Immunosuppression in Solid Organ Transplantation: A Review

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The success of organ transplantation has improved progressively over the past decades as a consequence of better understanding of organ dysfunction and graft loss, advances in perioperative care, and, in particular, more effective immunosuppressive regimens. With the introduction of cyclosporin A (CsA) at the beginning of the 1980s, a major improvement in patient and graft survival after solid organ transplantation was observed. Despite >80% 1-year functional survival of most grafts, the ultimate goal—to provide long-term treatment for irreversible organ failure—has not yet been achieved. Although recurrent disease, de novo infections, and malignancies may contribute to late graft deterioration, chronic rejection remains the most important risk factor.[1,2] In recent years, new immunosuppressive drugs with different modes of action and side effects have become available. At the same time, differing donor and recipient demographics and criteria based on the extension of patients on waiting lists and the acceptance of so-called "marginal donor organs" require modified immunosuppressive therapies. Immunosuppression today does not represent a single regimen applicable to all patients. In selecting the best immunosuppressive protocol, individual drug-related toxicity, recipient-related risk factors, and donor organ characteristics have to be taken into account, aiming at individualized immunosuppressive therapy.

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Mechanisms of Graft Rejection

Rejection is defined as an immune response that mediates injury and destruction of transplanted tissue. The hallmark of acute rejection is a cellular infiltrate, predominantly of small and activated lymphocytes and monocytes/macrophages, leading to inflammation and subsequent destruction of the organ. The pathophysiology of acute rejection has been conceptualized as a result of both antigen-dependent and antigen-independent stimuli.[3] Immunological events are of primary importance: differences in human leukocyte antigen (HLA) matching between donor and host, the influence of early acute rejection episodes, and persistent host alloresponsiveness over time all participate. Indeed, it recently became obvious that beside the immunological host response, the immunological status of the prospective graft is crucial for short- and long-term function. The donor organ quality, and therefore the immunogenicity of the graft, is primarily defined by donor-related factors such as gender, age, diseases, and the cause of death.[4] Clinical and experimental observations have proven that the brain death of the donor, as well as the transplantation of so-called marginal donor organs, is itself a risk factor for the outcome after transplantation, leading to increased immunogenicity of prospective transplants.[5,6] Changes associated with ischemia and reperfusion are antigen-independent events that may contribute not only to early delayed graft function but also to late allograft deterioration. The events surrounding these phenomena may also cause upregulation of major histocompatibility complex (MHC) antigens, thereby increasing graft immunogenicity and enhancing host alloreactivity against the graft. Those effects lead to T cell activation and early rejection episodes. Donor organ quality, ischemic insults, and the events of postischemic reperfusion contribute to tissue injury. Early expressed adhesion molecules appear to trigger subsequent events. Selectins, which are not expressed under resting conditions, are upregulated rapidly after injury and are responsible for initiating neutrophil binding, leukocyte sticking, and

cellular infiltration. The adhesion molecule–cytokine cascade is then amplified. Adherent leukocytes express other classes of adhesion molecules (ICAM, VCAM, LFA-1) and release pro-inflammatory lymphokines (TNFα, IFNγ). Expression of MHC class I and II molecules is increased. The expression of MHC on graft cells is primarily mediated by IFNγ, which is also increased by ischemia/reperfusion injury. Although MHC antigen expression alone does not lead to allograft rejection, it plays a dominant role in the T cell recognition process when the antigen is presented by antigen-presenting cells as signal 1, thus increasing the immunogenicity of the graft. A further step leading to T cell activation is the interaction with proteins on antigen-presenting cells, triggering receptors on T cells that provide costimulation (signal 2, CD 28, B7). After receiving both signals, protein tyrosine kinases are activated and initiate 3 key signaling pathways: the calcium-calcineurin pathway, the ras-MAPkinase pathway, and the activation of protein kinase C. After the initiation of preformed transcription factors, cytokines, cytokine receptors, and clonal expanding cells start acute rejection processes. Individual T cells develop effector functions such as delayed-type hypersensitivity, the ability to help B cells to produce antibodies, or T cell cytotoxicity.

Nonspecific injury with resultant upregulation of antigen expression and increased graft immunogenicity therefore may also increase the frequency of early acute rejection after transplantation. The number of such early episodes also appears as a critical risk factor for chronic rejection.[7,8]

ACUTE REJECTION:

Graft rejection that usually begins within 10 days after a graft has been transplanted into a genetically dissimilar host. Lesions at the site of the graft characteristically are infiltrated with large numbers of lymphocytes and macrophages, which cause tissue damage.

Immunosuppressants

The basic immunosuppressive protocol used in most transplant centers involves the use of multiple drugs, each directed at a discrete site in the T cell activation cascade and each with distinct side effects. The most common drugs used are cyclosporine A, tacrolimus, azathioprine, corticosteroids, mycophenolate mofetil, and rapamycine. Polyclonal or monoclonal antibodies directed at cell surface proteins are being used in the clinical setting as induction therapy and/or antirejection drugs. In recent clinical trials, mizoribine, deoxyspergualin (DSG), leflunomide (FK 778), and FTY 720 are being explored.[9,10] The immunosuppressants can be classified on the basis of their primary site of action and considered as inhibitors of transcription (cyclosporin A, tacrolimus), inhibitors of nucleotide synthesis (azathioprine, mycophenolate mofetil, mizoribine, leflunomide), inhibitors of growth factor signal transduction (rapamycin, leflunomide), and inhibitors of differentiation (15-deoxyspergualin). When an appropriately processed and presented antigen interacts with the T cell receptor, the resting T cell is activated, leading to synthesis and secretion of IL-2 and expression of high-affinity IL-2 receptors. The immunosuppressive properties of CsA (a small fungal cyclic peptide) and tacrolimus (a macrolide) are mainly linked to inhibition of the activity of calcineurin, a serine-thyronine phosphatase that activates intracellular gene-promoting transcription factors involved in IL-2 and other cytokine activation. To interrupt these actions, CsA binds to the intracellular cyclophyllin, and tacrolimus interacts with another cytoplasmic protein called FK-binding protein (FKBP); therefore, both drugs are formatting a heterodimeric complex that exists of the drug and its respective cytoplasmic receptor protein. Both of these complexes bind calcineurin and inhibit its phosphatase activity, thereby inhibiting the de novo expression of nuclear regulatory proteins and T cell activation genes. As both drugs block the stimulated T lymphocyte at the same level, immunosuppressive potencies as well as side effects are comparable.

Azathioprine and corticosteroids have been used as successful immunosuppressants since the beginning of clinical transplantation. The final step in the T cell activation cascade is the intracellular RNA and DNA synthesis. Purine synthesis inhibitors such as azathioprine block this step. After administration, azathioprine is converted to 6-mercaptopurine and further to 6-thio-inosine monophosphate. The immunosuppressive effects result from its capability to alkalyte DNA precursors and by inhibiting various enzyme systems, including the conversion of the central inosine monophosphate to adenosine monophosphate and guanosine monophosphate. In lymphocytes, 6-mercaptopurine inhibits lymphocyte proliferation primarily by depletion of adenosine rather than guanosine. The resulting side effects are rapidly dividing tissues,

particularly bone marrow-derived cells. Azathioprine is potentially mutagenic and may induce damage in chromosomes. Steroids, well-known hormones that are synthesized in the adrenal cortex, mediate their immunosuppressive potencies mainly by inhibiting the first enzyme of arachidonic acid metabolism, phospholipase A2, and thereby the whole arachidonic acid cascade, including the cyclooxygenase (thromboxane, CO) and 5-lipoxygenase (5-LO) pathways. Evidence also exists that steroids reduce cytokine gene expression by interacting with transcription factors (NFKB). Steroids also reduce IFNγ-dependent expression of adhesion molecules and MHC class II expression.[11] In most immunosuppressive protocols, steroids are commonly used, at least in the early period after transplantation and in the treatment of acute rejection episodes. They also seem suitable for the reduction of unspecific inflammatory changes in organs before transplantation.[12] The long-term therapy is controversial because of the well-known side effects of the drug. In addition, newer, alternative immunosuppressants may enable a long-term treatment without the side effects of steroids.

IMMUNOGENICITY:

The property of being able to evoke an immune response within an organism. Immunogenicity depends partly on the size of the substance in question and partly on how unlike host molecules it is. Highly conserved proteins tend to have rather low immunogenicity.

Mycophenolate mofetil, another purine synthesis inhibitor, is a semisynthetic derivate of a fungal antibiotic and inhibits the de novo synthesis of guanosine. This inhibition shows some selectivity for lymphocytes, which depend extensively on the nucleotide pathway, both on the de novo and salvage pathways, for purine nucleotide synthesis. Beside diminished proliferation of T and B lymphocytes, mycophenolate mofetil decreases the generation of cytotoxic T cells and suppresses antibody formation. In clinical trials, mycophenolate mofetil has been proven to be effective in combination with calcineurin inhibitors and to reduce the frequency of acute rejection episodes significantly.[13,14] Chronic rejection may be influenced by long-term treatment with this drug.[15] Side effects following a therapy with mycophenolate mofetil are diarrhea, esophagitis, and gastritis. In addition, altered wound healing, leukopenia, and opportunistic infections have been described.[16]

Rapamycine (sirolimus), like tacrolimus, binds to FKBP immunophilins, but regardless of the close similarity in structure and the binding site to tacrolimus, the immunosuppressive effect of rapamycine is entirely different. In contrast to tacrolimus, rapamycine has no affinity for the calcineurin-calmodulin complex and therefore does not affect the early activation genes of T cells. Instead, rapamycine blocks the second set of phosphorylation events (kinase-mediated TOR1 and TOR2 inhibition) following binding of IL-2 to its receptor- and cytokine-mediated signal transduction pathways in the late phase of the T cell cycle. Despite the fact that rapamycine and tacrolimus as pharmacological antagonists compete for the occupation of the same cellular receptor, they seem to interact in vivo in an additive or synergistic fashion. Experimental data demonstrated that therapeutic concentrations of tacrolimus bind only a small part of the intracellular FK-binding protein, leaving enough molecules available for binding to rapamycine. Those interactions seem to enable both drugs to develop their immunosuppressive potencies.[17-20] Rapamycine does not have nephrotoxic side effects, but experimentally, hypomagnesemia and tubular injury were observed. The most common side effect associated with rapamycine is hypertriglycerinemia, which has been reported in association with short-and long-term use of rapamycine. In addition, changes in blood glucose levels, headache, and nausea have been observed.

FTY720, a synthetic derivate of sphingosine myosine, has been proven, at least experimentally, to prolong organ survival after transplantation significantly.[10] In contrast to most known immunosuppressants, FTY720 does not inhibit lymphocyte proliferation or interfere with IL-2 synthesis. The immunosuppressive potencies of FTY720 seem to be linked to a potential reduction of circulating lymphocytes due to apoptosis or by altered lymphocyte homing.[21] So far, side effects of FTY720 in human recipients have not been defined and need to be investigated.

Mizoribine brequinar sodium, 15-desoxyspergualin, and leflunomide mediate their immunosuppressive potencies by different interactions with cell differentiation and proliferation.[22] These drugs have been proven to be highly effective in experimental studies.[23] Recent evaluations in clinical trials have been performed.[24,25] Mizoribine blocks the purine biosynthetic pathway and therefore inhibits mitogen-stimulated T and B cell proliferation.

Unlike azathioprine, mizoribine seems not to be myelotoxic or hepatotoxic. 15-Deoxyspergualin is unique in preventing the differentiation of T and B cells into mature effector cells. This drug has been reported to be effective as rescue therapy in renal graft recipients who have not responded to high-dose corticosteroid therapy. Leflunomide is an orally bioavailable prodrug that was originally used in the treatment of autoimmune diseases. In this context, it seems to be effective and free from side effects commonly associated with currently approved immunosuppressive drugs.[9] The active metabolite of leflunomide is A771726, which interferes with the de novo pathway of pyrimidine biosynthesis and therefore has some anti-proliferative potency. Experimental data demonstrated that A771726 is an inhibitor of tyrosine kinases, associated with growth factor synthesis, which plays an important role in triggering fibrotic changes in transplanted organs. Due to the long half-life of leflunomide, structurally similar alternative drugs with a more favorable pharmacokinetic profile, called malononitiloamides, are currently being evaluated for clinical use. Phase II clinical trials are currently under way.[26]

In the mid-1970s, several polyclonal antibodies were produced by immunizing rabbits or horses with T cells, thymocytes, or T cell lines. Today, these antibodies are predominantly genetically engineered and humanized. The utilization of antibodies seems to be limited by the recipients' response, leading occasionally to severe life-threatening side effects, including anaphylactic shock or thrombotic complications. Antibodies engineered on a human immunoglobulin background might minimize those problems.

Biological immunoactive agents such as polyclonal antilymphocyte antibodies (ALG, ATG) are used by many centers as induction or rescue (OKT3, monoclonal antibody) therapy. Antibody induction protocols have reduced the incidence of early rejection and are particularly beneficial for patients at high risk for immunological graft failure—in general, presensitized or retransplant patients.[27,28] Anti-IL-2Rα mABs such as the chimeric antibody basiliximab (Simulect, Novartis, Basle, Switzerland, $t_{1/2}$ = 6.5 days) and the humanized anti-Tac antibody daclizumab (Zenapax, Roche, Basle, Switzerland, $t_{1/2}$ = 20 days) block the interaction of IL-2 with its receptor. The interaction results in a rapid proliferation of the antigen-activated T cell with helper, suppressor, or cytotoxic abilities. A variety of antibodies with a broad variation of effectiveness are currently being evaluated. Monoclonal antibodies or small molecules specific for cell surface proteins implicated in the generation of costimulatory signals are also being explored for their efficacy in the clinic. Anti-LFA1 (CD11A), anti-ICAM-1 (CD54), anti-selectin (rPSGL, Genetics Institute, Cambridge, MA), OKT4A (anti-CD4), and Campath-1H (CD52) are being tested in solid organ transplant recipients.[29] In addition, soluble fusion proteins blocking the CD-28-B7 costimulatory pathway appear promising based on results from experimental transplantation.[30]

Individualized Immunosuppression

The availability of a variety of immunosuppressive agents with different modes of action and side effect profiles offers the opportunity for tailoring immunosuppressive therapy with regard to recipient-related and donor-associated characteristics and risk factors. The selection of immunosuppressants is based on efficacy, side effect profiles, and recipient- and donor-related factors. Clearly, the transplant clinician now has a greater choice in the selection and application of immunosuppressants, with the opportunity for an individualized immunosuppression. This approach may improve long-term graft function and reduce side effects, including significantly decreased rates of malignancies with the duration of immunosuppression.[31,32]

Since the introduction of calcineurin inhibitors, the frequency of acute rejection episodes has significantly decreased. Long-term graft outcome is predominantly determined by late patient death caused by cardiovascular complications and chronic graft deterioration.[33-35] In the past, standard immunosuppressive protocols were based on the calcineurin inhibitors tacrolimus and cyclosporin A. Both drugs were combined in most instances with steroids or other concomitant immunosuppressants. Beside nephrotoxicity, calcineurin inhibitors seem to be associated with hyperlipidemia and hypertension. Therefore, combination therapies and the utilization of a variety of drugs in low dosages may offer

the opportunity to balance the required immunosuppressive effect with diminished or absent side effects, taking into account the risk profile of the organ recipient. The absence of nephrotoxicity and its antiproliterative potencies makes rapamycine an attractive alternative for the widely used calcineurin inhibitors. On the other hand, the pronounced hyperlipidemia seems to be an additional risk factor for those categories of patients with an already increased cardiovascular morbidity and mortality. As drug-related side effects are also developing based on patients' susceptibility, even the conversion of the basic immunosuppressive regimen should be considered due to drug-related side effects. Indeed, patients with pronounced side effects obviously caused by a calcineurin inhibitor benefit in most instances from the conversion to the alternative immunosuppressive drug.[36]

DELAYED-TYPE HYPERSENSITIVITY:

An increased reactivity to specific antigens mediated not by antibodies but by cells.

Patients with an expected strong immune response, such as strongly mismatched recipients or those who lost their first graft due to acute rejections with high titers of circulating antibodies, should receive induction therapies with antibodies. In these instances, some centers recommend using tacrolimus as a long-term suppression because it is proven to have a greater immunosuppressive potency, which can even reverse rejections occurring after CsA application.[37]

Beside the recipients' immune status, the quality of the transplanted graft seems to be crucial for short- and long-term survival after transplantation. Recent clinical and experimental findings have demonstrated that organs from marginal donors, as well as those from brain-dead donors, are immunologically activated. Those organs show increased damage as a consequence of ischemia/reperfusion injury and a higher frequency of rejection episodes with reduced long-term graft function.[38-40] Donor treatment in terms of immunosuppressants applied to the organ donor may improve the success of the graft outcome. These findings have been shown in experimental models with improved kidney survival and long-term function after donor treatment with calcineurin inhibitors, steroids, or adhesion molecule-blocking antibodies. Recent clinical investigations have demonstrated beneficial effects after high-dose steroid and hormone applications in brain-dead organ donors.[12,41]

It seems recommendable that immunosuppressive therapies should start before transplantation, with treatment of the organ donor, as inflammatory changes occur early after brain death, and immunosuppressive drugs administered with the transplant procedure are frequently not sufficient to prevent unspecific damages.

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