominal cavity as a novel tool to study intestinal transplant immunobiology. Clin Exp Immunol 2010;162(1):138–145.
[94] Bingaman AW, Farber DL. Memory T cells in transplantation: generation, function, and potential role in rejection. Am J Transplant 2004;4(6):846–852.
[95] Jones ND. Memory T cells: how might they disrupt the induction of tolerance? Transplantation 2009;87(9 Suppl):S74–S77.
[96] Hale DA. Biological effects of induction immunosuppression. Curr Opin Immunol 2004;16(5):565–570.
[97] Tchao NK, Turka LA. Lymphodepletion and homeostatic proliferation: implications for transplantation. Am J Transplant 2012;12(5):1079–1090.
[98] Neujahr DC, Chen C, Huang X, et al. Accelerated memory cell homeostasis during T cell depletion and approaches to overcome it. J Immunol 2006;176(8):4632–4639.
[99] Pantenburg B, Heinzel F, Das L, et al. T cells primed by Leishmania major infection cross-react with alloantigens and alter the course of allograft rejection. J Immunol 2002;169(7):3686–3693.
[100] Brehm MA, Markees TG, Daniels KA, et al. Direct visualization of cross-reactive effector and memory allo-specific CD8 T cells generated in response to viral infections. J Immunol 2003;170(8):4077–4086.
[101] Welsh RM, Selin LK. No one is naive: the significance of heterologous T-cell immunity. Nat Rev Immunol 2002;2(6):417–426.
[102] Heeger PS, Greenspan NS, Kuhlenschmidt S, et al. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. J Immunol 1999;163(4):2267–2275.
[103] Gondolesi G, Fauda M. Technical refinements in small bowel transplantation. Curr Opin Organ Transplant 2008;13(3):259–265.
[104] Pirenne J, Kawai M. Tolerogenic protocols for intestinal transplantation. Transpl Immunol 2004;13(2):131–137.
[105] Sudan DL, Chinnakotla S, Horslen S, et al. Basiliximab decreases the incidence of acute rejection after intestinal transplantation. Transplant Proc 2002;34(3):940–941.
[106] Koshiba T, Kitade H, Waer M, et al. Break of tolerance via donor-specific blood transfusion by high doses of steroids: a differential effect after intestinal transplantation and heart transplantation. Transplant Proc 2003;35(8):3153–3155.
[107] Kawai M, Kitade H, Mathieu C, et al. Inhibitory and stimulatory effects of cyclosporine A on the development of regulatory T cells *in vivo*. Transplantation 2005;79(9):1073–1077.
[108] Starzl TE, Zinkernagel RM. Transplantation tolerance from a historical perspective. Nat Rev Immunol 2001;1(3):233–239.
[109] Starzl TE. Chimerism and tolerance in transplantation. Proc Natl Acad Sci USA 2004;101(Suppl 2):14607–14614.
[110] Starzl TE. Immunosuppressive therapy and tolerance of organ allografts. N Engl J Med. 2008;358(4):407–411.
[111] Leventhal J, Abecassis M, Miller J, et al. Chimerism and tolerance without GVHD or engraftment syndrome in HLA-mismatched combined kidney and hematopoietic stem cell transplantation. Sci Transl Med 2012;4(124):124ra28.
[112] Leventhal J, Abecassis M, Miller J, et al. Tolerance induction in HLA disparate living donor kidney transplantation by donor stem cell infusion: durable chimerism predicts outcome. Transplantation 2013;95(1):169–176.
[113] Scandling JD, Busque S, Dejbakhsh-Jones S, et al. Tolerance and withdrawal of immunosuppressive drugs in patients given kidney and hematopoietic cell transplants. Am J Transplant 2012;12(5):1133–1145.
[114] Pirenne J, Kawai M. Tolerogenic protocol for intestinal transplantation. Transplant Proc 2006;38(6):1664–1667.
[115] Opelz G, Vanrenterghem Y, Kirste G, et al. Prospective evaluation of pretransplant blood transfusions in cadaver kidney recipients. Transplantation 1997;63(7):964–967.
[116] Gruessner RW, Nakhleh RE, Harmon JV, et al. Donor-specific portal blood transfusion in intestinal transplantation: a prospective, preclinical large animal study. Transplantation 1998;66(2):164–169.
[117] Dummer CD, Carpio VN, Goncalves LF, et al. FOXP3+ regulatory T cells: from suppression of rejection to induction of renal allograft tolerance. Transpl Immunol 2012;26(1):1–10.

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[118] Louis S, Braudeau C, Giral M, et al. Contrasting CD25hiCD4+T cells/FOXP3 patterns in chronic rejection and operational drug-free tolerance. Transplantation 2006;81(3):398–407.
[119] Moreau A, Varey E, Bouchet-Delbos L, Cuturi MC. Cell therapy using tolerogenic dendritic cells in transplantation. Transplant Res 2012;1(1):13.
[120] Casiraghi F, Perico N, Remuzzi G. Mesenchymal stromal cells to promote solid organ transplantation tolerance. Curr Opin Organ Transplant 2013;18(1):51–58.
[121] Sun XJ, Li R, Sun X, et al. Unique roles of Schistosoma japonicum protein Sj16 to induce IFN-gamma and IL-10 producing CD4(+)CD25(+) regulatory T cells *in vitro* and *in vivo*. Parasite Immunol 2012;34(8–9):430–439.

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