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Tolerance in Renal Transplantation

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1. Introduction

Tolerance in human transplantation can be defined in two ways [1]. Clinical tolerance (also referred to as clinical operational tolerance [2]) is the survival of a foreign organ or tissue (allogeneic or xenogeneic) in a normal recipient in the absence of immunosuppression [1]. Immune tolerance is the absence of a detectable immune response against a functional organ or tissue in the absence of immunosuppression [1].

Early evidence demonstrating that adult mice could be tolerant of skin grafts after the induction of neonatal tolerance by the introduction of splenocytes intraperitoneally was shown by Brent and Medawar, in 1953 [3]. The central role of the thymus in mediating cellular immunity and graft rejection was established by JFAP Miller, who showed that nude mice tolerated skin allografts because of a marked deficiency of lymphocytes [4]. Conversely, there have been recent studies that show that spleen transplantation in pigs or dogs has a tolerogenic effect on renal transplantation [5, 6]. On the basis of the promising results obtained in these animal models, several tolerogenic protocols have been attempted in humans, but most have failed to achieve robust and stable tolerance after renal transplantation. This is due to that the transplantation immunobiology is very complex, because of the involvement of several components such as antibodies, antigen presenting cells, helper and cytotoxic T cell subsets, immune cell, surface molecules, signaling mechanisms and cytokines; which play a role in the alloimmune response.

2. The alloimmune response

The allogeneic immune response has largely been attributed to the recognition of donor antigens, presented in the context of human leukocyte antigen (HLA) molecules to T cells,

which in turn direct a huge array of cellular and humoral responses, causing tissular damage and graft rejection. This type of response is mediated by the adaptative branch of the immune system [7].

The immune system can be divided in two components, the innate and adaptative immunity. The innate immunity, refers to a nonspecific response that involves the recruitment of diverse components of the immune system such as, macrophages, neutrophils, natural killer cells (NK cells), cytokines, several cellular receptors, complement components, cytokines, Toll-like receptors (TLRs), and antimicrobial peptides (AMP's). The adaptative immunity, which involves recognition of specific antigen, conferring both specificity and a memory effect [8]. Data suggest that initial allograft injury (such as ischemia) may initiate an innate immune response (Figure 1A), thus contributing to acute and chronic allograft rejection. Furthermore, this inflammatory response may initiate and expand the adaptive immune response to the point where the different HLA antigens come into play for the first time [9]. Some immunologist choose not to divide the alloimmune response in adaptative and innate branches; nevertheless, they are closely related and dependent on each other.

The main and strongest responses to alloantigens are mediated by host T cells, which recognize peptide antigens presented by antigen presenting cells (APCs) in the context of HLA. The phenomenon by which the recipient immune system reacts with donor antigens that are considered to be "non-self" is called allorecognition. Foreign or donor antigen presentation to T cells may occur by either direct or indirect pathways [10] (Figure 2A).

2.1. Direct allorecognition pathway

The direct allorecognition pathway involves recognition of intact donor HLA molecules on the donor cells, usually APCs. This seems to contradict the classic self-HLA restriction property of T cells, since the peptide being recognized is presented in a non-self HLA, and to date, two models have been proposed to explain this discrepancy [11].

The "high determinant density" model proposes that the transplanted organ carries a variable number of passenger APCs in the form of interstitial dendritic cells (DCs). Such APCs have a high density of allo-HLA molecules and are capable of directly stimulating the recipient's T cells. Given the very high ligand density, the affinity of alloreactive T cell receptors required to generate an optimal alloimmune response can be significantly lower compared to that required for self-HLA peptide complex [12].

In the "multiple binary complex" model, peptides derived from endogenous proteins that are bound into the groove of donor HLA molecules play a role. These peptides are derived from the same normal cellular proteins that are present even in the recipient. However, the differences in the allo-HLA groove causes a different set of peptides to be presented from homologous proteins. These peptides can be recognized by the recipient T cells. Therefore, even a single HLA mismatch between the donor and the recipient would be able to stimulate a large number of alloreactive T cells [13].

This pathway is thought to be the dominant pathway involved in the early alloimmune response (acute graft rejection), as the relative number of T cells that proliferate on contact with

allogeneic or donor cells is extraordinarily high compared with the number of clones that target antigen presented by self-APCs [14].

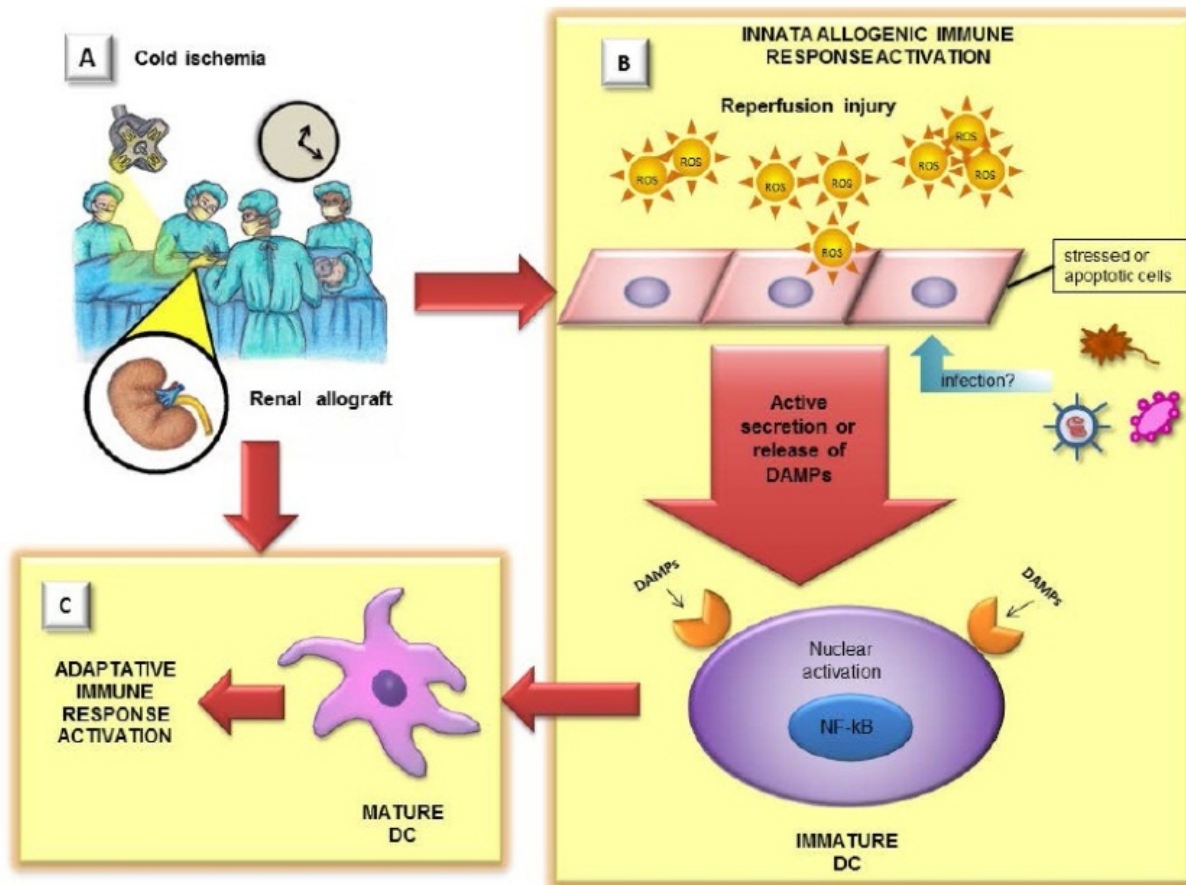


Figure 1. The alloimmune response: (A) ischemia may initiate an innate immune response, (B) which contributes to acute and chronic allograft rejection. The initial allograft injury, during reperfusion, is associated with generation of DAMPs for maturation of donor-derived and recipient-derived dendritic cells, (C) which represents the bridge to the development of an adaptive alloimmune response that results in rejection. Abbreviations: DAMPs, Damage-Associated Molecular Patterns; NF- κ B, Nuclear Factor- kappa beta; DC, Dendritic Cell.

2.2. Indirect allorecognition pathway

In the indirect pathway, T cells recognize processed alloantigen presented as peptides by self-APCs (host-APCs) [11]. The basic premise for indirect allorecognition as a mechanism involved in allograft rejection is shedding of donor HLA molecules from the graft. These HLA molecules are then taken up by recipient APCs and presented to CD4⁺ T cells. Interestingly, there is also evidence that demonstrates that recipient DCs can acquire and process intact donor HLA molecules from donor cell debris and stimulate CD8⁺ T cells by cross priming. Therefore, both CD4⁺ and CD8⁺ T cells mediate indirect allorecognition [11]. The indirect pathway is postulated to play a dominant role in chronic allograft rejection [15].

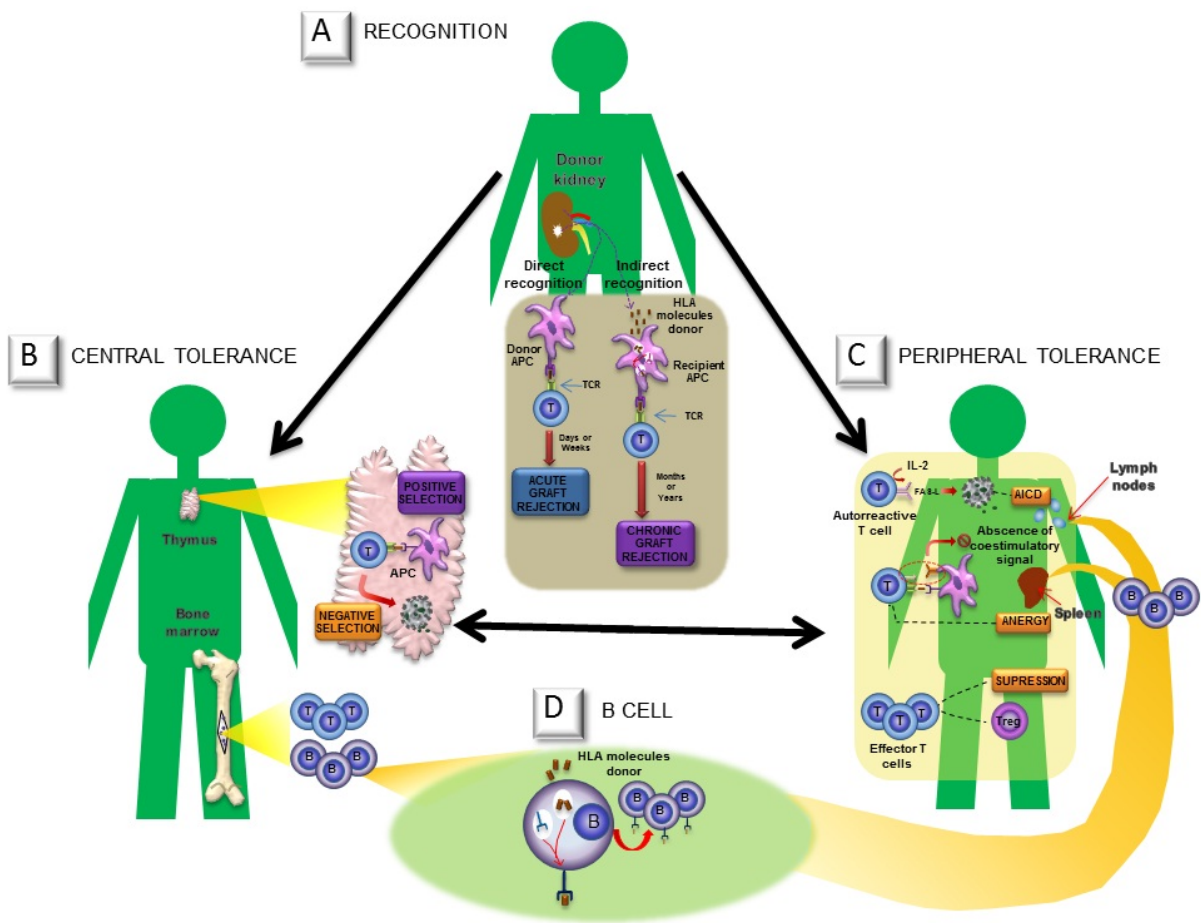


Figure 2. A). Allorecognition process. Two pathways lead to T-cell activation, the direct pathway and the indirect pathway. The mechanisms of tolerance are: (B) Central tolerance in which T cells migrate from the bone marrow to the thymus where they are educated, such that those recognizing self-antigens are deleted, and (C) Peripheral mechanisms of tolerance for self-reactive T cells including AICD, anergy, and suppression by T_{reg} . (D) B-cell awaiting the proper stimulus of a T-cell to initiate the production of alloantibodies. Two possible scenarios ensure tolerance: deletion of these self-reactive B cells and receptor editing, which is a process by which a new receptor with altered specificity is generated through another sequence of B cell receptor gene rearrangements. Abbreviations: HLA, Human Leukocyte Antigen; APC, Antigen Presenting Cells; TCR, T-cell Receptor; T, T-cell; T reg, regulatory T cells; B, B-cell; IL-2; Interleukin-2; AICD, Activation-Induced Cell Death.

2.3. Other allorecognition pathways

A third mode of allorecognition, which Lechler's group has termed the "semi-direct" pathway, has been recently proposed [16]. This model is based on the transfer of intact HLA molecules between cells. DCs have been shown to acquire intact HLA class I and II molecules from exosomes secreted by other DCs and to prime both naïve $CD8^+$ and $CD4^+$ T cells, thereby inducing an alloimmune response [17,18].

Another mechanism of allorecognition involves NK cells. NK cells may recognize HLA classical and non-classical type I molecules through interactions with cell surface receptors called killer cell immunoglobulin-like receptors (KIR, formerly named killer inhibitory

receptors) that recognize classical HLA class I molecules [19] and CD94/NKG2 receptors that recognize non-classical HLA class I molecules. Currently, the role of NK cell-mediated cytotoxicity in allograft rejection remains controversial, but recent data shows that NK cells are potent alloreactive cells when fully activated with IL-15 and can mediate potent acute skin rejection, at least in a murine model [20]. While reports continue to provide evidence supporting a role for NK cells in promoting rejection, there are a growing number of studies that illustrate an alternative role for NK cells in promoting allograft survival and tolerance [21].

2.4. Activation of T cells

Through their specific antigen receptors, T cells are capable of recognizing external antigens and initiating immune responses. These reactions may be characterized predominantly by cell-mediated reactions in which effector immune cells play a major role; or by humoral reactions in which the stimulation of B cells (Figure 2D) may induce antibody responses. The T cells orchestrate both the initiation and the propagation of immune responses, largely through the secretion of protein mediators termed cytokines and chemokines. Moreover, recent findings suggest that a novel subtype of T cells, named regulatory T cells, have an important role in achieving allograft tolerance [22]. These facts make T cells important targets for immunosuppressive therapy and tolerance induction protocols.

T cells require two separate signals before activation occurs. The first signal is antigen specific and is provided by the interaction of a T cell receptor (TCR) with a peptide antigen presented within the antigen binding groove of HLA molecules on the surface of APCs (Figure 2A). These are HLA class I molecules in the case of CD8⁺ T cells and class II molecules in the case of CD4⁺ T cells. The second, costimulatory, signal is provided by the interaction of T cell surface molecules with their ligands on APCs, being the most important the B7¹-CD28 and CD40-CD154 interactions. The first signal in the absence of the second signal may lead to T cell inactivation, anergy, or failure of a Th1 (T helper cell-1) response with a switch to a Th2 (T helper cell-2) response [23].

The Th1/Th2 response refers to the pattern of cytokines produced by T helper cells. Th1 cells produce interleukin-12 (IL-12) and interferon gamma (IFN-gamma) inducing macrophage activation leading to delayed-type hypersensitivity responses. The Th1 response has been implicated in acute allograft rejection. Th2 cells produce IL-4, IL-5, IL-10, and IL-13, and provide help for B cell function [24]. IL-4 is a growth factor for B cells and antibody production, and also can directly inhibit T cell maturation along the Th1 pathway [25]. Such responses have been associated with allograft tolerance, but are mainly implicated in clearing parasitic infections and the presentation of allergic diseases.

Once the binding of CD4/CD8 co-receptors stabilizes the immunologic synapse between the T cell and the APC, tyrosine-based activation motifs on the CD3 complex leads to the phosphorylation of a series of intracellular proteins, resulting in the activation of a variety of enzymes including calcineurin, and the activation of transcription factors, such as nuclear factor of activated T cells (NFAT) and NF- κ B, permitting the transcription of different genes,

1 B7-1 (or CD80) and B7-2 (or CD86).

including HLA class I and IL-2 [26]. There are other important events implicated in the activation of T cells, including leukocyte migration and the interaction of chemokines with their receptors.

3. Transplantation tolerance

The alloimmune response can be divided into central and peripheral tolerance, according to the mechanisms that induce a tolerance state. These are related and not exclusive [27] (Figure 2).

3.1. Central tolerance

Central tolerance is the most important means by which T and B autoreactive lymphocytes are eliminated in a process termed clonal deletion. T and B cells mature and are educated in the thymus and the bone marrow, respectively (Figure 2B).

Immature T lineage cells emerge from hematopoietic progenitors in the bone marrow and enter the thymus without expressing either the TCR or coreceptors. Since they lack CD4 and CD8 antigens, these cells are called double-negative (DN) cells or thymocytes. T cell selection begins after DN cells have undergone a TCR-mediated rearrangement process and up-regulated both CD4 and CD8 antigens, thus becoming double-positive (DP) cells [28]. From here, the thymocyte's fate is determined by the nature of its interaction with self-peptides that are presented on the self-HLA molecules of thymic stromal cells. This process is called "the affinity-avidity model". If a T cell reacts too strongly with self-antigens presented on bone marrow-derived APCs, it is eliminated by apoptosis or negative selection in the thymus [29]. Thymocytes with TCRs that interact with self HLA/peptides with lesser avidity, are positively selected and evolve into mature T cells that express either the CD4 or CD8 receptor (single positive T cells). The cells with very low avidity interactions fail to induce survival signals and die within the thymus. At the end of the process, only 3% of the total number of CD4⁺CD8⁺ DP cells are exported from the thymus, having developed into single positive CD4⁺ or CD8⁺ cells [30].

Currently, it is not completely understood how many peripheral tissue-specific antigens are expressed and presented in the thymus to ensure central T-cell tolerance to antigens that will be encountered in the periphery eventually. The expression of peripheral proteins in the thymus (such as insulin, thyroglobulin, and renal autoantigens) is driven in part by a gene called AIRE (autoimmune regulator). Mutations in the AIRE gene result in a disease known as autoimmune polyglandular syndrome type I. Interestingly, only certain organs and systems are involved, and within these, only particular parts of the organ tend to be affected, confirming that additional mechanisms must be involved to maintain systemic tolerance [31].

B cells undergo a similar process, as they are tested for reactivity to self-antigens before they enter the periphery. Immature B cells, developing in the bone marrow, test antigen through their antigen receptor, a surface IgM called the B cell receptor (BCR). If signaling through the BCR is sufficiently weak, immature B cells can be rendered permanently unresponsive or

anergic. However, if immature B cells are strongly self-reactive, there are two possible scenarios to ensure tolerance. The first is deletion of these self-reactive B cells. The second is receptor editing, a process by which a new receptor with altered specificity is generated through another sequence of B cell receptor gene rearrangements [32].

3.2. Peripheral tolerance

Besides the deletion process of autoreactive cells occurring during central tolerance, some T or B cells with self-reactivity may escape from the thymus or bone marrow, making the loss of self-tolerance easier. However, several mechanisms, collectively named peripheral tolerance, can control or eliminate such cells. Peripheral tolerance involves deletion and apoptosis, anergy, and regulation or suppression (Figure 2C).

3.2.1. Deletion and apoptosis

This mechanism is used to eliminate activated T cells specific for self-antigen. The programmed cell death, or apoptosis, is also termed activation-induced cell death (AICD). This process is mediated by the interaction of Fas (CD95) with its ligand (Fas-L or CD95L) on T cells, and can occur in developing thymocytes as well as mature T cells [33]. IL-2 can activate the STAT 5 signaling pathway through the IL-2 receptor (IL-2R), which in turn potentiates the up-regulation of Fas-L and the down-regulation of Bcl2 expression on T cells, thus promoting AICD. Conversely, IL-15 acts as a growth and survival factor for T cells [34, 35]. Since augmented AICD can induce tolerance through elimination of populations of reactive lymphocytes [36], certain tolerogenic models which use IL-15 antagonists and IL-2 agonists during transplantation have resulted in donor-specific tolerance [37]. Further research on this topic is needed before considering this peripheral mechanism as a therapeutic approach.

3.2.2. Anergy

The hyporesponsiveness of T or B cells to further antigenic stimulation, also called anergy, is a process that can result from antigenic stimulation in the absence of costimulation. In the case of T cells, complete activation requires the presentation of peptide on the HLA molecule to the TCR (first signal), and costimulatory signals, such as the B7-CD28 and CD40-CD154 interactions (second signal). The second signal is required to induce the multiple pathways that will lead to the activation of IL-2 gene transcription, ultimately inducing T cell activation and proliferation. However, it has been shown that IL-2 production and subsequent signaling through its receptor, IL-2R, is necessary for T cells to escape anergy, since blocking IL-2/IL-2R engagement even after stimulation through the TCR and CD28 still results in induction of T cell anergy [38].

As with T cell activation, B cell activation requires two signals. In this context, naïve B cells can be anergized if their surface immunoglobulins bind to self-antigens (first signal) in the absence of the additional necessary T cell signals (second or costimulatory signal) [39].

3.2.3. Regulation or suppression

A third mechanism of peripheral tolerance is regulation or suppression of immune responses to self or foreign antigens. Perhaps, the regulatory T cells (Treg cells) are the most important and well documented effectors of this mechanism to date. These cells control the type and magnitude of the immune response to foreign antigen to ensure that the host remains undamaged. Treg cells are also integral to maintaining a lack of response to self-antigens or tolerance [40].

There are two subsets of Treg cells. "Natural" Treg cells, are a thymus-derived population that constitute about 10% of the CD4 population. Natural Treg cells express CD4, CD25, CTLA4, and GITR on their surface [41], and express transcription factor Foxp3 intracellularly [42]. The importance of Foxp3 as the orchestrator of the molecular programs involved in mediating Treg function has been highlighted by diseases such as IPEX syndrome (immune dysfunction, polyendocrinopathy, enteropathy and X-linked inheritance), in which a mutation in the Foxp3 gene has been described [43].

The other subset of T_{reg} cells, commonly termed "adaptive" T_{reg} cells, develops in the periphery, in a thymic-independent manner, following antigen encounter under particular circumstances, namely exposure to transforming growth factor- β (TGF- β). This leads to the expression of Foxp3; the hallmark of T_{reg} cells [44]. Data suggesting the role of these cells in immunologic tolerance has been obtained from different studies in which patients with normal graft function reportedly possess a smaller T_{reg} population compared with patients having chronic allograft rejection, suggesting that T_{reg} cells may prevent damage and graft loss [45]. Other groups have shown that certain immunosuppressive protocols are more permissive than others in generating these populations [46].

The mechanisms by which Treg cells exert their effects are not completely understood. There have been two main mechanisms proposed. One mechanism requires cell contact between CD4+CD25+ Treg and responder cells and interaction between CTLA-4 and GITR molecules [47], while the other mechanism involves the induction of suppression or regulation by newly generated suppressor T cells in a cytokine-dependent manner through IL-10 and/or TGF β [48, 49]. Although promising, there is still too much to learn, before using this subset of cells for tolerance induction in renal transplantation.

In addition to T_{reg} cells, there are other cell phenotypes with regulatory properties, such as CD8⁺ T cells and certain NK populations [50]. CD8⁺ T cells with regulatory/suppressive properties have been named "veto cells". Such cells maintain peripheral tolerance by attacking alloreactive T cells which are present in bone marrow with increased frequency, and may be responsible in part for the reduction in graft versus host disease and the induction of chimerism seen in some bone marrow transplant models [51].

4. Tolerogenic strategies in renal transplantation

Tolerance in renal transplantation is an exceptional finding. Approximately 100 cases of tolerance in renal transplantation have been reported to date, mainly in patients who

were not compliant with their immunosuppressive regimens or in individuals who had previously received a bone marrow transplant for hematological disorders [52]. At the present time, in looking for tolerance in renal transplantation, physicians in clinical practice have implemented protocols and surgical procedures in which tolerance was the planned objective before the transplant.

4.1. Strategies and protocols

Protocols in which tolerance in renal transplantation was the planned objective before the transplant may be divided into three subgroups, namely molecule-based, cell-based, and total lymphoid irradiation.

4.1.1. Molecule-based protocols

The molecule-based group includes all cases in which the induction of tolerance was attempted through administration of presumed tolerogenic drugs. These tolerogenic drugs include polyclonal antithymocyte globulin antibodies and anti-CD25 monoclonal antibodies. Anti-CD25 monoclonal antibodies competitively inhibit IL-2R-dependent T cell activation, while the polyclonal antithymocyte globulin antibodies are directed against lymphocyte antigens. The goal of the induction treatment was the nonspecific removal of clones of immune cells responsible for rejection before contact with foreign donor antigens occurred. Once the donor antigens were in place after implantation of the new kidney, repletion of immune cells occurred, favored by the homeostatic expansion triggered by leukocyte depletion. In addition, minimization of maintenance immunosuppression was implemented to further reduce the anti donor response with just enough treatment to prevent irreversible immune damage to the graft, but not with such heavy treatment that the donor specific clonal exhaustion-deletion process was precluded [53].

4.1.2. Cell-based protocols

In the cell-based group, patients received a donor-cell infusion of highly enriched CD34+ hematopoietic progenitor cells mixed with CD3+ T cells, [54] ie, patients received heavy conditioning regimens in association with the perioperative infusion of immunomodulatory cells, such as transplant-acceptance inducing cells. Afterward, maintenance immunosuppression was given for a few months until complete withdrawal, when possible. Overall, although these trials demonstrated that the infusion of transplant-acceptance inducing cells is feasible, major concerns remain regarding the efficacy and safety of such an approach. Whether this approach confers any benefit in the establishment of minimal immunosuppression in renal transplantation patients when compared with the protocols currently in use is unclear. Lastly, the optimal dose and timing of cell infusions, along with the most appropriate concomitant immunosuppression regimen, remains to be determined [55,56].

Patients who received renal transplantation after bone marrow transplantation from the same donor are also included in this group. Bone marrow transplantation, when successful, generally results in the total replacement of the recipient's bone marrow with the do-

nor's bone marrow hematopoietic cells, a condition referred to as full chimerism [57]. Experimental data have confirmed that the infusion of donor-derived bone marrow cells can prolong allograft survival by still incompletely understood mechanisms [58]. However, the translation of this model from animals to humans has remained a very challenging task. In particular, an immunosuppression-free state has been achieved only sporadically after living-related donor renal transplantation, whereas similar findings have never been documented after deceased donor renal transplantation [57,59–63]. In some studies, the perioperative infusion of donor bone marrow seems to reduce the incidence of acute and chronic rejection, [57,60,61] and to improve graft function when infused not only systemically but also intrathymically [62,63].

4.1.3. Total lymphoid irradiation protocols

Total lymphoid irradiation was originally developed as a nonmyeloablative treatment for Hodgkin disease [64]. This treatment modality was first used about 40 years ago to induce prolonged renal allograft survival. However, total lymphoid irradiation has significant short- and long-term effects on lymphocyte subpopulations through suppression of activated T cells and the IL-2 pathway. Importantly, as the doses of radiation required for total lymphoid irradiation to be effective are high, with 10 doses of total lymphoid irradiation (80 to 120 cGy) targeted to the lymph nodes, spleen, and thymus, [54] its clinical application is limited by the toxicity that occurs with such high doses. With the advent of more effective immunosuppressive drugs and cytolytic therapy with antithymocyte globulin and monoclonal antibodies, the use of total lymphoid irradiation has declined considerably and is mainly applied, as stated earlier, as a nonmyeloablative preparative regimen of total lymphoid irradiation in combination with the infusion of donor-derived cells to induce a state of lymphohematopoietic chimerism [65-71].

4.2. Surgical procedures

Currently, Japan has a serious shortage of cadaveric organs. As a result ABO incompatible living kidney transplantation is being performed [72–76].

Between 2001 and 2004, the ABO-incompatible living kidney transplantation procedure used a 1-week pretransplant immunosuppression with tacrolimus/mycophenolate mofetil/methylprednisolon. During this period, splenectomy was performed in all cases and the short-term outcome was excellent [77]. Graft survival was 93.5% at three years and 91.3% at five years in these patients [78].

The spleen is involved in the production of B lymphocytes and IgM, so splenectomy can result in decreased antibody content and increased tolerance [79]. This effect could be considered analogous to the effect of rituximab (anti-CD20+ monoclonal antibody), [80,81] which prevents acute rejection mediated by antibodies, resulting in a tolerogenic effect. Conversely, recent studies show the important role of the spleen for the induction and maintenance of regulatory CD4+CD25+ T cells, which are important for self-tolerance [82,83]. This immune regulatory mechanism is known as non-specific suppression of acti-

vation and differentiation, and is the result of the release of anti-inflammatory cytokines [84,85]. Therefore, upon splenectomy, the activity of regulatory T cells is presumably affected, and this may simulate the mechanisms of action of some currently used immunosuppressant drugs, such as basiliximab and daclizumab (chimeric monoclonal antibodies that selectively affect T lymphocytes) [86].

5. Conclusion

Despite advances in understanding the cellular and molecular mechanisms of the alloimmune response, tolerance induction in renal transplantation remains an important clinical challenge. In clinical practice, prevention of graft rejections has combined tolerance mechanisms, such as suppression of activated T cells, inhibition the IL-2 pathway, decreased antibody production, and t chimerism. However, no completely satisfactory results have been achieved. The reason for these seemingly insurmountable challenges stems from the properties of the alloimmune response, which are not yet completely understood.

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