Immune Responses in Transplantation: Application to Composite Tissue Allograft

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ABSTRACT

After announcements of successful hand, larynx, knee, muscle, nerve, and, most recently, face transplantation, composite tissue allografts (CTAs) have been introduced into the armamentarium of plastic and reconstructive surgery. Because the microsurgical techniques required to perform CTA transplants are well established and used in daily practice by plastic surgeons, the immunologic aspects of transplantation remain of great interest to plastic surgeons. CTAs offer a unique potential for coverage of large multitissue defects; however, compared with the relatively homogenous tissue of solid organ transplants, the heterogenicity of tissue components of CTA may generate high immunologic responses. Although modern immunosuppressive agents significantly improve successful allograft acceptance, chronic allograft rejection as well as immunosuppressive drug toxicity remain major problems in the clinical practice of transplantation. The major goal of transplantation immunology is to develop tolerance to allograft transplants and long-term drug-free survival. Several experimental protocols have been designed to develop tolerance; however, none of them have been proved to induce tolerance in clinical transplantation. This review outlines the mechanisms of allograft acceptance and rejection and describes the barriers to transplantation tolerance based on our current knowledge as it applies to solid organs and CTA transplants. The review also describes innovative immunosuppressive protocols.

KEYWORDS: Composite tissue allograft, immune response, immunosuppression

Composite tissue allograft (CTA) is currently accepted as a method of choice in plastic and reconstructive surgery. Because the microsurgical techniques required to perform CTA transplants are well established and used in daily practice by many plastic surgeons, the immunologic aspect of transplantation surgery remains of great interest to plastic surgeons. The key issues of interest pertain to theories regarding graft acceptance and rejection and to the bases of action of new immunosuppressive agents and protocols.

The first successful hand transplant, under modern immunosuppressive drugs, was performed in Lyon, France, in 1998 and demonstrated that CTA is a clinical reality.[1] According to the International Registry on Hand and Composite Tissue Transplantation, 35 hand/forearm/digit transplantations have been performed in 27 patients worldwide since 1998.[2] One of the most technically challenging CTAs is the laryngeal transplant, which was successfully performed by Dr. Marshall Strome in 1998.[3] Also, flexor tendon apparatus and nonvascularized nerve allografts have been introduced in

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clinical practice.[4,5] Allografting may also be performed to cover a large abdominal wall defect.[6] Most recently, a surgical team from France performed the first partial face transplant.[7] This posed a great challenge not only surgically but also medically because skin grafts are particularly susceptible to rejection.[8]

Because CTA transplantations are not lifesaving procedures, much consideration is devoted to the issue of minimizing or withdrawing immunosuppression. Inducing immunologic tolerance is the ultimate objective of CTA transplants; if reduction of immunosuppression proves feasible, there is no doubt that CTAs will hold great potential in plastic and reconstructive surgery.

Composite tissue allograft transplants differ from solid organ transplants in that solid organs present a relatively homogenous structure, whereas CTAs are histologically heterogenous; that is, they are composed of different tissues types such as skin, muscle, bone, bone marrow, lymph nodes, nerve, and tendon. They also express a distinct immunogenicity of transplanted elements. A hierarchy of antigenicity has been established, with the skin being the most antigenic; cartilage, tendon, and vessels being the least antigenic; bone of lower immunogenicity; and muscle being intermediate.[8]

To test the efficacy of immunosuppression and skin allograft acceptance, a series of experiments addressing surgical and immunologic aspects of face transplant were recently performed in a rat model.[9–11] The results indicated that long-term survival in a face allograft model is possible, without side effects, under a low maintenance dose of immunosuppression.[10,11] In an experimental model of limb allograft, long-term survival and tolerance were achieved across a major histocompatibility complex (MHC) barrier using a 7-day protocol of αβ-TCR mAb and cyclosporin A.[12,13]

Recent papers devoted to the use of CTA in clinical practice discuss the clinical and functional outcome of CTAs, donor-recipient matching for CTAs, the risk of immunosuppression and chronic rejection, and generation of clinical tolerance.[2,14]

Based on the experience gained from solid organ transplants, this review discusses immunologic aspects of graft acceptance and rejection in CTA transplants. An overview of transplant immunology and of immunosuppressive protocols in CTA transplants would most certainly interest plastic surgeons, for in the near future the application of CTA transplants may well become routine in plastic and reconstructive surgery.

IMMUNOLOGIC FACTORS IN GRAFT ACCEPTANCE AND REJECTION

MHC Molecules and the Rejection Process

Genetic disparities of MHC molecules between the donor and recipient impel the recipient's immune system to perceive grafted antigens as foreign; accordingly, it destroys grafted tissue. MHC molecules are not equally distributed on all cells of the body. MHC class I molecules are constitutively expressed on the surface of most nucleated cells in the body, whereas MHC class II molecules are restricted to the professional antigen-presenting cells (APCs), for example, dendritic cells (DCs) and activated macrophages; and to B cells, activated T cells, and vascular endothelial cells. The expression of MHC molecules on cells is controlled by various cytokines and may be upregulated by IFN-γ and TNF, which are powerful inducers of MHC expression on many cell types. MHC class II molecules are targets for rejection because they bind antigen into their peptide-binding site and present this antigen to T cells.[15]

The Role of T-Helper Cells and Their Subpopulations Th1 and Th2 in Allograft Rejection

T-lymphocyte precursors acquire the ability to recognize antigens through the development of specific surface receptors.[16] The definitive T-cell lineage marker is the T-cell antigen receptor (TCR). Based on the TCR marker, T-cells are subdivided into two different types: αβ-T cells and γδ-T cells. αβ-T cells are responsible for most of the immune responses, whereas γδ-T cells usually respond to such antigenic challenges as viral and bacterial infections; they also function in antitumor immunity.[16]

Conventional T-cell responses against foreign proteins require the presentation of the foreign antigen to the APC in association with MHC molecules by direct or indirect pathways. The role of the T cell–mediated process was demonstrated in a mouse model lacking T cells. The mice that lacked the T cells accepted fully mismatched allografts, but reconstitution of the T cells immediately induced the rejection process.[17]

Rejection responses have a molecular basis in TCR-MHC interaction. The first step in activation of T cells is accomplished via TCR receptors through antigen recognition of donor-derived peptides in association with the MHC antigens expressed on the graft. The second step of T-cell activation entails delivery of costimulatory signals through the binding of T-cell molecules such as CD28 to its ligands CD80 or CD86 expressed on donor APCs.[18] After the costimulatory signal is completed, the T cells are able to secrete IL-2, which interacts with its TCR to generate a third signal in T-cell activation, which leads to T-cell differentiation. Activated T cells reject transplanted grafts via an immunologic mechanism including the release of proinflammatory cytokines.[15]

$CD4^+$ T cells are distinguished as either T-helper-1 (Th1) or Th2 based on the particular cytokines that are secreted upon activation.[19] Th1 cells

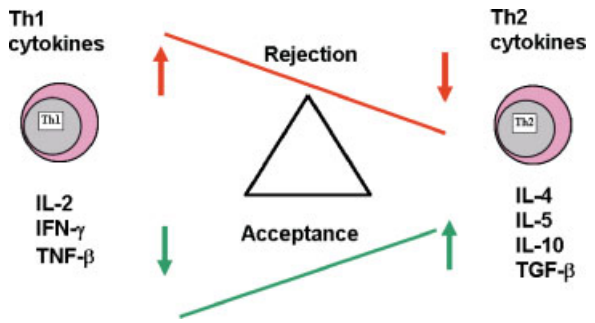

Figure 1 The balance of cytokines determines graft acceptance or rejection.

produce IL-2, which is involved in cellular rejection and is required for activation of T cytotoxic (Tc) cells. Th1 cells exclusively produce IFN-γ, which induces MHC expression, increases the activity of APCs, activates large granular lymphocytes, and finally, in concert with TNF-β, activates macrophages. Moreover, IFN-γ and TNF-β also upregulate the expression of adhesion molecules on vascular endothelium, which facilitates adhesion of leukocytes to the walls of blood vessels prior to their migration across the endothelium into the tissues.

Th2 cells produce IL-4, IL-5, IL-6, IL-10, IL-13, and TGF-β; and this profile of cytokines favors the acquisition of tolerance.[19] Cytokines IL-4, IL-5, and IL-6 are also required for B-cell activation, leading to the production of alloantibodies and humoral immunity. In a simplistic model, Th1 cytokines are involved in cellular immunity and in allograft rejection, whereas Th2 cytokines promote humoral immunity and allograft acceptance (Fig. 1).[19]

APCs Involved in Allorecognition

APCs are a heterogenous population of leukocytes that are present in the skin, lymph nodes, spleen, and thymus and within or underneath most mucosal epithelia. Lymphoid organs are a rich source of professional APCs that can select lymphocyte trafficking through the B- and T-cell areas. The T-cell area is rich in antigen-processing DCs, termed interdigitating cells. The B-cell area is enriched with another type of cell called follicular dendritic cells.[16] In the skin epidermis, active cells able to present antigen to primed T cells and named Langerhans cells were identified. APCs are rich in class II MHC molecules, which are important for presenting antigen to Th cells.

The APCs activating rejection can come from either the donor or the recipient. Allograft rejection may occur via two distinct pathways: the direct and indirect mechanisms of allorecognition. T cells reacting via the direct pathway recognize donor MHC molecules on the surface of donor-derived APCs. Donor APCs migrate out of the allograft to the draining lymph nodes and spleen and stimulate recipient T cells directly. In contrast, indirect recognition requires that the recipient APCs process the donor MHC antigens that have been continuously shed from the graft before presenting it to the recipient T cells in a self-restricted manner.[20]

It has been suggested that the direct pathway predominates during early acute rejection, whereas the indirect pathway provides a continuous supply of alloantigen responsible for chronic rejection.[20] Accordingly, direct activation is a more powerful stimulus of rejection than the indirect route.

THE DYNAMICS OF REJECTION

The rejection process is an inflammatory process developed by a specific immunologic response and is characterized by different effector mechanisms. CTA represents diverse tissue components with varying antigenicity. Although there are no standardized criteria for characterizing the severity or type of rejection of CTA at the clinic, a rudimentary classification system for acute rejection of the skin component of CTA transplants has been recently introduced.[21] CTA rejection is assumed to be mediated by mechanisms similar to those of solid organ transplantation.[21] Based on understanding of the rejection process in solid organ transplants, brief descriptions are presented below of the various mechanisms that underlie the rejection process in CTA.

Hyperacute Rejection

Hyperacute rejection develops very rapidly within a few minutes after transplantation and is a cause of graft function failure. Hyperacute rejection represents the classic example of the antibody-mediated rejection process associated with preexisting antibodies against the MHC of donor cells. Anti-MHC antibodies may be present in the recipient's circulation due to prior blood transfusions, rejection of the previous transplants, or multiple pregnancies. In addition, the presence of antibodies against the ABO blood group system can induce hyperacute rejection.[22] Most of the damage that occurs during the hyperacute rejection process affects the endothelial cells of capillary vessels and small arterioles. Immunologic complexes on the vessel endothelial cells activate the complement and coagulation cascade, which leads eventually to damage of the vascular endothelium and ultimately to graft dysfunction.[22]

Accelerated Rejection

Development of accelerated rejection takes place between 24 hours to 5 days after transplantation. The increased risk of developing an accelerated phase of rejection arises from sensitization of the allograft recipient, for example by the first allograft. Accelerated

rejection is associated with interstitial hemorrhages, vascular thrombosis, and leukocyte infiltrations. Graft destruction is accompanied by extensive endothelial deposition of humoral mediators IgM, IgG, C3, fibrin and leukocyte infiltrations, and by alloantibody production as well. Proinflammatory cytokines such as IFN-γ and IL-6 are present within the graft and contribute to graft damge.[23] Accelerated rejection is considered a classic humoral mediated response; it precedes T cell–dependent mechanisms.

Acute Rejection

Acute rejection occurs within a few days to a few weeks after transplantation and is usually complete within 1 to 6 months. Acute humoral rejection is clinically determined by the following factors: morphologic evidence of tissue injury, the presence of capillary C4 deposition as the immunopathologic evidence for antibody-mediated action, and serologic evidence of circulating antibodies to donor human histocompatibility antigens (HLA) or other endothelial antigens.[24] This process may be related to changes in the graft arteries or parenchymal cells. During acute rejection, fibrinoid necrosis of media arteries and aggregation of platelets and fibrin in lumen of vessels lead to vascular obstruction. In addition, leakage and focal necrosis occur, and inflammatory cells accumulate in the interstitium of the graft. Acute rejection arises from the primary activation of T cells and the consequent triggering of various effector mechanisms (Fig. 2).

Cellular components in acute human CTA rejection include vasculitis, dermatitis, myositis, and perineural involvement—confirmed through the evaluation of skin biopsy specimens from the first human hand and face recipients[25,26] and from skin biopsies provided by multicenter hand and abdominal wall transplant cases.[21] However, severe vascular rejection in solid organ allograft recipients has been attributed to a humoral mechanism of the alloantibody-mediated

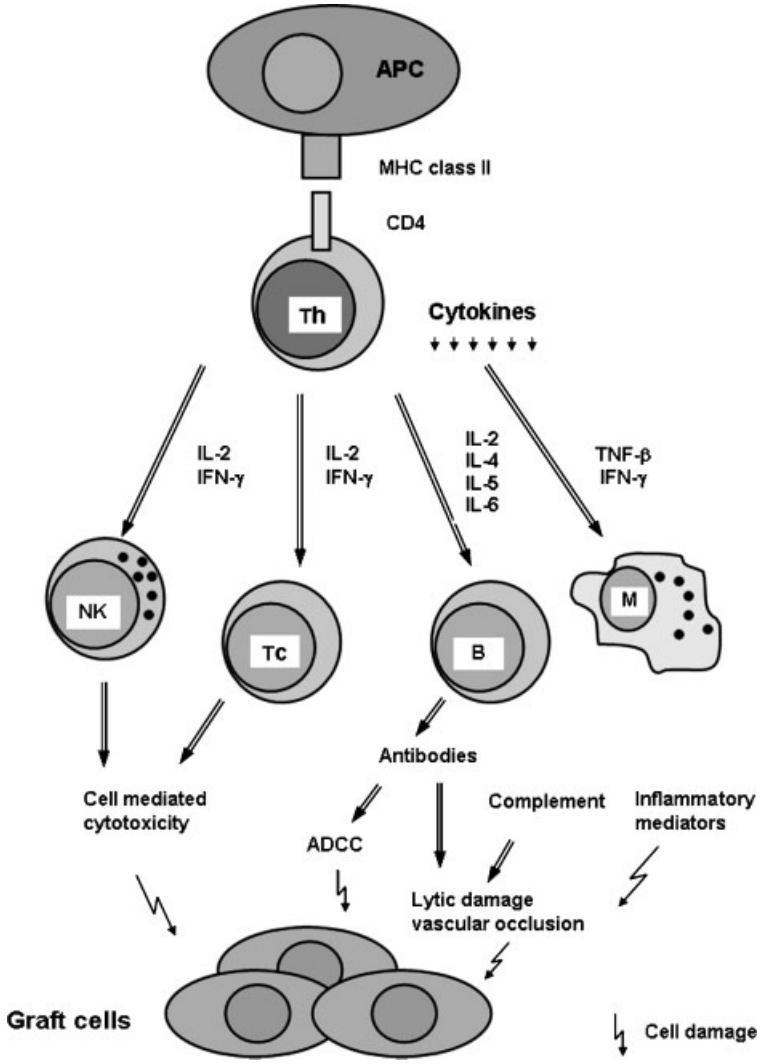

Figure 2 Schematic overview of immunologic mechanism of acute allograft rejection.

syndrome as a consequence of alloantibody production against donor HLA.[27] In contrast with solid organ transplant recipients, anti-HLA antibodies were never detected in human hand transplant recipients.[28] Clinical results suggested that a humoral mechanism was not responsible for the acute CTA rejection process in hand transplant recipients. This contrasts with preclinical observations in the model of non-human primates, where alloantibodies were detectable after limb allograft rejection.[29] Future studies are therefore needed to clarify the role of alloantibodies in CTA rejection.

Chronic Rejection

Depending on the genetic disparity between the donor and recipient and the use of immunosuppressive treatment, chronic graft rejection can be a slow process occurring over months or years. Chronic allograft destruction thus shares a characteristic with all solid organ and CTA transplants. The frequency of chronic rejection is correlated with several risk factors, for example, acute rejection episodes, recipient sensitization, insufficient MHC-matched antigens, recipient age and race, inadequate immunosuppression, hypertension, cytomegalovirus (CMV) infection/reactivation, prolonged ischemic time of the graft before transplantation, smoking, and hyperlipidemia.[30] Chronic rejection is the result of graft injury by immunologic and nonimmunologic factors.

Immunologic Factors of Chronic Rejection

The chronic rejection process is still not fully understood in CTA transplants. Current investigations of CTA rejection focus on clinicopathologic and histologic evaluation.[26] In the case report of a hand allograft recipient, the allograft was rejected 29 months after transplantation, in the absence of regular maintenance immunosuppression because of noncompliance on the part of the patient. In this case, clinicopathologic and histologic examination revealed features resembling the chronic lichenoid cutaneous graft-versus-host disease process[26]; however this single case is insufficient to identify the factors underlying chronic rejection in CTA transplants. From the similarity of histopathologic lesions observed in CTA specimens when compared with the histologic specimens of solid organ transplants, the possible mechanism of chronic rejection in CTAs can be inferred. These observations are reflected in the following discussion and are based on the well-established criteria of chronic rejection in solid organ transplants.

The hallmark of chronic graft rejection is found in injury to the endothelium of vessels. Initial vascular injury initiates an inflammatory cascade, which in turn leads to allograft artheriosclerosis and graft fibrosis. The cardinal feature of chronic rejection is luminal obliteration caused by blockage of the blood vessels in the graft. Blockage occurs because of the proliferation of smooth muscle cells that have migrated from the vessel wall and deposited matrix proteins. Ultimately, interstitial fibrosis

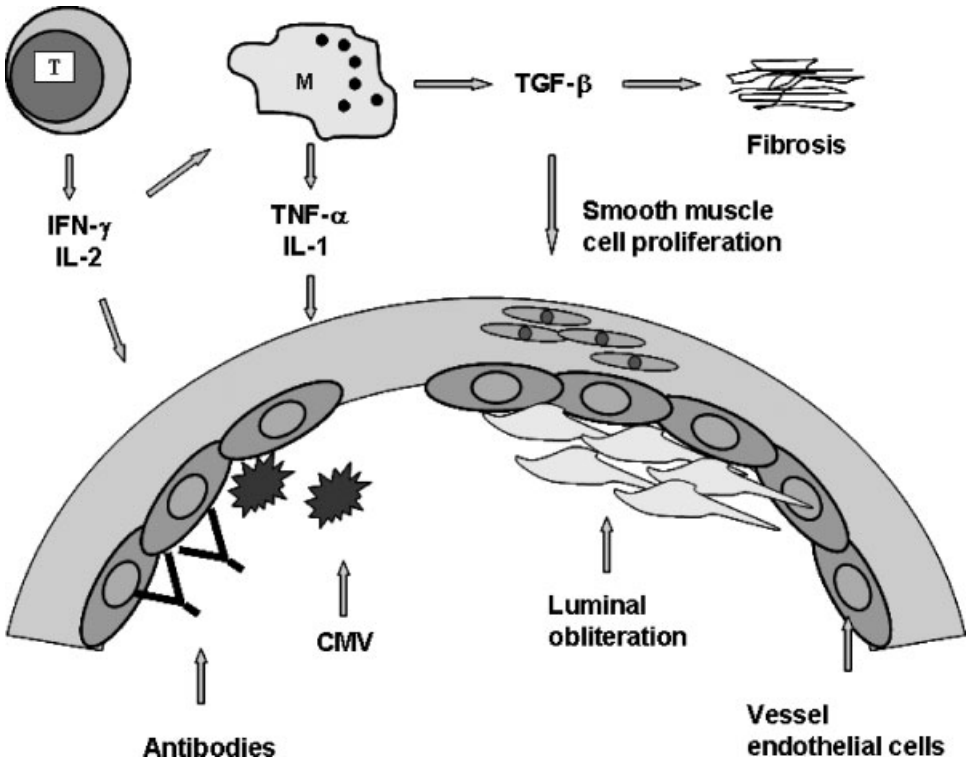

Figure 3 Schematic overview of immunologic mechanism of chronic allograft rejection.

leads to allograft dysfunction (Fig. 3). Many studies emphasize that both direct and indirect allorecognition of donor antigens promotes development of transplant vasculopathy.[20,31]

Nonimmunologic Factors of Chronic Rejection

The fact that organs from living donors are superior to those of cadaver donors suggests that brain death can influence graft quality. Brain death involves a syndrome that includes rapid swings in blood pressure, hypotension, coagulopathy, pulmonary changes, hypothermia, and electrolyte abnormalities.[30] Animal models reveal that after explosive brain death, levels of catecholamines and cytokines increase and MHC class I and II antigens are upregulated, suggesting that immunogenicity is increased in the peripheral organs.[32] Increasing levels of proinflammatory cytokines in the transplanted organs and in the serum were also confirmed in brain death human donors.[33]

A second nonimmunologic factor that may increase the immunologic response is ischemia/reperfusion injury. Ischemia/reperfusion may contribute not only to early delayed graft function but also to late allograft dysfunction, and it may be a risk factor for chronic rejection. Total ischemia time of an allograft includes transient warm ischemia time before or during allograft removal from the donor, cold ischemia time associated with preservation, and ischemia time during the period of revascularization.[31] The factors associated with organ removal, storage, and engraftment may increase the immunogenicity of allografts by upregulating MHC antigens and activating the cytokine adhesion molecule cascade, which in turn leads to leukocyte infiltration and ultimately to obliterative vasculopathy and fibrosis.[34] The mechanisms by which these insults may contribute to ultimate graft dysfunction remain unclear.

IMMUNOMODULATION AND TRANSPLANTATION TOLERANCE

Transplantation of CTAs was developed after 1990 when modern immunosuppression with calcineurin inhibitors (cyclosporin A [CsA], tacrolimus), antiproliferative agents (mycophenolate mofetil [MMF], azathioprine), antibodies (anti-thymocyte globulin [ATG], anti-CD25 monoclonal antibody [MoAb], anti-CD3 MoAb), and steroids (methylprednisolone, prednisolone) was shown to improve the effectiveness of transplantation surgery. Current studies are focused on immunosuppressive agents that effectively induce allograft acceptance with minimal toxic side effects. Immunosuppressive drugs are used in transplantation procedures for induction and maintenance and for treatment of the rejection process.[35]

The goal of induction therapy is to inhibit the immune system so that it becomes unable to marshal an immediate immunologic response leading to acute rejection (Fig. 4). Currently for induction therapy, polyclonal antibody ATG, and MoAb anti-CD3 (OKT-3) and against IL-2 receptor (daclizumab and basiliximab) are used in clinical practice.[35,36]

The immunosuppressive agents currently used for maintenance therapy in solid organs and CTA transplants are calcineurine inhibitors such as CsA and tacrolimus, which inhibit activation of T cells due to suppression of IL-2 production by T cells.[35] Treatment with MMF causes death by apoptosis in a large proportion of activated T cells by inhibiting DNA synthesis and suppressing antibody formation by B cells. Sirolimus provides immunosuppression by inhibiting activation signals; it does this by affecting the G_1 phase of the cell cycle and blocking the second signals of activation delivered by IL-2, IL-4, and IL-6 to T cells.[35]

Because of the different degree of antigenicity of the CTA components (in contrast with solid organ transplants), the rejection process in CTA is mediated by different cellular and humoral mechanisms. Skin being the most immunogenic component of the CTA, it therefore requires a stronger degree of immunosuppression in comparison with solid organ transplants.

Clinical experience with immunosuppressive protocols in CTA including hand transplants[1,37] abdominal wall transplant,[6] and very recently partial face transplant[7] were based on the protocols used in solid organ transplant. In hand and face transplant recipients, induction therapy with ATG, tacrolimus, MMF, and prednisone was used. In addition, some hand transplant recipients were treated with anti-CD25 MoAb, whereas the face transplant recipient received donor bone marrow transplantation (BMT) as a supportive therapy.[7] Maintenance therapy consisted of prednisone, MMF, and tacrolimus. Abdominal wall recipients received anti-CD52 MoAb and tacrolimus as an induction therapy without use of corticosteroids during maintenance therapy.[6] All CTA recipients received antiviral, antibacterial, and antifungal prophylaxis. Episodes of rejection were treated with increased dosages of corticosteroids.

Immunosuppressive agents significantly improved the life of transplant recipients; however, the risk of serious side effects persists as a major problem in the field of transplantation. Modern immunosuppressants, which are used in the clinic, may generate side effect such as nephropathy hypertension, hyperglycemia, hyperlipidemia, and posttransplant diabetic complications that are associated with calcineurin inhibitors therapy.[35] MMF can cause gastrointestinal upset and may induce leukopenia but demonstrated antitumor effect.[38,39] Steroids are associated with several complications such as hypertension, diabetes, weight gain, osteoporosis, gastrointestinal lesions, and poor wound healing. Prolonged immunosuppressive therapy gives rise to the recurring

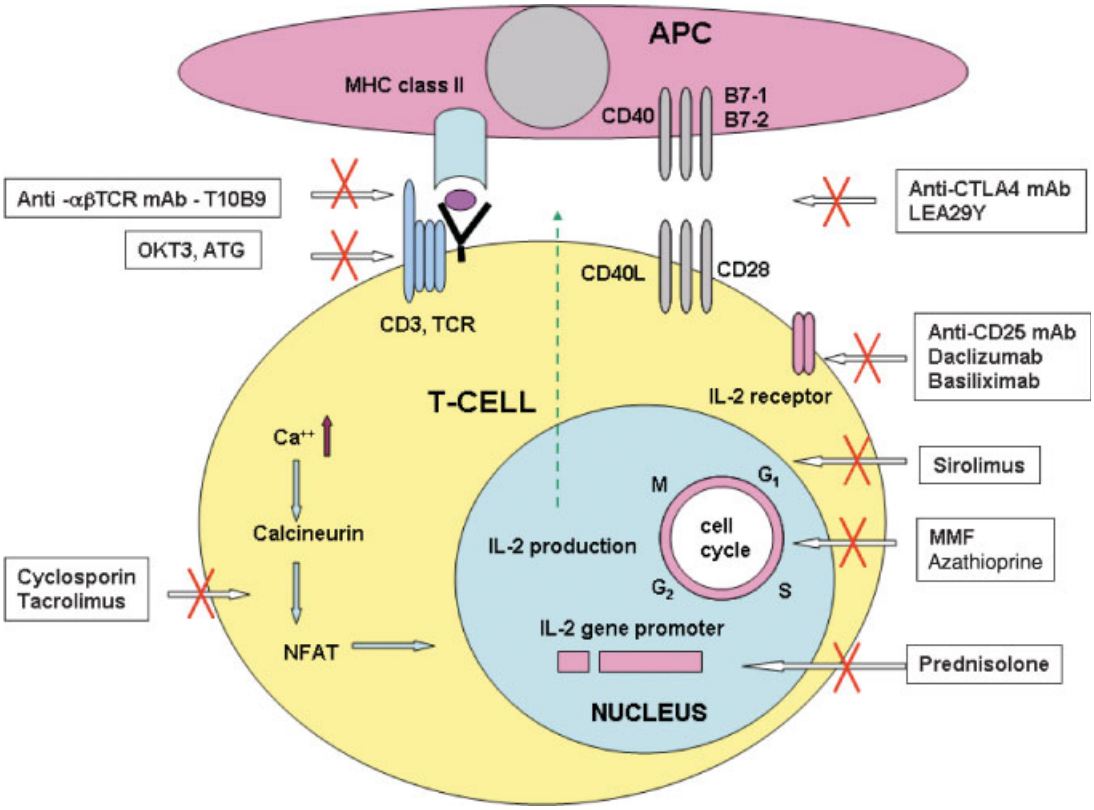

Figure 4 Current immunosuppressive agents and schematic overview of mechanism and sites of action.

problem of opportunistic infections, which can lead to allograft failure. The most serious complication of chronic immunosuppression lies in the risk of malignancy, which may be an effect of prolonged calcineurin inhibitors or antimetabolites therapy.[38] Induction therapy with anti-CD3 MoAb or antilymphocyte antibody is associated with the development of posttransplant lymphoproliferative disorder (PTLD). In contrast, induction therapy with anti IL-2 receptor agents did not increase the risk of PTLD.[39]

A combination of immunosuppressive drugs is often used for maintenance therapy to reduce the immune system's ability to recognize and therefore reject transplanted tissue. Combination therapy often has a more powerful immunosuppressive effect than monotherapy because each drug inhibits the immunologic response using a different mechanism. Administering low doses of each individual agent reduces drug-related side effects without totally compromising the immune system's ability to protect itself against opportunistic infections and malignancies. The same drugs used for induction and maintenance therapy are administered to patients during episodes of acute rejection.[35]

CONCLUSION

This overview of the immunologic aspects of allograft transplantation clearly indicates that although CTAs differ immunologically from solid organ transplants, our knowledge of allograft acceptance, rejection, and immunosuppressive protocols can nevertheless be directly applied to CTA transplants. Indeed, CTAs already constitute a significant component in the armamentarium of plastic and reconstructive surgery.

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