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Review on the Influence of Immune System on Organ Transplantation and its Therapeutic Strategy

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Abstract: Preserving immunity by minimizing immunosuppression or inducing tolerance is one of the major goals of the transplant immunologist. The immune responses against transplanted organs arise from several genetics barriers such as blood group incompatibility and human leukocyte antigen matching. After organ transplantation an immunosuppressive regimen is required to prevent graft rejection. Immunosuppressive drugs inhibit immune function by targeting both T- and B-cell responses through blockage of cellular proliferation induced by alloantigen stimulation and by inhibition of the cytokine production necessary for such stimulation. However, the absence of discrimination between the immune response against alloantigen from the transplanted organ and the immune response against environmental antigens renders transplanted patients strongly. Optimizing the immunosuppressive drug regimen to balance mandatory immunosuppression while preserving immunity is a difficult challenge for clinicians in charge of transplanted patients. The development and optimization of assays to monitor the current state of an immune response is of great interest.

Key words: Allograft · Alloimmune · Immunosuppressant · Chimaerism

INTRODUCTION

The living animals' body contains all the components necessary to sustain life. Tissues of living animals are resistance to microbial invasion. These resistances are due to multiple interlinked defense mechanisms known as immune systems and physical barriers. Immune systems are also divided in to two these are innate immunity and acquired immunity. Although the immune response first attracted the attentions of scientist because of the body's ability to fight organ grafts leads to much broader view the function of the immune system [1].

The immune response against transplanted organs arises from several genetic barriers; such as blood group incompatibility and human leukocyte antigen (HLA) mismatching [2]. The alloimmune responses against transplanted organs are activated by direct and indirect path ways. It has been demonstrated that regulatory cells are essential for induction and maintenance of tolerance [3]. However the exact mechanism by which the allograft rejection can occurs is still not fully understood because of the complex immune mechanisms involved in graft rejection [4].

The immunosuppressive regimens used after organ transplantation are efficient for allograft survival, but as a result of none specific mechanisms of action it makes the recipients susceptible to viral and bacterial infections. Experience in solid organ transplantation (SOT) is less extensive, although renewed efforts are under way to detect the mechanisms of tolerance and rejection [5].

Therefore the objectives of this paper are:

- To review the mechanisms of alloimmune response against transplanted organs and consequence of immune suppression for recipients' immunity
- To review the development of therapeutic strategies for monitoring immune response after organ transplantation.

Immune Response Against Transplanted Organs Genetic Barriers

Blood Group Incompatibility and Human Leukocyte Antigen Antigens: The immune response against transplanted organs arises from several genetic barriers. Blood group incompatibility is the first and if organ

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transplantation across the blood barrier is performed in selected cases (e.g. kidney); ABO-compatible transplantation is the rule. The second genetic barrier is formed by the highly polymorphic human leukocyte antigens (HLA) expressed by almost all nucleated cells. The HLA effect is most pronounced in allogeneic HSCT, where compatibility for HLA at a high resolution level has been clearly shown to be associated with better survival and a lower rate of GVHD [2].

In organ transplantation the effect of HLA matching on clinical outcome varies greatly with the organ being transplanted. In the case of liver transplantation for autoimmune disease, donor matching may actually be detrimental [6]. In renal transplantation, the benefits of HLA matching are still evident even with modern immunosuppression [7]. The better survival of transplanted kidneys with good matching is explained by the reduction of rejection episodes, leading to a reduction of the total "load" of immunosuppressive drugs, most of which have renal side effects. Good HLA matching should therefore result in less immunosuppression and better immunity for transplant patients [8].

In Switzerland 10% of patients on the waiting list for a first kidney transplant are immunized with anti-HLA antibodies. This number rises to 55% for those awaiting re-transplantation. In these patients HLA matching is still important and is mandatory for a specific locus, to avoid humeral rejection. In this specific context of hyper immunization strategies have been optimized to desensitize patients before transplantation or to define acceptable mismatches [9, 10].

Direct and Indirect Pathway of Alloimmune Response: The alloimmune response against the transplanted organ is activated by two pathways. With the direct pathway, the donor antigen presenting cell (APC) transplanted with the graft presents donor antigen (HLA molecules or minor antigen) to the CD4+ and CD8+ T cells which are activated in the secondary lymphoid organ. The second pathways is recipient APCs migrate to the graft, process donor Ag and activate CD4+ and CD8+ T cells, which recognize the alloantigen presented by the self-APC in the secondary lymphoid organ. Activation is mediated by the TCR which recognizes the HLA and peptide in the

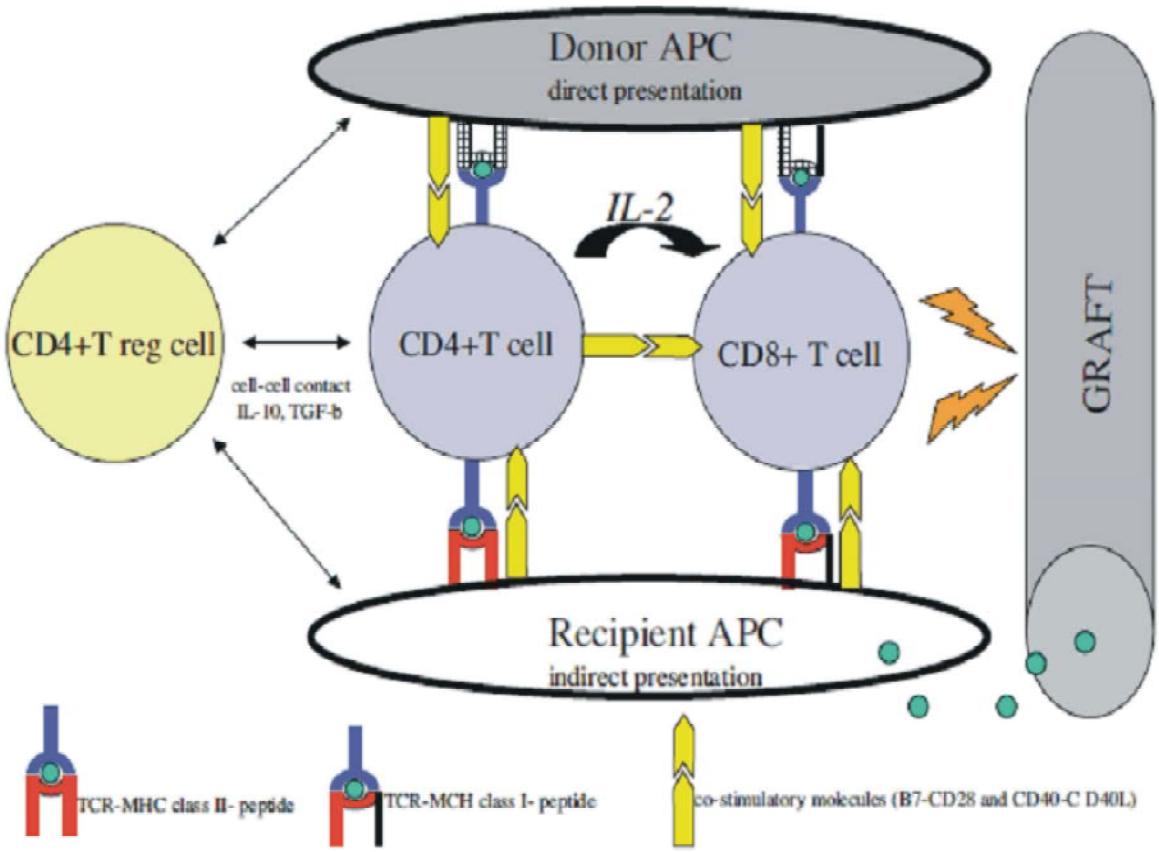

Fig. 1: Immune response against a transplanted organ [12]

presence of co stimulation such as B7 (APC)-CD28 (T cell) and CD 40 (APC)-CD40 ligand (T cell). The presence of IL-2 is required by a mechanism that is still unclear (cell-cell contact or inhibition by cytokines such as TGF-b or IL-10), regulatory cells such as CD4+CD25+ are able to suppressCD4+ and CD8+ T-cell activation [11].

More recently, the role of regulatory cells in controlling and suppressing self-antigen activation has been recognized and the same cell population has also been shown to have regulatory activities on alloantigen after transplantation. It has been demonstrated that the regulatory cells are essential for induction and maintenance of tolerance. Many types of regulatory cells have been described in a number of different systems; these include CD25+CD4+, CD8+CD28– T cell (Liu *et al.*, 1998) and T-cell receptor (TCR)+ CD4,CD8, cells [13] as well as natural killer cells (NKC) [14].

Organ transplantation has become an accepted form of treatment for end-stage kidney, liver, heart, pancreas and lung disease and to prevent immune response against the transplanted organ as described above, patients receive a combination of immunosuppressive drugs for the rest of their lives. Classical immunosuppressive regimens are based on glucorticoids, calcineurin inhibitors such as cyclosporine or tacrolimus and more recent drugs such as mycophenolate mofetil, sirolimus or monoclonal antibody which block IL- 2 receptor have contributed to the impressive one year graft survival figures achieved by most transplant centers worldwide. Under these regimens, T-cell responses are globally impaired through blockage of cellular proliferation after antigen stimulation, as well as inhibition of the cytokine production necessary for such stimulation [15].

These drugs have little direct B-cell effect but by inhibiting T-cell response most of these regimens also have a T- dependent B-cell inhibition. Corticosteroids are potent cytokine inhibitors (interleukin-1, interleukin-2, interleukin- 6, tumor necrosis factor and interferon-g) and block antigen-induced T-cell proliferation. Calcineurin inhibitors directly inhibit interleukin-2- dependent T-cell proliferation and blocking interleukin- 4 and interleukin-5 production by cells has an inhibitory effect on B-cell function and antibody production. Azathioprine and mycophenolate mofetil, also used as third-line agents, in different steps, blocking both T- and B-cell proliferation. More recently developed, sirolimus inhibit T-cell activation. The combination of these mechanisms leads to significant impairment of the immunological cascade following alloantigen presentation to immune cell [16].

Allograft Rejection: Allograft rejection remains the single largest impediment to the success in the field of transplantation. Graft rejection is different from other immune responses as two different sets of antigen presenting cells are involved, one from the donor and other from the recipient. Exact mechanism by which allograft rejection can occur is still not fully understood because of the complex immune mechanisms involved in the graft rejection. Rejection episodes lead to adverse immune response and affect the allograft survival. The immune response following an allograft is primarily against major histocompatibility complex (MHC) molecules of the donor from which recipients differ. As many as 8-10% of the normal adult T cell repertoire is capable of recognizing and responding to the foreign MHC molecules [4].

Immunological Mediators Involved in Allograft Rejection: T-cell mediated rejection: - Thymus derived T cells have an essential role in acute allograft rejection. If the host is naturally or experimentally deprived of T cells (eg. nude mice, SCID mice, thymectomized mice) it is unable to reject allograft in the first set. If the passive transfer of T cells into athymic mice is done vigorous graft rejection will take place. In clinical transplantation, the role of T cells has been confirmed by the dramatic effects of anti-T cell antibodies, including monoclonal anti-CD3 antibody, anti-thymocyte globulin and anti-lymphocyte globulin, the effectiveness of which is often limited by the side effects of non-specific immunosuppression. Treatment of rhesus monkey by CD3 immunotoxin just before transplantation resulted in long-term graft acceptance in more than 50% of the monkeys. The allograft differs from host at class I and class II loci. Both CD8+ and CD4+ T cells are activated by recognition of alloantigen of the grafts; the CD8+ T cells recognize foreign MHC class I molecules, which are expressed by all the cells in the graft. The differentiation of cytotoxic T lymphocytes (CTLs) is largely dependent on CD4+ T helper cells being stimulated by allogenic class II molecules present on antigen presenting cells (APCs) in the allograft. Several lines of evidences suggest that the CD4 subset and its lymphokine products are the principal mediators of rejection [17].

There are evidences, which suggest that some CD8+ T cells can also provide sufficient help to allow cytotoxic T lymphocytes to differentiate independent of CD4+ T cells. However, these CD8+ T cells appear to depend upon the same professional APCs, as those required by

conventional CD8+T cell. The most important APCs stimulating an anti-graft response may be dendritic cells residing in the interstitial of the graft. Dendrite cells are now regarded as critical instigators and regulators of immune reactivity, which play a key role in both the direct and indirect pathways of allorecognition. Molecular signaling between dendritic cells and Th cells directs the differentiation of naive (Tho) cells into either Th1 or Th2 cells. Specific cytokines such as IL –10 and other factors can inhibit IL-12. Experimental dendritic cell targeted approaches to the therapy of organ allograft rejection include administration of co-stimulation of blocking agents together with donor dendritic cells or genetic engineering of the dendritic cells to express tolerance promoting molecules[18].

Antibody Mediated Rejection: The role of antibody in hyper acute rejection has been clearly established [8]. A direct correlation is seen between positive re-transplant cross match which detects anti- MHC class I antibodies and the development of hyper acute rejection [19]. Anti-graft antibodies can be eluted from donor kidneys after hyper acute rejection. The passive transfer of anti-graft antibodies in experimental models can provoke hyper acute rejection. It is likely that antibodies also play a role in other types of rejection; however, their mechanisms remain incompletely understood and also controversial especially in chronic rejection [20].

The scanty cellular infiltrate in most cases of chronic rejection is antibody mediated rejection. However, direct evidence for antibody-mediated damage in chronic dysfunction is inconclusive. The antibodies causing hyper acute rejection may be preformed or they may develop under the influence of immunosuppressive drugs, which could modulate their rate of production. Antibodies can bind to the graft, making the detection of soluble ant graft antibody difficult. Thus the role of antibody in the pathogenesis of chronic dysfunction remains undetermined [21].

Cytokines Mediated Rejection: Cytokines are soluble mediators secreted by one cell that acts on another cell or organ; the term is generally reserved for protein mediators. Naïve T cells could be converted into either Th1 or Th2 type cells. Th1 produces high levels of interferon IFN and TNF. Both IFN and TNF which may promote cell mediated cytotoxicity and delayed type hypersensitivity reactions, Th2 cells produce high levels of IL –4, IL-5, IL-10 and IL-13 which promote humoral response. Both Th1 and Th2 responses counter regulate one another. Th2 cytokines may evoke allograft rejection by recruitment of alternate effector mechanisms. Hence the exact mechanisms underlying this phenomenon in general are not yet defined and both Th1 and Th2 clones can reject skin grafts. Th1 to Th2 immune deviation can induce islet allograft tolerance across multiple minor histocompatibility antigen barriers. Apoptosis may promote the development of immunoregulatory T cells and facilitate active immunosuppression [22].

Adhesion Molecule Expression in Allograft Rejection: It is important to know the role of various molecules in transplantation. As after knowing various regulatory mechanisms the drugs can be designed to block the positive signals for induction of allograft tolerance, it is known that cyclosporine blocks positive signals required for T cell proliferation and apoptosis. Antigens specific lymphocyte immune response requires at least 2 stimuli from the antigen presenting cells. If the second stimulus (co-stimulation) does not occur, tolerance ensues. Two signals are: TCR-peptide - MHC recognition (specific response). T-cells ligand (CD28 and B7 binding (non specific co-stimulation provide + or -support). CD28 / B7 may not be the only co-stimulator pair. In addition other cell - cell interactions [9].

In bone marrow transplantation exvivo manipulation (graft engineering) is being attempted more frequently using a variety of methods, including co-stimulation blockade to prevent graft-versus-host-disease by tolerating donor T cells. This technique also lends itself to solid organs transplantation, where graft tissue is not amenable to prolonged exvivo manipulation. In this case, host T cells are tolerated to alloantigens using cytokines such as IL-10 and TGF to induce regulatory T cells exvivo, it should be possible to induce antigen specific suppression for allo and auto antigens if known. Recently gene therapies in clinical transplantation have a potential future [23].

Role of Anti HLA Antibodies in Rejection: Preexisting HLA antibodies against the donors are associated with acute rejection in case of renal Transplantation, which have a worse prognosis, often requiring aggressive early treatment with anti-CD3 cell antibodies. Flow cytometry is shown to be a better technique for detecting these antibodies [2].

Role of Panel Reactive Antibodies: Panel reactive antibodies are not against individual HLA gene products but are expressed as percentage positivity against a panel of cells. High prevalence of PRA shows that a patient is sensitized. Highly sensitized patients are one at increased risk of early graft loss. It is recommended [19] that patients waiting for transplant should be tested for PRA, if need be erythropoietin should be given as it causes reduction in the sensitization. HLA gene products consist of private and public determinants both of which are defined by antibodies reacting to a single epitope. Antibodies reacting to a single epitope define private and public determinants.

Private determinant is unique to a single HLA gene product; where as a public determinant is shared by multiple HLA gene products. It is therefore, possible to reduce the large number of HLA alleles to a small number of closely related groups that share common HLA derived antigenic targets; these groups are known as cross reactive groups (CREG). If matching is at CREG the chances of finding matched donor increases, even beneficial effect of CREG matching have been reported [24].

Role of Minor Histocompatibility: Minor histocompatibility antigens may play an important role in the graft rejection and are defined as cell surface antigens other than the MHC antigens. These antigens may not be universally present on all the cells and they don't interact functionally with MHC antigens. However, the role of these antigens is not well defined in humans. Experimental data obtained from studies of congenic strains of mice suggests that polymorphism of minor HLA antigens may be similar to that of the MHC. The Immune mechanism in transplantation important difference between them are that minor histocompatibility antigens mi-HAgs are less potent and immunogenic and they don't initiate the immune response independently, while, MHC antigens are more immunogenic and can trigger the antibody production against incompatible alloantigens. These mi-HAgs accounted for comparatively slower and more chronic rejections. Goulmy have been the first reported the possible involvement of mi-HAgs in human transplantation[25].

Role of Tissue Specific Antigens: Tissue specific antigens are defined as an antigen system that is expressed only on one type of organ, tissue or cell. These tissue specific antigens are independent from the systemic antigens such as HLA antigens, which have a wide distribution throughout the body. Clane [26] first described the phenomenon of differential allograft survival between organs from the same donor [26]. Whereas skin and kidneys were acutely rejected and, liver allograft survival seemed to be prolonged in unrelated pigs. Several cases of multiple organ transplants have been reported in which one organ is rejected while other continues to function. One possible explanation for this observation is the affect of tissue specific antigens. Poindexter, have characterized a kidney specific peptide, which recognize kidney cell line but not MHC identical B-lymphoblastic cell line [27].

Role of Heat Shock Protein: Heat shock proteins may be involved in the pathogenesis of chronic rejection. This hypothesis was tested with a rat cardiac allograft model in recipients pretreated with donor bone marrow cells. Chronic rejection was manifested in this group by obliterate arteriopathy and the epicedium and endocardium containing lymphocytic infiltrates [28]. Current experimental evidences support the concept that during cellular rejection, graft-infiltrating cells induce a stress response within the allograft which increases the expression of heat-shock proteins and triggers the recruitment and activation of hsp-dependent lymphocytes. A variety of stress proteins exhibit higher tissue levels during the different phases of allograft rejection [27].

Therapeutic Strategies for Preservation of Immunity after Organ Transplantation: The immunosuppressive regimens used after organ transplantation are efficient but, as a result of their non-specific mechanism of action, they fail to prevent chronic graft rejection, life-threatening infections and malignancy. The "perfect" immunosuppressive regimen would specifically inhibit anti-graft alloimmunity but preserve immunity against bacteria and viruses. This objective is close to the definition of tolerance, which was originally defined as long-term allograft survival in the absence of immunosuppressive drugs.The donor specific unresponsiveness observed in the tolerant state goes together with the persistence of third party response in functional assays, meaning that the immune response against any foreign antigen (except those expressed by the graft) is preserved. The immunological mechanisms of

tolerance induction towards an allograft are basically the same as those which maintain tolerance to self-antigen: central or peripheral deletion, anergy, regulation/ suppression and ignorance [29].

Central deletion can be achieved by direct injection of donor cells into the thymus but in clinical practice colonization of the thymus by donor hematopoietic cells ensures a continuous supply of donor antigen in the thymus leading to negative selection of the immature T cell. Peripheral tolerance can be achieved by the depletion of T lymphocytes with monoclonal antibody to remove alloreactive T cells without specificity. Blocking the co-stimulatory molecules prevents T cells from activation, leading to anergy. Regulatory T cells inhibit T-cell activation by cell-cell contact and/or secretion of anti-inflammatory cytokines such as TGF-b or IL-10. Regulatory T cells could also maintain dendritic cells in an immature and tolerogenic state. Ignorance is achieved in very specific conditions such as non-vascularized organ transplantation i.e corneal allograft [30].

Tolerance Induction Mechanism

Peripheral Tolerance Induction: The first is induction of peripheral tolerance by depletion of lymphocytes. Because graft rejection is mainly mediated by CD4+ and CD8+T cells, lymphocyte depletion at the time of organ transplantation has been advocated by some as a strategy for reducing the rate of rejection [5]. This strategy began many years ago with total lymphoid irradiation (TLI) and was then combined in animal studies with anti-CD3 or anti-CD4. Nonhuman primate studies have also suggested that T-cell depletion at the time of transplantation may substantially promote long-term unresponsiveness [31].

In humans, TLI was used in combination with anti-thymocytes globulin (ATG) in a small number of patients and a few became tolerant. The more common experience of T-cell depletion in kidney transplantation is with Campath-1H (alemtuzumab), a monoclonal antibody directed against the CD52 protein expressed at the surface of T cells. Campath has now been used in more than 100 kidney transplanted patients in combination with other immunosuppressive drugs [32].

Lymphocyte depletion with Campath-1H appears to be effective in preventing rejection and so far has been quite safe from the infection/malignancy standpoint. However, cellular and also strong humoral rejection episodes were observed in several patients and it was important to realize that intensive T-cell depletion did not induce tolerance. Other promising depleting strategies using anti-CD3 coupled with an immunotoxin are under investigation. To induce peripheral anergy, co stimulation blockade is another strategy for promotion of graft acceptance in transplantation and one which has the advantage of being associated with very few toxicities. A recent study shows that belatacept, an investigational selective co stimulation blocker of the B7-CD28 pathway, did not appear to be inferior to cyclosporine as a means of preventing acute rejection after renal transplantation. Belatacept was used with other immunosuppressive drugs in this study [32].

Promising initial studies with a monoclonal antibody which blockaded the CD40-CD40L pathway (anti-CD154) were performed in non-human primates. Graft survival was greatly prolonged [34] but true tolerance was not achieved. In humans, anti-CD154 has begun testing in clinical trials but this monoclonal antibody was associated with an increased incidence of thrombotic side effects [35].

Coinfusion Hematopoietic Stem Cell: Other strategies based on coinfusion of hematopoietic cells and organ transplantation has been proposed for induction of tolerance. Reports on donor lymphocyte infusion and infusion of cadaveric bone marrow have been published and in some studies a tendency to better long-term survival of the graft is observed, with a significant reduction of immunosuppressive drugs in some patients [36].

The idea of hematopoietic stem cell infusion is based on the hypothesis that donor-derived hematopoietic cells can reach the recipient thymus and promote negative selection of newly generated donor reactive T cells leading to central tolerance. The animal models developed to set up this strategy have demonstrated that mixed allogeneic chimaerism may induce a reliable and robust form of tolerance. In the patient, bone marrow or peripheral stem cell infusion could be acceptable only if low toxicity regimens for achieving mixed chimaerism are developed [37].

Trial to Identify Regulatory T-cells: Trials to identify regulatory T cells (Treg) in long-term kidney transplant recipients have already started [38]. Tracking the expansion or depletion of Treg in transplant patients may therefore enable immunosuppression protocols to be reevaluated in the near future. Ex vivo strategies for

generation and/or clonal expansion of the regulatory T cells from transplant recipients is another exciting approach which highlights the future potential for cellular therapeutic agents. In animal models, treating GVHD with expanded regulatory cells seems a promising approach [39], but careful study of Treg generated by these strategies in in vivo models, together with clinical trials, is essential to ensure safe and smooth induction of tolerance to donor alloantigen in the future. In the emerging field of cellular therapy the preservation of antiviral immunity by immunotherapy with large scale culture and amplification of virus specific CD8+ T-cells has shown promising results, but this approach will be confined to a small number of patients who have escaped antiviral therapy without cellular immune protection and have a potentially life-threatening viral infection [40].

Finally, with a view to preserving immunity one should bear in mind the simpler approaches which can be applied to a large cohort of transplanted patients in order to minimize the amount of immunosuppressive drugs after organ transplantation. Due to the plethora of evidence implicating steroids in complications following organ transplantation, many trials have been performed with the goal of either withdrawing steroids after a long period of use or after only short-term use, or avoiding them altogether in transplants. For the same reason and also in view of their financial cost, clinical trials designed to withdraw calcineurin inhibitors have been published and have been associated with an acceptable incidence of rejection following withdrawal [41].

CONCLUSION

Preserving immunity by minimizing immunosuppression or inducing tolerance is one of the major goals of the transplant immunologist. Studies in transplantation center have illustrated the difficulties in translating non-human primate model success into the clinical area. Redundancy of the immune system, species differences that make tolerance more difficult to achieve in higher species and species-specific complications have contributed to the difficulties in introducing such new approaches in the clinic.

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