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Clinical Immunosuppression

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1. Introduction

Organ transplantation is the act of transferring organs from donors to recipients. Thus, the malfunction of an organ system can be corrected with the transplantation of an organ; organs that have successfully been transplanted include the kidney, liver, heart, lung, and pancreas. Over the past 50 years, the medical community has witnessed great advances in the care of liver transplant recipients, including new immunosuppression modalities, therapies for chronic rejection, and improved operative and preservation techniques. However, the immune system remains the most formidable barrier for clinicians to perform transplantations as a routine form of medical treatment[1].

The immune system has developed elaborate and effective mechanisms to combat foreign agents, including antigens derived transplantation; thus, in transplant recipients, transplantation elicits a complex series of immunological processes. Rejection results when a pathologic and intense inflammatory response develops, or when repair and remodeling of tissues fails. Knowledge of these mechanisms is important to understand the clinical features of rejection, which enables early diagnosis and the delivery of appropriate treatments[1]. These mechanisms are generally categorized as inflammation, immunity, tissue repair, and structural reinforcement of damaged tissues. Comprehension of these mechanisms is also critical for the development of novel drugs, treatments, and strategies to minimize rejection and inhibit the effects of the immune system on transplanted organs, helping to extend the survival and functionality of transplanted organs[2].

The introduction of routine pretransplant screening of graft recipients for anti-donor antibodies has made hyperacute rejection rare. However, no accepted therapeutic strategy to treat chronic rejection is currently recognized. The control of acute rejection has been the primary aim of immunosuppression, thereby allowing tissue repair to progress[3].

The use of combination immunosuppressive therapy has evolved over time. With all the successes of immunosuppressive therapies comes the obligation to tailor treatments to meet the individual patient's characteristics and to balance the risks and benefits of these medications.

Transplantation tolerance could eliminate many of the adverse events associated with immunosuppressive agents. Safe and reliable strategies for the induction of full tolerance have not yet been developed. However, methods to induce states of "partial tolerance" have

been discovered, where lower-than-conventional amounts of ongoing pharmacologic immunosuppression are needed[3, 4]. Although the induction of immunologic tolerance has been achieved and studied in numerous laboratory animal models, immune tolerance remains an elusive goal of transplantation immunology and clinical organ transplantation.

2. History of immunosuppression

Early efforts at transplantation were unsuccessful because of inadequacies in surgical techniques and a fundamental lack of knowledge regarding the immune system. The development of immunosuppressive drug treatments enabled organ transplantation and improved the survival of transplanted organs since the first liver transplant by Dr. Thomas E. Starzl in 1963. Since then, many new and progressively more selective immunosuppressive agents and treatment strategies have been developed. As knowledge of the immune system evolved, therapies that targeted specific immunoregulatory organs enabled the ability to prolong life through organ transplantation. Initial attempts at immunosuppression were with total body radiation, but all of the patients died. In 1949, corticosteroids were used to treat autoimmune disorders and to prevent allograft rejection. Since then, many new and progressively more selective immunosuppressive agents have been developed. These therapies have enabled organ transplantation and improved the survival of transplanted organs. In 1959, cyclophosphamide was demonstrated to suppress antibody production and was used for bone marrow transplantation. In the 1960s, azathioprine (AZA) was found to delay organ graft rejection and was used to suppress the rejection of transplanted kidneys. The first polyclonal anti-lymphocyte globulin was used in 1967, and it spawned the development of other polyclonal and monoclonal antibodies for immunosuppressive therapy. After the first initially successful series of transplantations were performed between 1962 and 1964 in Denver, Colorado, the combination of azathioprine and steroids came into widespread use, becoming part of the primary immunosuppressive regimen for the next 20 years. The T cell-inhibiting properties of cyclosporine, a calcineurin inhibitor, were discovered in 1976. Subsequently, cyclosporine (Sandimmune and later, Neoral) was introduced in the 1980s, when it was used in combination with azathioprine and steroids to prevent rejection in allograft transplants. Its use was credited with a dramatic improvement in graft survival. In 1969, methotrexate was shown to inhibit antibody production and the development of delayed hypersensitivity in guinea pigs[5].

The development of mycophenolate mofetil (MMF), an inosine 5'-monophosphate dehydrogenase (IMPDH) inhibitor, began in 1982, and research continues on other IMPDH inhibitors. Mycophenolate mofetil rapidly replaced azathioprine almost universally. The next advance occurred in 1987, with the introduction of tacrolimus (FK506) to inhibit interleukin (IL)-2 production and lymphocyte proliferation. Tacrolimus has gradually supplanted cyclosporine in many transplant centers. Interest in the antibiotic sirolimus (SRL), which is also known as rapamycin, was renewed in the 1980s when it was shown to prevent allograft rejection.

Other immunosuppressive agents and their dates of discovery include mizoribine in 1981, leflunomide in 1978, deoxyspergualin in 1981, muromab-CD3 (OKT3) in 1985, brequinar in 1986, azodicarbonamide in 1989, vitamin D analogs such as MC1288 in 1991, and bisindolylmaleimide VIII in 1999. Other agents that were developed include Minnesota anti-lymphocyte globulin and anti-thymocyte globulin (ATG)[6].

3. Clinical stages of rejection

Rejection is the consequence of the recipient's alloimmune response to the non-self antigens expressed in donor tissues[7]. Clinically, it can be divided into the following three stages:

3.1 Hyperacute rejection

In hyperacute rejection, the transplanted tissue is rejected within minutes to hours after graft implantation because transplant patients are serologically presensitized to nonself graft antigens, which are known as alloantigens. Histologically, numerous polymorphonuclear leukocytes (PMNs) exist within the graft vasculature and are associated with widespread microthrombi formation and platelet accumulation. Little or no leukocyte infiltration occurs. Hyperacute rejection is humorally mediated and occurs because the recipient has pre-existing antibodies against graft-derived antigens, which can be induced by prior blood transfusions, multiple pregnancies, prior transplantation, or xenografts. The antigen-antibody complexes activate the complement system, causing massive thrombosis in the capillaries, which prevents graft vascularization. The liver is relatively resistant to hyperacute rejection. Although this may be due to its dual blood supply, it is more likely because of incompletely understood immunologic properties. Hyperacute rejection has become relatively rare since the introduction of routine pretransplantation screening of graft recipients for anti-donor antibodies.

3.2 Acute rejection

Acute rejection can occur within 24 hours of graft implantation and continue over a period of days to weeks. Acute rejection commonly manifests within the first 6 months after transplantation.

Acute cellular rejection is mediated by lymphocytes that have been activated against donor antigens, primarily in the lymphoid tissues of the recipient. Graft antigens are recognized by T cells and the resulting release of cytokines eventually leads to tissue distortion, vascular insufficiency, and cell destruction. Histologically, leukocytic infiltration that is dominated by equivalent numbers of macrophages and T cells is observed within the interstitium. The donor dendritic cells, which are also called passenger leukocytes, enter the circulation and function as antigen-presenting cells (APCs).

The primary aim of immunosuppression has been to control acute rejection by allowing time for tissue repair to occur. Most combination therapies block T-cell activation by providing intense immunosuppression during the immediate post-transplantation period known as the induction phase. Later, during the immunosuppressive maintenance phase, multiple-drug cocktails are administered to maintain a state of low- or nonresponsiveness to the allograft.

3.3 Chronic rejection

Chronic rejection develops months to years after acute rejection episodes have subsided. Chronic rejections are both antibody- and cell-mediated. The use of immunosuppressive drugs and tissue-typing methods has increased the survival of allografts in the first year, but chronic rejection remains unpreventable in most cases[5].

Although chronic rejection appears as fibrosis and scarring in all transplanted organs, the specific histopathological picture depends on the organ transplanted. In liver transplants, chronic rejection is characterized by vanishing bile duct syndrome. Histologically, progressive neointimal formation occurs within large and medium arteries and, to a lesser extent, within the veins of the graft. Leukocyte infiltration usually is mild or absent. These processes result in reduced blood flow, with subsequent regional tissue ischemia, fibrosis, and cell death. In chronic rejection, pathologic tissue remodeling results from peritransplant and posttransplant trauma. Cytokines and tissue growth factors induce smooth muscle cells to proliferate, migrate, and produce new matrix material. Interstitial fibroblasts are also induced to produce collagen. The factors that can increase the risk of chronic rejection include previous episodes of acute rejection, inadequate immunosuppression, extended periods of cold ischemia, the development of posttransplant infections such as cytomegalovirus (CMV), initial delayed graft function, and organ reperfusion injury.

Currently, unless inadequate immunosuppression is the cause of rejection, no accepted therapeutic strategies exist for the reversal of chronic rejection. The CD40 and CD28 pathways have been proposed as being important in initiating T-cell responses and lowering T-cell activation thresholds, respectively. Therefore, blocking T-cell costimulation has been proposed to improve long-term outcomes.

4. Mechanisms of rejection

The immune response to a transplanted organ consists of both cellular (lymphocyte-mediated) and humoral (antibody-mediated) mechanisms. The evolving understanding of liver allograft rejection was reviewed by Eksteen[23].

4.1 Acute humoral rejection

Humoral rejection is a form of allograft injury and subsequent dysfunction that is primarily mediated by antibodies and complement. The antibodies involved are either preformed antibodies or anti-donor antibodies that develop following transplantation. Proteinuria is associated with donor-specific antibody detection; it is likely an important factor in the rapid decline in glomerular filtration rates and early graft failure in patients that develop de novo anti-HLA antibodies. The presence of even low levels of donor-specific antibodies, which may not be detected by complement-dependent cytotoxic and flow cytometry crossmatches, have been shown to be associated with inferior allograft outcomes. These patients may require augmented immunosuppression.

Following transplantation, the inactive product C4d from the classical complement activation pathway is deposited in peritubular capillaries (PTC); immune detection of this product in allograft biopsies is used for the diagnosis of antibody-mediated rejection. However, one study reported substantial fluctuations in C4d Banff scores in the first year following transplantation[8]; these authors suggested that these results may reflect the dynamic and unpredictable nature of the humoral process. Thus, C4d by itself may not be a sufficiently sensitive indicator, and detection of microvascular inflammation utilizing donor-specific antibodies may be more useful for diagnosing antibody-mediated rejection.

4.2 T cell-mediated rejection

Although other cell types are also involved, T cells are central in graft rejection. The rejection reaction consists of the sensitization stage and the effector stage.

Sensitization stage

In this stage, CD4 and CD8 T cells, via their T-cell receptors, recognize the alloantigens expressed on the cells of the foreign graft. Two signals are needed for antigen recognition; the first is provided by the interaction of the T cell receptor with the antigen presented by an MHC molecule, while the second signal is provided by a costimulatory receptor/ligand interaction. Of the numerous costimulatory pathways, the interaction of CD28 on the T cell surface with its APC surface ligands, B7-1 or B7-2 (commonly known as CD80 or CD86, respectively), has been most studied. In addition, cytotoxic T lymphocyte–associated antigen-4 (CTLA4) on T cells also binds to these ligands, providing an inhibitory signal. Other costimulatory molecules include CD40 and its ligand, CD40L (CD154)[9, 10].

Typically, the helices of the MHC molecules form the peptide-binding groove and are occupied by peptides derived from normal cellular proteins. Thymic or central tolerance mechanisms (clonal deletion) and peripheral tolerance mechanisms, such as anergy, ensure that these self-peptide MHC complexes are not recognized by T cells, thereby preventing autoimmune responses.

At least 2 distinct, but not necessarily mutually exclusive, pathways of allorecognition exist: the direct and indirect pathways. Each leads to the generation of different sets of allospecific T cell clones.

4.3 Direct pathway

In the direct pathway, host T cells recognize intact allo-MHC molecules on the surface of the donor cells or APCs. Mechanistically, host T cells see allo-MHC molecule + allo-peptide as being equivalent in shape to self-MHC + foreign peptide; therefore, host T cells recognize the donor tissue as foreign. This pathway is presumably the dominant pathway involved in early alloimmune responses.

The transplanted organ carries a variable number of passenger APCs in the form of interstitial dendritic cells. Such APCs have a high density of allo-MHC molecules and are capable of directly stimulating recipient T cells. The relative number of T cells that proliferate on contact with allogeneic or donor cells is extraordinarily high compared to the number of clones that target antigens presented by self-APCs. Thus, this pathway is important in acute allorejection.

4.4 Indirect pathway

In the indirect pathway, T cells recognize processed alloantigens that are presented as peptides by self-APCs. Secondary responses such as those that occur in chronic or late acute rejection are associated with T cell proliferative responses to a more variable repertoire, including peptides that were previously immunologically silent. Such a change in the pattern of T cell responses has been termed epitope switching or spreading.

A link between self-MHC + allopeptide-primed T cells and the development of acute vascular rejection has been demonstrated to be mediated in part by accelerated alloantibody production. In addition, chronic allograft vasculopathy may be mediated by T cells primed by the indirect pathway[11].

4.5 Molecular mechanisms of T cell activation

During T cell activation, membrane-bound inositol phospholipid is hydrolyzed into diacylglycerol (DAG) and IP3. This increases the concentration of cytoplasmic calcium. The elevation in calcium promotes the formation of calcium-calmodulin complexes that activate a number of kinases as well as protein phosphatase IIB or calcineurin. Calcineurin dephosphorylates cytoplasmic nuclear factor of activated T cells (NFAT), permitting its translocation to the nucleus, where it binds to the IL-2 promoter sequence and stimulates transcription of IL-2 mRNA. Numerous other intracellular events, including protein kinase C (PKC) activation by DAG and the activation of nuclear factor kappa B (NF-κB) also occur at the molecular level[10].

Effector stage

Alloantigen-dependent and -independent factors contribute to the effector mechanisms. Initially, nonimmunologic "injury responses" (ischemia) induce a nonspecific inflammatory response. Because of this, antigen presentation to T cells is increased because of the upregulated expression of adhesion molecules, MHC class II, chemokines, and cytokines. It also promotes the shedding of intact, soluble MHC molecules that may activate the indirect allorecognition pathway. After activation, CD4 T cells initiate macrophage-mediated delayed type hypersensitivity (DTH) responses and provide help to B cells to initiate antibody production.

Various T cells and T cell-derived cytokines such as IL-2 and IFN-γ are upregulated early after transplantation. Later, ß-chemokines, including regulated upon activation, normal T cell expressed and secreted (RANTES), IP-10, and MCP-1 are expressed, promoting intense macrophage infiltration of the allograft. IL-6, TNF-α, inducible nitric oxide synthase (iNOS) and growth factors also play a role in this process. Growth factors, including TGF-ß and endothelin, cause smooth muscle proliferation, intimal thickening, interstitial fibrosis, and, in the case of the kidney, glomerulosclerosis.

Endothelial cells activated by T cell–derived cytokines and macrophages express MHC class II, adhesion molecules, and costimulatory molecules. Therefore, these cells can present antigen and recruit more T cells, amplifying the rejection process. CD8 T cells mediate cell-mediated cytotoxicity reactions by inducing cell lysis or apoptosis.

4.6 Apoptosis

The final common pathway for cytolytic processes is the triggering of apoptosis in the target cell. After CTL activation, CTLs produce cytotoxic granules that contain perforin and granzymes. At the time of target cell identification and engagement, these granules fuse with the effector cell membrane and extrude their content into the immunological synapse. By a yet unknown mechanism, the granzymes are inserted into the target cell cytoplasm where granzyme B triggers apoptosis through several different mechanisms, including the

direct cleavage of procaspase-3 and the indirect activation of procaspase-9. This has been shown to play the dominant role in apoptosis induction in allograft rejection.

Alternatively, CTLs can also use the Fas-dependent pathway to induce cytolysis and apoptosis. The Fas pathway is also important in limiting T cell proliferation in response to antigenic stimulation; this is known as fratricide between activated CTLs. Cell-mediated cytotoxicity has been shown to play an important role in acute, but not chronic, allograft rejection[12].

4.7 Role of natural killer cells

Natural killer (NK) cells are important in transplantation because of their ability to distinguish allogenic cells from self and their potent cytolytic effector mechanisms. These cells can mount maximal effector responses without any prior immune sensitization. Unlike T and B cells, NK cells are activated by the absence of MHC molecules on the surface of target cells, which is commonly referred to as the "missing self" hypothesis. Recognition is mediated by various NK inhibitory receptors triggered by specific alleles of MHC class I antigens on cell surfaces.

In addition, NK cells also possess stimulatory receptors, which are triggered by antigens on nonself cells. NK cell effector responses include both cytokine release and direct toxicity mediated through perforin, granzymes, Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL). Through this "double negative" mode of activation, they are thought to play a role in the rejection of both bone marrow transplants and transplanted lymphomas in animal models.

NK cells also provide help to CD28-positive host T cells, thereby promoting allograft rejection. Their importance in bone marrow transplants has been recognized for years. In humans, the NK cell-mediated graft-versus-host alloresponse has been used for its potent graft-versus-leukemia effect, contributing to an increase in the rate of sustained remission in patients with acute myelogenous leukemia[11].

NK cells are now being recognized as active participants in acute and chronic rejection of solid tissue grafts. Recent studies have indicated that NK cells are present and activated following infiltration into solid organ allografts. They may regulate cardiac allograft outcomes. Studies have also shown that humans with killer cell immunoglobulin-like receptors that are inhibited by donor MHC have a decreased risk of liver transplant rejection. In cases of renal transplantation, NK cells are not suppressed by the current immunosuppressive regimens[13].

4.8 Role of innate immunity

Although T cells play a critical role in acute rejection, the up-regulation of proinflammatory mediators in the allograft is now recognized to occur prior to T cell-mediated responses. This early inflammation following engraftment is due to the innate response to tissue injury that is independent of the adaptive immune system. Several recent studies have examined the role of Toll-like receptor (TLR) agonists and TLR signals in allorecognition and rejection.

The activation of innate mechanisms alone does not appear to be sufficient to lead to graft rejection itself. However, they are important for optimal adaptive immune responses to the

graft and may play a major role in resistance to tolerance induction. The development of methods to blunt innate immune responses, which has potential implications for a wide variety of diseases, is likely to have a significant impact on transplantation as well.

5. Minimizing rejection

Rejection is the consequence of the recipient's alloimmune response to the nonself antigens expressed in donor tissues. Although rejection cannot be completely prevented, a degree of immune tolerance to the transplant can develop. Several concepts have been postulated to explain the development of partial tolerance. They include clonal deletion, the development of anergy in donor-specific lymphocytes, development of suppressor lymphocytes, and the production of factors that down-regulate the immune response against the graft. Other hypotheses include the persistence of donor-derived dendritic cells in the recipient that promote an immunologically mediated chimeric state between the recipient and the transplanted organ.

Tissue typing or crossmatching is performed prior to transplantation to assess donor-recipient compatibility for human leukocyte antigen (HLA) and ABO blood group.

The ABO blood group compatibility is tested first because incompatibility between the blood groups leads to rapid rejection. In the lymphocytotoxicity assay, patient sera are tested for reactivity with donor lymphocytes. A positive crossmatch is a contraindication for transplantation because of the risk of hyperacute rejection. The lymphocytotoxicity assay is used mainly for kidney transplantation.

The panel-reactive antibody (PRA) test screens the serum of a patient for lymphocytic antibodies against a random cell panel. Patients with prior transfusions, transplants, or pregnancies may have a high degree of sensitization and are less likely to have a negative crossmatch with a donor. A reduced risk of sensitization at the time of a second transplant has been observed when a more potent immunosuppression therapy comprised of rabbit anti-thymocyte globulin, tacrolimus, and mycophenolate mofetil/sodium is utilized for nonsensitized primary kidney or kidney/pancreas transplant patients. Mixed lymphocyte reactions (MLR) can be used to assess the degree of major histocompatibility complex (MHC) class I and class II compatibility. However, this test is not rapid and can be used only in cases involving living related donors. Therefore, this test is rarely used at present.

The primary aim of immunosuppression has been to control acute rejection by allowing tissue repair to develop. Immunosuppressive drugs are used in 2 phases: the initial induction phase, which requires much higher doses of these drugs, and the later maintenance phase.

Immunosuppression can be achieved by several mechanisms that affect lymphocytes including lymphocyte depletion, diversion of lymphocyte traffic, or blocking lymphocyte response pathways[14, 15].

Most combination therapies block T-cell activation by providing intense immunosuppression during the immediate posttransplantation period (induction phase).
Immunosuppressive agents exert their effects through the following mechanisms.

- Regulators of gene expression: The classic examples are glucocorticoids; others include vitamin D analogs and deoxyspergualin. However, recent studies have shown that glucocorticoids affect inflammation by other nongenomic mechanisms.

- Alkylating agents: Cyclophosphamide and other deoxyribonucleic acid (DNA) alkylating agents are mutagenic and can increase the risk of developing cancer.
- Kinase and phosphatase inhibitors: These include cyclosporin A (CsA), tacrolimus (FK506), and sirolimus (SRL), which inhibit kinase cascades.
- Inhibitors of de novo purine synthesis: The first-generation inhibitors are 6-mercaptopurine and azathioprine; the second-generation inhibitors are mizoribine and MMF. Potential third-generation enzyme targets include inosine monophosphate dehydrogenase and T lymphocyte-specific purine nucleoside phosphorylase. The polygentamate derivatives of methotrexate are antifolate compounds that inhibit de novo purine synthesis.
- Inhibitors of de novo pyrimidine synthesis: These inhibitors include brequinar, leflunomide, and the structurally related malononitrilamides that inhibit dihydroorotate dehydrogenase.

6. Clinically relevant immunosuppressive agents

Immunosuppressive agents are drugs that inhibit or block the activity of the immune system to prevent the rejection of transplanted organs and tissues.

These drugs are not without side effects and risks. Because the majority of immunosuppressive agents act non-selectively, the immune system is less able to resist infections and the spread of malignant cells. There are also other side effects, which include hypertension, dyslipidemia, hyperglycemia, peptic ulcers, and liver or kidney injury. Immunosuppressive drugs also interact with other medicines, affecting their metabolism and action. Actual or suspected immunosuppressive agents can be evaluated in terms of their effects on lymphocyte subpopulations in tissues using immunohistochemistry [16, 17].

Immunosuppressive drugs can be classified into the following groups:

6.1 Corticosteroids

Steroids have been the cornerstone of immunosuppression and remain important in treating episodes of acute rejection. However, newer treatment regimens are trying to minimize the use of steroids to avoid or minimize their adverse effects.

In pharmacologic or supraphysiologic doses, glucocorticoids are used to suppress various allergic, inflammatory, and autoimmune disorders. They are also administered as posttransplant immunosuppressants to prevent acute transplant rejection and graft-versus-host disease. Nevertheless, they do not prevent infection and also inhibit later reparative processes.

Glucocorticoids suppress cell-mediated immunity; they act by inhibiting the genes that encode the cytokines interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, and TNF-α. The inhibition of IL-2 is particularly important because it reduces T cell proliferation.

Glucocorticoids also suppress humoral immunity, causing B cells to express reduced amounts of IL-2 and IL-2 receptors. This diminishes both B cell clonal expansion and antibody synthesis.

Glucocorticoids also stimulate the release of lipocortin-1 into the extracellular space, where it binds to leukocyte membrane receptors and inhibits the following inflammatory events:

epithelial adhesion; emigration; chemotaxis; phagocytosis; respiratory burst; and the release of various inflammatory mediators including, but not limited to, lysosomal enzymes, cytokines, tissue plasminogen activator, and chemokines from neutrophils, macrophages, and mast cells.

6.2 Cytostatics

Cytostatics inhibit cell division. In immunotherapy, they are used in smaller doses than when used to treatment malignant diseases. They affect both T and B cell proliferation. As they are the most effective, purine analogs are most frequently administered.

Alkylating agents

The alkylating agents used in immunotherapy include cyclophosphamide (nitrogen mustards), nitrosoureas, platinum compounds, and others. Cyclophosphamide is probably the most potent immunosuppressive compound. In small doses, it is very efficient for the treatment of systemic lupus erythematosus, autoimmune hemolytic anemias, Wegener's granulomatosis, and other immune diseases. However, high doses cause pancytopenia and hemorrhagic cystitis.

Antimetabolites

Methotrexate is a folic acid analogue. It binds dihydrofolate reductase and prevents the synthesis of tetrahydrofolate. In addition to its use in transplant patients, it is used for the treatment of autoimmune diseases such as rheumatoid arthritis and Behcet's disease.

It is extensively used to control transplant rejection reactions. Azathioprine is nonenzymatically cleaved to mercaptopurine, which acts as a purine analogue and an inhibitor of DNA synthesis. Additionally, mercaptopurine itself can also be administered directly.

By preventing the clonal expansion of lymphocytes in the induction phase of the immune response, it affects both the cell and the humoral immunity. It is also efficient in the treatment of autoimmune diseases.

6.3 Antibodies

Antibodies are sometimes used as a quick and potent immunosuppressive therapy to prevent acute rejection episodes; additionally, antibodies such as monoclonal anti-CD20 antibodies are used in targeted treatment of lymphoproliferative or autoimmune disorders.

Antibodies interact with lymphocyte surface antigens, resulting in the depletion of circulating thymus-derived lymphocytes and interference with cell-mediated and humoral immune responses. Lymphocyte depletion occurs either by complement-dependent lysis in the intravascular spaces or by opsonization and subsequent macrophage phagocytosis. Adverse effects such as fever, chills, thrombocytopenia, leukopenia, and headache typically occur with the first few doses.

Heterologous polyclonal antibodies are obtained from the serum of animals (e.g., rabbit, horse) that were injected with the patient's thymocytes or lymphocytes. Currently, anti-lymphocyte (ALG) and anti-thymocyte antigens (ATG) are used. They are part of the

treatment strategy for cases of steroid-resistant acute rejection reaction and grave aplastic anemia. However, they are added primarily to other immunosuppressives to diminish their dosage and toxicity. They also enable transition to cyclosporine therapy.

Polyclonal antibodies inhibit T cells and cause their depletion through either complement-mediated cytolysis or cell-mediated opsonization that is followed by macrophage phagocytosis and degradation. In this way, polyclonal antibodies inhibit cell-mediated immune reactions, including graft rejection, delayed hypersensitivity, and graft-versus-host disease (GVHD); additionally they also influence thymus-dependent antibody production.

In 2005, two preparations became available: Atgam, obtained from horse serum, and Thymoglobulin, obtained from rabbit serum. Polyclonal antibodies affect all lymphocytes and cause general immunosuppression, possibly leading to post-transplant lymphoproliferative disorders (PTLD) or serious infections, such as those caused by cytomegalovirus. To reduce these risks, treatment is provided in a hospital, where adequate isolation from infection is available. Atgam and Thymoglobulin are usually administered for five days intravenously in the appropriate quantity. Patients stay in the hospital as long as three weeks to give the immune system time to recover to a point where there is no longer a risk of serum sickness.

Monoclonal antibodies are directed towards precisely defined antigens. Therefore, they cause fewer side effects. Especially significant are the antibodies directed against IL-2 receptor- (CD25-) and CD3. They are used to prevent organ transplant rejection, but also to track changes in the lymphocyte subpopulations. It is reasonable to expect the development of similar new drugs in the future.

T-cell receptor directed antibodies

Muromonab-CD3 is a murine anti-CD3 IgG2a monoclonal antibody that prevents T-cell activation and proliferation by binding the T-cell receptor complex that is present on all mature T cells. As such, it is one of the most potent immunosuppressive substances; it is commonly administered to control episodes of steroid- and/or polyclonal antibody-resistant acute rejection. Because it acts more specifically than polyclonal antibodies, it is also used prophylactically in transplantations.

However,the mechanism of action of muromonab-CD3 is only partially understood. It is known that the molecule binds the TCR/CD3 receptor complex. In the first few administrations, this binding non-specifically activates T-cells, leading to a serious syndrome 30 to 60 minutes later that is characterized by fever, myalgia, headache, and arthralgia. Sometimes the syndrome develops into a life-threatening reaction of the cardiovascular system and the central nervous system that requires lengthy therapy for recovery. After this period, CD3 blocks TCR-antigen binding and causes either conformational changes or the removal of the entire TCR3/CD3 complex from the T-cell surface. This lowers the number of available T-cells, perhaps by sensitizing them for uptake by tissue-resident macrophages. Also, cross-linking of CD3 molecules activates an intracellular signal that causes T cell anergy or apoptosis unless the cells receive another signal through a co-stimulatory molecule. Additionally, anti-CD3 antibodies shift the balance from Th1 to Th2 cells.

However, patients can develop neutralizing antibodies that reduce the effectiveness of muromonab-CD3. Muromonab-CD3 can also cause excessive immunosuppression. Although

anti-CD3 antibodies act more specifically than polyclonal antibodies, they lower the cell-mediated immunity significantly, predisposing patients to opportunistic infections and malignancies.

IL-2 receptor directed antibodies

Interleukin-2 is an important immune system regulator that is necessary for the clonal expansion and survival of activated T lymphocytes. Its effects are mediated by the trimeric cell surface IL-2 receptor, which is comprised of α, β, and γ chains. IL-2 receptor α (CD25, T-cell activation antigen, TAC) is expressed only by activated T lymphocytes. Therefore, it is of special significance for selective immunosuppressive treatment, and research has been focused on the development of effective and safe anti-IL-2 antibodies. Through the use of recombinant gene technology, murine anti-TAC antibodies have been modified, leading to the introduction of two chimeric mouse/human anti-Tac antibodies in 1998: basiliximab (Simulect) and daclizumab (Zenapax). These drugs act by binding the IL-2 receptor α chain, preventing IL-2-induced clonal expansion of activated lymphocytes and shortening their survival. Basiliximab and daclizumab are currently being used for the prevention of acute organ rejection after bilateral kidney transplantation; they are similarly effective and are associated with minimal side effects.

Other monoclonal antibodies

Efalizumab is a humanized monoclonal antibody that targets the lymphocyte function-associated antigen-1 (LFA-1) receptor through the CD11a side chain. Efalizumab (Raptiva), a drug indicated for psoriasis, was withdrawn from the US market on June 8, 2009 because of potential risks for progressive multifocal leukoencephalopathy (PML). PML is a rapidly progressive infection of the central nervous system caused by the JC virus that leads to death or severe disability. Demyelination associated with PML results from the JC virus infection. JC virus belongs to the genus *Polyomavirus* of the *Papovaviridae* family. PML should be considered in any patient exhibiting new-onset neurologic manifestations who has taken efalizumab. For more information, please see the Food and Drug Administration MedWatch Safety Alert.

Monoclonal antibodies against B7-1 (CD80) and B7-2 (CD86) have been developed to block T-cell CD28 activation and proliferation. In a recent trial, one of these antibodies, belatacept, was not inferior to cyclosporine as a means of preventing acute rejection after renal transplantation. Cytotoxic T lymphocyte antigen 4 immunoglobulin (CTLA4Ig) can simultaneously inhibit B7-1 and B7-2 interactions with CD28 and has been used successfully in animal models, demonstrating a beneficial effect on chronic allograft rejection. Monoclonal anti-CD45-RB, leflunomide, FK778, FTY720, alemtuzumab (anti-CD52 antibody), and rituximab are some of the other agents that are currently in different phases of evaluation. Other antibodies targeting CD28 are also in development.

6.4 Immunophilin-binding agents

The available immunophilin-binding agents are cyclosporine and tacrolimus. These agents are calcineurin inhibitors that primarily suppress T lymphocyte activation by inhibiting IL-2 production. They are associated with numerous toxicities that are often dose-dependent. Nephrotoxicity occurs with both the drugs. Hirsutism, gingival hypertrophy, hypertension, and hyperlipidemia develop more often with cyclosporine than tacrolimus.

Calcineurin inhibitors and azathioprine have been associated with post-transplant malignancies and skin cancers in organ transplant recipients. The results of several studies suggest that calcineurin inhibitors have oncogenic properties that are predominantly linked to the production of cytokines that promote tumor growth, metastasis, and angiogenesis. This drug has been reported to reduce the frequency of regulatory T cells; additionally, after converting from CNI monotherapy to a mycophenolate monotherapy, patients were found to have increased graft success and T-Reg frequencies.

Cyclosporine

Since its introduction in 1983, Cyclosporine has become one of the most widely used immunosuppressive drugs. It is a cyclic fungal peptide that is comprised of 11 amino acids. Cyclosporine is thought to bind to the cytosolic protein cyclophilin (an immunophilin) of immunocompetent T lymphocytes. The complex of Cyclosporine and cyclophilin inhibits the phosphatase calcineurin. The drug also inhibits lymphokine production and cytokine release, leading to the reduced functionality of effector T-cells. The adverse events of cyclosporine would be hair growth as well as trembling and shaking of hands. For cyclosporine, the target conventional trough levels (C0) levels were adapted as 300–400 ng/mL during the first postoperative month, 100–200 ng/mL for up to 1 year, and approximately 100 ng/mL or less thereafter. Cyclosporine is used to treat acute rejection reactions, but has been increasingly been replaced by newer, less nephrotoxic immunosuppressants.

Tacrolimus

Tacrolimus (trade name Prograf) is a product of the bacterium *Streptomyces tsukubaensis*. It is a macrolide lactone that acts by inhibiting calcineurin. Although tacrolimus is used particularly for liver and kidney transplants, in some clinics it is used for heart, lung and heart/lung transplants. It binds to the immunophilin FKBP1A; the tacrolimus-FKBP1A complex then binds to calcineurin and inhibits its phosphatase activity. In this way, Tacrolimus prevents the cell from transitioning from the G0 into G1 phase of the cell cycle. While tacrolimus binds to a different intracellular protein (FKBP-12) compared to cyclosporine, it has the same mechanism of action of cyclosporine, is more potent than Cyclosporine and is associated with less pronounced side effects compared to cyclosporine. Tacrolimus treatment was started orally to maintain a C0 whole blood level of 10–15 ng/mL initially with reduction of the dose to obtain C0 levels between 3-10 ng/mL at 1 year after liver transplant. However, neurotoxicity, alopecia, and posttransplant diabetes mellitus develop more frequently with tacrolimus compared to cyclosporine.

Advagraf

Advagraf is a new oral formulation of tacrolimus with prolonged-release characteristics compared to the currently authorised product Prograf(t). Advagraf therapy requires careful monitoring by adequately qualified and equipped personnel. Because the later product is nationally authorised, the invented name may vary depending on the country of authorisation. Advagraf is the first calcineurin inhibitor formulated to enable once daily oral dosing and it is expected that it may help to improve compliance with dosing and cause less interference with the daily life activities of the patient. This medicinal product should only be prescribed, and changes in immunosuppressive therapy initiated, by physicians experienced in immunosuppressive therapy and the management of transplant patients.

Voclosporin

Voclosporin is a calcineurin inhibitor that is under development by Isotechnika as of 2010. This company uses calcineurin as a surrogate marker to assess the amount of immunosuppression achieved using drugs in this category. Other companies also have next generation drugs in this class in their pipelines.

6.5 Antiproliferative agents

Antiproliferative agents inhibit DNA replication and suppress B cells and T cells proliferation. Azathioprine and MMF are commonly used antiproliferative agents. MMF is an organic synthetic derivative of the natural fermentation product mycophenolic acid (MPA) that causes the noncompetitive reversible inhibition of inosine monophosphate dehydrogenase, which interferes with purine synthesis. Adverse effects of MMF include nausea, diarrhea, leukopenia, and thrombocytopenia. Invasive CMV infection has also been rarely associated with MMF. The introduction of MMF has been shown to be associated with improvement or stabilization of renal function, even several years after transplantation.

Other antiproliferative agents, such as cyclophosphamide and, more recently, leflunomide, have also been used.

6.6 Mammalian target of rapamycin (mTOR) inhibitors

Sirolimus is a macrocyclic antibiotic produced by *Streptomyces hygroscopicus* fermentation. It is used to prevent rejection reactions. Although it is a structural analogue of tacrolimus, it acts somewhat differently and has different side effects. Sirolimus binds to FKBP-12 and modulates the activity of mTOR, which inhibits IL-2-mediated signal transduction and results in T- and B-cell cycle arrest in the G1-S phase. Sirolimus is associated with numerous adverse effects including leukopenia, thrombocytopenia, anemia, hypercholesterolemia, hypertriglyceridemia, proteinuria, ae well as leg oedema. Contrary to Cyclosporine and tacrolimus, drugs that affect the first phase of T lymphocyte activation, sirolimus affects the second phase of T lymphocyte activation, signal transduction and lymphocyte clonal proliferation. So sirolimus is not used early after transplant because of wound dehiscence. Also sirolimus carries a black box warning that cautions against possible development of early postttransplant hepatic artery thrombosis. Although sirolimus binds to FKBP-12 like tacrolimus, the complex inhibits mTOR, not calcineurin. Therefore, sirolimus acts synergistically with Cyclosporine, and when used in combination with other immunosuppressants, it has few side effects. Also, it indirectly inhibits several T lymphocyte-specific kinases and phosphatases, preventing their transition from G1 to the S phase of the cell cycle. In a similar manner, sirolimus prevents plasma cell differentiation, reducing the production of IgM, IgG, and IgA antibodies. It has also been associated with mucositis, delayed wound healing, lymphocele formation, pneumonitis, and prolonged delayed graft function. It is also active against tumors that are PI3K/AKT/mTOR-dependent.

6.7 Everolimus

Everolimus is the 40-O-(2-hydroxyethyl) derivative of sirolimus and works similarly to sirolimus as an mTOR (mammalian target of rapamycin) inhibitor. It is currently used as an

immunosuppressant to prevent rejection of organ transplants. Everolimus may have a role in transplantation as it has been shown to reduce chronic allograft vasculopathy in such transplants. Because hypercholesterolemia and hypertriglyceridemia have been reported, monitoring of blood lipid level is recommended.

6.8 Other drugs

Many other agents are used to interfere with secondary signaling, and may therefore aid in tolerance induction[18].

Interferons

IFN-β suppresses the production of Th1 cytokines and monocyte activation; it is used to slow down the progression of multiple sclerosis. IFN-γ can trigger lymphocyte apoptosis.

Opioids

Prolonged use of opioids can cause immunosuppression of both innate and adaptive immune responses. Decreased proliferation and function have been observed in macrophages and lymphocytes. It is hypothesized that these effects are mediated by opioid receptors expressed on the surface of these immune cell populations.

Small biological agents

Fingolimod is a new synthetic immunosuppressant that is currently in phase 3 of clinical trials. It increases the expression or changes the function of certain lymphocyte adhesion molecules, such as α4/β7 integrin, causing their accumulation in the lymphatic tissues and their subsequent removal from circulation. In this respect, it differs from all other known immunosuppressants.

The use of any immunosuppressive drug requires a balance between the risk of loss of transplanted organ and the toxicity of the agent. The goal is to balance an appropriate level of immunosuppression with the long-term risks, which include the development of infections, cancer, and metabolic complications.

7. Therapeutic management

7.1 Phases

Immunosuppressive treatment of the transplanted patient begins with the induction phase, which begins perioperatively and continues immediately after transplantation. Maintenance therapy then continues for the life of the allograft. Induction and maintenance strategies use different medicines at specific doses or at doses adjusted to achieve target therapeutic levels to give the transplanted patient the best hope for long-term graft survival[19].

Induction strategy

The induction strategies include antibody-based therapy and aggressive early immunosuppression to avoid early acute rejection.

Antibody-based therapy:
This therapy uses monoclonal or polyclonal antibodies and is administered in the early posttransplant period (up to 8 wk). Antibody-based therapy allows for avoidance of or

dose reduction of calcineurin inhibitors, possibly reducing the risk of nephrotoxicity. All agents are effective for preventing acute rejections, although the anti-CD25 antibodies may require concurrent administration with calcineurin inhibitors. The adverse effect profiles of polyclonal and monoclonal antibody therapies limit their use in some patients. Patients at a high risk of rejection may receive rabbit anti-thymocyte globulin (Thymoglobulin).

Aggressive early immunosuppression:
This therapy uses maintenance drugs at higher doses to achieve the strongest immunosuppressive effect directly following transplantation. Approximately 50% of patients do not receive antibody therapy at the time of transplantation. The highest doses of calcineurin inhibitors place patients at increased risk of nephrotoxicity and may not be the best strategy for patients at the highest risk for rejection.

Maintenance strategy

Maintenance of immunosuppression is the key for the prevention of acute and chronic rejections throughout the life of the graft.

After induction therapy, whether this involves high-dose steroids that are then tapered off or an anti-thymocyte globulin preparation, maintenance therapy involves maintaining the program of conventional immunosuppression in order to prevent graft rejection[21]. Conventional maintenance therapy has evolved over the years and now includes multiple immunosuppressive agents that are given in non-toxic doses. Historically, corticosteroids and azathioprine were used to maintain grafts after induction therapy. Cyclosporine was then added to the armamentarium for maintenance therapy. Triple-drug therapy using cyclosporine, azathioprine, and prednisone is the most common maintenance regimen for many transplant recipients. Triple-drug therapy permits lower doses of cyclosporine and azathioprine to be given, as well as enabling the use of low-dose steroids or every-other-day steroid therapy.

In stable liver transplant patients, cyclosporine can be discontinued and the recipient maintained on azathioprine or MMF and every-other-day steroids. Alternatively, patients have been maintained on cyclosporine monotherapy with complete withdrawal of MMF and steroids.

Following the clinical introduction of tacrolimus, this potent agent increased rapidly in popularity for maintenance therapy in liver transplant recipients. Tacrolimus-based therapies usually include very-low-dose prednisone, but may also utilize azathioprine or MMF. The potent activity of tacrolimus enables more rapid steroid withdrawal, and many patients can be maintained off steroids altogether with the use of tacrolimus.

7.2 Anti-rejection strategies

Acute rejection

A number of strategies are available for patients who experience an acute rejection episode. For typical patients, alteration in clinical graft function prompts a liver biopsy and pathological evaluation of the graft for rejection. The 3 agents used to treat acute rejection are (1) steroids, (2) anti-thymocyte globulin, and (3) muromonab-CD3.

Steroids are the first-line treatment for rejection. These agents are the mainstay of therapy for acute rejection episodes, preventing macrophage IL-1 release and blocking T cell synthesis of IL-2. Steroids also have anti-inflammatory properties. The typical dosage is 10 mg/kg/d for 3-5 days, which is then tapered down to a maintenance dose. Steroids reverse 60-75% of rejection episodes.

Anti-thymocyte globulin: This agent binds all circulating T and B lymphocytes, which are then lysed or phagocytosed by macrophages and neutrophils. The efficacy of anti-thymocyte globulin is similar to muromonab-CD3. Anti-thymocyte globulin treatment is generally reserved for steroid-resistant acute rejection because of its cost, toxicity, and the development of anti-drug antibodies.

Muromonab-CD3: This agent displaces the T3 molecule from antigen receptors, binds all mature T cells, and prevents alloantigen recognition. The reversal rate of first acute rejection episodes is 94% for patients treated with muromonab-CD3. Muromonab-CD3 is sometimes used as the first-line agent for severe vascular rejections. The development of human anti-murine antibodies allows for the reappearance of CD3 T cells, which may decrease muromonab-CD3 efficacy and necessitate higher doses, possibly increasing the risk of infection. A second course of muromonab-CD3 treatment may be given for recurrent rejection, although repeated treatments can be associated with complications from the development of anti-murine antibodies. The success rate in recurrent episodes is approximately 40-50%.

Chronic rejection

For patients with chronic rejection, newer agents may be on the horizon to slow or reverse the rejection process. Unless inadequate immunosuppression is the cause of rejection, changes in immunosuppressive therapy are generally not effective in reversing chronic rejection. In liver transplant recipients whose organs have significant regenerative abilities, the use of high-dose tacrolimus appears to have some effect in reversing chronic rejection; however patients must be treated early in the course of chronic rejection. The addition of sirolimus to MMF is currently being studied to determine efficacy. Long-term data on transplanted patients treated with sirolimus demonstrated that the chronic rejection rates are much lower compared to rates traditionally reported for cyclosporine-based regimens. Blood pressure management, treatment of hyperlipidemia, and diabetes management are the current mainstays of treatment for graft preservation[20].

7.3 Primary immunosuppressive agents

Calcineurin inhibitors combine with binding proteins to inhibit calcineurin activity. This works to inhibit IL-2, which is critical for T helper cell proliferation. Calcineurin normally exerts phosphatase activity on the nuclear factor of activated T cells. This factor then migrates to the nucleus to initiate IL-2 transcription. Although studies have shown that cyclosporine and tacrolimus were associated with similar rates of graft survival, several studies have shown lower rates of rejection episodes with tacrolimus.

Levels of both cyclosporine and tacrolimus must be carefully monitored. Trough levels appear to correlate well with drug exposure in patients receiving tacrolimus. Initially, levels can be kept in the range of 10-20 ng/mL; however, after 3 months, levels are kept lower

(5-10 ng/mL) to reduce the risk of nephrotoxicity. Controversy continues regarding the best method to monitor cyclosporine levels.

7.4 Adjuvant agents

These agents are usually combined with a calcineurin inhibitor and include steroids, azathioprine, MMF, and sirolimus. Currently, most protocols use a calcineurin inhibitor and steroids with or without MMF. The use of adjuvant agents allows clinicians to achieve adequate immunosuppression while decreasing the dose and toxicity of individual agents.

In kidney transplant recipients, mycophenolate mofetil has assumed an important role in immunosuppression after several clinical trials reported a marked decrease in the prevalence of acute cellular rejection compared to azathioprine; furthermore, a reduction in 1-year treatment failures was also reported for MMF. Ongoing long-term studies suggest MMF also reduces the prevalence of chronic rejection.

Sirolimus has shown great promise for its potential to combat acute cellular rejection and to provide rescue immunosuppression. Current work shows that sirolimus causes a significant decrease in acute rejection and improvement in patient and graft survival compared to azathioprine[21, 22].

8. Complications of immunosuppression

Clinical immunosuppression strategies involve striking a balance between freedom from rejection episodes and freedom from the toxicity and complications of immunosuppressive treatment regimens. As the number of rejection episodes decreases, the likelihood of opportunistic infections, late malignancy and drug toxicity increases.

The specific toxicities of the immunosuppressive drugs are described in the sections pertaining to each drug. In general, the goal of multidrug therapy is to decrease the toxicities that are seen with higher doses of individual drugs.

8.1 Infection and malignancy issues

Opportunistic infections remain an important risk for immunocompromised patient despite the use of prophylactic measures[24]. Exposure to viruses such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus, and human papillomavirus place the recipient at risk for infection and, potentially, later malignancy.

The incidence of CMV has been reduced with the use of antiviral prophylaxis in the first 3 months posttransplant. However, preemptive monitoring and initiation of treatment in the case of significant viremia after discontinuation of prophylaxis remains to be proven as a strategy for reducing the risk of late-onset CMV disease. Approximately 27% of patients who die with a functioning graft die from infectious or malignant complications. This highlights issues regarding the appropriate amount of immunosuppression required to balance aspects of graft function with complications related to therapy.
An increasingly recognized problem associated with immunosuppression is BK virus nephropathy. This virus, a member of the human papovavirus family, lives in the human genitourinary tract and replicates in some immunosuppressed patients, causing allograft

dysfunction. While antiviral agents such as cidofovir and leflunomide are active against the BK virus, the mainstay of therapy is a reduction in immunosuppression. The risk of acute allograft rejection with dose reduction is currently under investigation.

The most serious long-term effects of immunosuppression are the late malignancies that can develop in transplanted patients[25]. In addition to the post-transplantation lymphoproliferative diseases that are specific to chronically immunosuppressed patients, patients may also develop Kaposi's sarcoma. In addition, patients are at higher risk for malignancies that are common in non-immunosuppressed patients. The most common cancer observed in immunosuppressed patients is skin cancer, which mimics its frequency in the general population. A slightly higher incidence of Hodgkin's disease, non-Hodgkin's lymphoma, and breast, colon, lung, uterine and ovarian cancers has been observed in transplant recipients. For this reason, patients should undergo yearly cancer surveillance, including chest radiography and a general physical examination to look for new skin lesions; Pap smears and pelvic examinations are also suggested for women.

8.2 PTLD

Posttransplant lymphoproliferative disease (PTLD) is a growing concern in transplanted patients. Most cases of PTLD are of B-cell origin and are linked to EBV infections. Patients present with constitutional symptoms such as night sweats, fever, and weight loss. An acute rise in creatinine levels, similar to what occurs during acute allograft rejection, may also be seen. Risk factors for PTLD include primary EBV infection; the use of cyclosporine, tacrolimus, and MMF; and exposure to anti-thymocyte globulin (ATG) or OKT3. Treatment options include reduction or discontinuation of immunosuppression with an increase in prednisone to reduce rejection risk.

The long-term survival of liver transplant recipients requires lifelong treatment with immunosuppressive drugs. Despite the use of multiple agents in smaller doses, significant toxicities that are either directly or indirectly related to the immunosuppressive therapy can occur[27]. The primary long-term effects of corticosteroids include growth inhibition, Cushing's syndrome, osteoporosis, avascular femoral head necrosis, cataracts, glaucoma, cardiovascular disease and gastritis-peptic ulcer disease. The long-term effects of azathioprine include hepatitis, pancreatitis and red-cell aplasia. For cyclosporine, the long-term effects include hypercholesterolemia, arteriosclerosis, hypertension and nephrotoxicity. The long-term effects of tacrolimus may include hypertension and nephrotoxicity; however, it is too early to determine what other side effects may develop over time for this drug.

In summary, significant progress has been made in developing effective immunosuppressive protocols. These protocols rely on combination therapy using multiple drugs at low dosage to prevent rejection, treat established rejection episodes and minimize both the short-term toxicities and the long-term complications associated with immunosuppressive therapy.

9. Induction of tolerance

Transplant tolerance is defined as a state of donor-specific unresponsiveness without a need for ongoing pharmacologic immunosuppression. Safe, reliable strategies for the induction of full tolerance have not yet been developed[28]. However, during the study of achieving

immune tolerance, methods to induce states of "partial tolerance" have been discovered; in these cases, lower-than-conventional amounts of ongoing pharmacologic immunosuppression are required to prevent rejection. Nonetheless, immune tolerance remains the holy grail of transplantation immunology and clinical transplantation[29].

9.1 Induction of tolerance in transplant patients

Clinical allograft transplantation research has been conducted to identify methods to induce full or partial tolerance in transplant patients. These strategies are not ready for general clinical use until further evidence-based studies are available.

Full tolerance

The holy grail of organ transplantation is full immunologic tolerance, a state of indefinite survival of a well-functioning allograft that does not require maintenance immunosuppression. In addition, the host must retain a normal immune response and not suffer from immunosuppression-related infections, neoplasia, or other drug-related adverse effects. Rare cases of operational tolerance after transplantation, with complete cessation of immunosuppressive therapy have been observed and reported; these cases generally are associated with patient noncompliance regarding therapy.

Most studies concerning the intentional induction of immunologic tolerance have involved patients with hematologic malignancies. Full tolerance was achieved with myeloablative therapy prior to organ transplantation in combination with induced donor chimerism by means of bone marrow transplantation and excellent human leukocyte antigen (HLA) matching. Mixed chimerism retains a graft-versus-host T-cell effect that allows for transplant acceptance despite the subsequent disappearance of the donor chimerism.

Myeloablative therapy includes total body irradiation and lymphoablative methods, such as total body irradiation and the use of azathioprine and corticosteroids. However, the complications of full tolerance and the unpredictable timing organ transplantation with regards to the time required for myeloablative therapy prior to transplantation precludes the routine application of these therapies.

Cosimi and Sachs studied mixed chimerism in a small number of patients. They used nonmyeloablative conditioning, such as peritransplantation low-dose total-body irradiation or thymic irradiation plus anti-thymocyte globulin therapy combined with splenectomy. Donor-specific marrow infusion was given at the time of transplantation. Cyclosporine was given for about a month after transplantation and then stopped. Patients had transient chimerism for several weeks, and graft survival was approximately 70% over the long term.

Tregs are responsible for maintaining tolerance by broadening suppression through a mechanism termed linked-suppression, where tolerance to a specific epitope is spread to all epitopes of that protein; this tolerance is also spread to cohorts of naïve T-cells as they develop. Immunologist Herman Waldmann described this phenomenon as a process of infectious tolerance. Tregs from tolerant animals can be transferred to naive animals, in which they subsequently confer antigen-specific tolerance, including tolerance to skin and organ allografts. Although not completely understood, this is referred to as adoptive tolerance, and it has been recognized since the 1990s when Dr. Metcalfe at Cambridge published a landmark paper describing this phenomenon.

Tolerance induction through the expansion and transfer of donor Tregs to an allograft recipient or by means of the ex vivo development of Treg from recipient T cells are intriguing but yet-untested strategies in humans. Currently, in heart transplantation, analysis of whether FOXP3 gene expression in peripheral blood cells reflects anti-donor immune responses is underway. Overall, these possibilities represent exciting ways to broaden the translational approach of tolerance induction through the use of T regulatory cells.

Partial tolerance

At present, partial tolerance that requires minimal immunosuppression is possible. This allows for the minimal use of immunosuppressive drugs, which results in reduced risks for infection, neoplasia, and drug-related adverse effects. Partial, or incomplete, donor-specific tolerance has been termed minimal immunosuppression tolerance or prope tolerance from the Latin word for near.

Professor Sir Roy Calne postulated that prope tolerance preserves some of the transplant recipient's immune responses to infection and other antigens, reducing morbidity and mortality caused by immunosuppressive effects.

Although numerous researchers are investigating assays to monitor the degree of immunosuppression, no assays or tests are currently available to monitor tolerance. Dr. Sarwal at Stanford University is currently using exciting microarray technologies to describe genetic identifiers of allograft recipients that are rendered tolerant. This technology may be what is required to overcome the current barriers to allograft tolerance.

Identifying either prope or complete tolerance depends on the elimination, withdrawal, or reduction of maintenance immunosuppression followed by the observation of a favorable response. Allograft biopsies may or may not be helpful in identifying rejection at an early stage if the strategy is unsuccessful. Indeed, a specific directive of granting agencies, such as the National Institutes of Health and the Immune Tolerance Network, is to fund research to develop tolerance assays.

The demonstration of immune tolerance induction in many rodent models stands in stark contrast to the lack of success in humans and primates, with the exception of myeloablative therapy followed by donor-derived stem cell infusion. The specific pathogen-free environment in which rodents are housed for their lifetime limits the number of memory T cells that they develop. In contrast, humans and primates are exposed to many viruses during their long and less pathogen-free lives. In addition, they generate a considerable pool of self-renewing memory T cells; in fact, nearly half of circulating T cells in adult humans are memory T cells. Therefore, they are less immunologically naïve compared to experimental rodents. Many of these memory T cells can cross-react with foreign MHC. Therefore, the translation of tolerance induction strategies from the rodent laboratory models to large animals and then to humans may need to account for differences in previous specific and net immunologic memory.

9.2 Mechanisms for tolerance

Tolerance is generally accepted to be an active process and, in essence, a learning experience for T cells[30]. Tolerance is said to occur mechanistically at 2 levels: centrally and peripherally.

Central and/or Intrathymic Tolerance

The chief mechanism of T-cell tolerance is the deletion of autoreactive T cells in the thymus, rendering the organism tolerant to "self." Immature T cells migrate from the bone marrow to the thymus, where they encounter peptides derived from endogenous proteins that are bound to major histocompatibility complex (MHC) molecules on thymic epithelial cells.

Double-positive ($CD4^+$ and $CD8^+$) thymocytes initially undergo random generation of different T-cell receptors (TCRs). Positive selection, also called thymic education, ensures that only clones with TCRs that exhibit moderate affinity for self-MHC are allowed to develop. Negative selection by means of apoptosis occurs when T cells do not produce functional TCRs, when TCR rearrangement fails, when T cells have low affinity for MHC-self-peptide complexes, or when T cells have extremely high affinity for such complexes. Negative selection also results in the deletion of some thymocytes that interact with autoantigens presented by interdigitating cells and macrophages at the corticomedullary junction. The remaining cells lose either CD4 or CD8 and leave the thymus to function in the periphery as mature, functional $CD4^+$ and $CD8^+$ T cells[31].

Peripheral tolerance

Many potentially reactive T cells escape intrathymic deletion, reflecting the fact that many antigens are absent intrathymically or are present at insufficient levels to induce tolerance in the thymus. Several peripheral, nonthymic mechanisms that prevent autoimmunity by rendering peripheral T cell repertoires tolerant also exist[32].

9.3 Sequestration of antigens into privileged sites

Some antigens are sequestered into privileged sites away from the immune system because of physical barriers, such as tight junctions, or immunologic barriers, such as the expression of Fas ligand (FasL) or reduced MHC class I expression. Thus, antigen-presenting cells (APCs), and subsequently T lymphocytes, may never encounter these self-antigens. Therefore, immune cells remain ignorant of these antigens. At some of these sites, proinflammatory lymphocytes are controlled by apoptosis due to the expression of FasL or the secretion of cytokines such as transforming growth factor-beta (TGF-β) or interleukin (IL)-10. When T cells enter these sites, their Fas receptors interact with the FasL of these sites, and they undergo apoptosis. Privileged sites include the brain, the testes, and the anterior chamber of the eye. Transplanted tissues are most likely to survive in these privileged sites because of the tight control of proinflammatory lymphocytes.

9.4 Apoptosis of T cells due to persistent activation or neglect

Apoptosis, or programmed cell death, of lymphocytes is an important mechanism of immune control and homeostasis. Apoptosis contributes to the deletion of clones that are persistently activated and of activated lymphocytes when the immune response is no longer needed (e.g., after an infection clears). Cells that are persistently stimulated undergo activation-induced cell death involving Fas-FasL signaling or tumor necrosis factor. Most T cells that remain after antigen clearance are deprived of the stimuli required to survive and undergo passive cell death. Apoptosis of donor-reactive lymphocytes is also known as the "deletional" method to induce tolerance; in theory, this represents the most fail-safe

mechanism of tolerance induction. In the absence of donor-reactive lymphocytes, the response to donor antigens could not be induced no matter what antigens are encountered.

9.5 Clonal anergy

T lymphocytes require 2 signals to become activated, proliferate, and differentiate. The first is the recognition of an appropriate MHC-antigen complex by the TCR of the responsive lymphocyte. The second signal is delivered by costimulatory molecules also expressed by APCs; Costimulatory ligands are only able to engage once the first signal is activated. Lack of costimulation causes anergy, when T cells fail to respond to the MHC-peptide complex and remain unresponsive to subsequent challenges.

CD28 is the main costimulatory ligand expressed by naive T cells encountering antigen. CD28 signaling enhances T-cell proliferation by boosting T cell IL-2 production. It also enhances expression of CD40 ligand, which interacts with CD40 on APCs to induce the upregulation of the costimulatory molecules CD80 (B7-1) and CD86 (B7-2) to further enhance costimulatory signaling.

Recently, Rigby et al. used inhibition of T-cell costimulation as an effective means to prevent autoimmunity and allograft rejection in multiple animal models. They studied the effects of anti-CD28 and CTLA4-Ig on diabetes development and the requirements to induce tolerance in nod/scid mice after the transfer of transgenic beta-cell reactive BDC2.5.NOD T-cells. These authors were successful in this set of experiments and have helped to develop the understanding of natural regulatory mechanisms that may have a unique role in establishing targeted, long-standing immune protection and peripheral tolerance.

T lymphocytes also express CD152 (CTLA-4) after CD28 binds to its ligands B7-1 and B7-2 on APCs. The interaction of CTLA-4 and B7 molecules decreases opportunities for B7-CD28 binding and downregulates T-cell IL-2 production, which subsequently reduces T-cell proliferation. CD28 interacts with B7 molecules, first leading to T-cell activation. However, after this effect peaks, upregulation of CTLA-4 with its relatively high affinity for B7 molecules limits the degree of activation. Verbinnen et al. recently studied the involvement of regulatory T cells (Treg) and deletion of alloreactive cells in the induction and maintenance of tolerance after costimulation blockade (CTLA-4) in a mouse model of graft-vs.-host disease. The study showed that clonal deletion of host-reactive T cells was a major mechanism responsible for tolerance.

9.6 Regulatory T cells

Regulatory T cells (Tregs), also called suppressor T cells, suppress the activation of clone-specific T-cell activity. Tregs account for 10-15% of $CD4^+$ T cells and express the transmembrane protein CD25, which is the alpha chain of the IL-2 receptor. $CD4^+CD25^+$ Tregs are anergic to TCR-mediated activation but potently suppress the activation of other T cells. However, not all $CD25^+$ T cells are regulators. Some naive T cells upregulate CD25 in response to antigen, a change that represents the activation rather than the suppression of an immune response. The thymus produces anergic but suppressive $CD4^+$ 25^+ T cells, which are also identified by the expression of FoxP3, the transcription factor responsible for their development. These T cells suppress the activation and expansion of autoreactive $CD4^+CD25^-$ populations.

Studies in mice have shown that Tregs are antigen-specific and that they regulate peripheral tolerance by producing suppressive cytokines such as IL-10 and TGF-beta. They depend on continuous antigen exposure to stay capable of mediating suppression. Antigen removal reduces the quantity of cells.

In allograft rejection, direct stimulation of T cells in response to donor-derived antigens presented by donor APCs had been the focus of transplantation research for many years. However, indirect antigen presentation, in which self-APCs present donor peptides in an MHC-restricted fashion is responsible for the induction of antigen-specific Tregs that can directly and indirectly suppress other alloreactive T cells. Although positive costimulation with CD28 appears to be necessary for the development of intrathymically derived Tregs, costimulation blockade with CTLA-4 is required for the development of peripherally acquired suppressor Tregs.

10. Immunosuppression withdrawal

Although tolerance induction may allow for the withdrawal of immunosuppression in the future, at this time, immunosuppressive medications appear to be necessary for the life of the transplanted organ[33].

10.1 Steroid versus steroid-free protocols

The known toxicity of long-term steroid exposure has prompted the development of steroid-free immunosuppressive regimens. Benefits of the withdrawal or avoidance of steroids include normal growth in children, improved lipid profiles, improved blood pressure, better glycemic control, and a lower risk of bone disease.

The development of cyclosporine prompted attempts to develop steroid-free protocols. Initially, patients were doing well with cyclosporine monotherapy. Over time, 50% of these patients required steroids, usually for episodes of acute rejection. Strong randomized studies are undoubtedly needed to prove both the efficacy and the safety of these protocols.

Steroid withdrawal has been used as a strategy to avoid adverse steroid effects in transplanted patients. Recent data show that the risk of rejection is higher in patients withdrawn from steroids on a cyclosporine-based protocol. After tacrolimus became available, protocols with this drug showed that withdrawal of steroids after 6 months was successful 80% of the time. More recently, studies involving rapid steroid withdrawal (over 1-2 wk) in patients taking tacrolimus show similar graft survival rates compared with patients withdrawn after 3-6 months. Although the roles of sirolimus and MMF in steroid-free protocols have yet to be definitively determined, the future looks promising for greater use of steroid-free protocols.

10.2 Calcineurin inhibitor-free protocols

Because of the risk of both acute and chronic nephrotoxicity attributed to calcineurin inhibitors, the development of protocols free of these agents is desirable. The use of sirolimus, MMF, and anti-CD25 antibodies has been studied to determine whether graft survival and acute rejection rates can be maintained at the present rates in the absence of calcineurin inhibitors.

The withdrawal of cyclosporine has been investigated in several trials. While the long-term graft survival rates were similar in patients withdrawing from cyclosporine compared to those maintained on it, the incidence of acute rejection in the withdrawal group was higher. The addition of sirolimus has been used in these withdrawal protocols. Higher rates of acute rejection were again noted in the withdrawal group.

Many other protocols that minimize exposure to calcineurin inhibitors have been studied. Promising protocols include sirolimus, MMF, and steroids or the combination of anti-CD25 antibodies, sirolimus, MMF, and steroids. These protocols have shown acceptable graft survival rates and acute rejection rates; however, these studies were small in size and further research is warranted[34]. In short, multiple regimens have been shown to be effective.

10.3 Pregnancy

Current data suggest that protocols involving cyclosporine, azathioprine, and steroids are associated with low rates of birth defects. However, patients are treated with high-risk pregnancy strategies. However, children born to parents with previous transplants are often small for their gestational age. Preliminary data suggest the safety of tacrolimus. MMF animal data and some early human studies show adverse effects on fetal development. Presently, few data exist regarding sirolimus and pregnancy.

10.4 Length of treatment

Episodes of acute cellular rejection have occurred after the cessation of medication even 20 years after transplantation. For patients with stable graft function, individual components of the treatment regimen may be gradually diminished or completely discontinued; however, in most patients, some degree of immunosuppression must be continued. Some patients with severe resistant infections or malignancy related to immunosuppressants require the discontinuation of these medicines.

11. Future perspective

Immunity, regulation, graft rejection versus acceptance, and tolerance have proven to be extraordinarily complex. Indeed, currently used drugs and treatment protocols that are largely directed at inhibiting alloreactive T cells, have not optimally improved allograft survival or function. The tremendous progress made in understanding the molecular and cellular basis of allograft rejection has not yet been translated into durable modalities that have advanced clinical care and outcomes. Despite the many advances in both immunological knowledge and the practical application of clinical immunosuppression, the holy grail of indefinite graft survival with immune tolerance in clinical solid organ transplantation remains a distant dream. The current challenge is to integrate molecular, cellular, and anatomic concepts to achieve the equivalent of a unified field theory of the immune response to organ transplantation. A shift in emphasis, focusing on underappreciated immune pathways must now be considered to make further improvement. We highlight 3 areas of recent interest, complement, NK cells and lymphatics[35, 36], which reinforce the concept that the transplant community must direct attention on how the immune system as a whole responds to a transplant. Discoveries of

new molecules, cell populations, functions or pathways have each led to the hope that the field has finally reached the point that reliable immune manipulation can now be achieved. Once that perspective is gained, we may finally be poised to make the major leaps forward in clinical care and outcomes.

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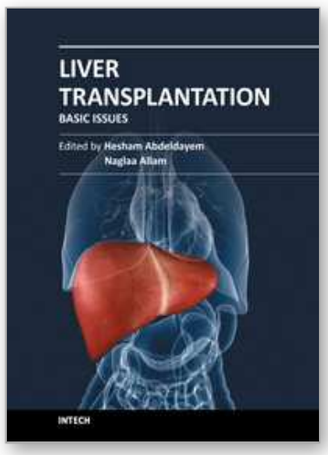

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This book covers a wide spectrum of topics including history of liver transplantation, ischemia-reperfusion injury, immunology of liver transplantation, viral hepatitis and liver transplantation, other indications for liver transplantation, prognostic factors and perioperative period. The authors of the chapters are experts in their respective fields. They are proponents covering different aspects of liver transplantation and come from many centers across the world. The interdisciplinary approach and the authority of the contributors resulted in a valuable reference to anyone interested in developing a global view in liver transplantation including medical students, residents, fellows, nurses, and practicing physicians and surgeons as well as researchers in the field of liver transplantation.

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