Immunology Insights Into Cardiac Allograft Rejection

Marcello Savasta, MD, Salvatore Lentini, MD

Cardiovascular and Thoracic Department, Policlinico G. Martino University Hospital, University of Messina, Italy

Despite long-term complications from chronic immunosuppressive therapy, the phenomenon of chronic rejection is still a limitation in cardiac allograft recipients. In this review, starting from basic immunologic concepts, we analyze the mechanisms involved in rejection following heart transplantation, with particular emphasis on chronic rejection manifested as cardiac allograft vasculopathy (CAV). Etiopathogenesis of CAV and diagnostic imaging studies are also discussed.
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Heart transplantation represents an effective treatment of patients with end-stage cardiac disease that is nonresponsive to conservative medical or surgical treatment. Immunosuppressive therapy, technology innovations, increased experience, better patient selection, and improved organ preservation all have played a major role in this achievement. The introduction of cyclosporin A in immunosuppressive therapy in 1978 by Roy Calne represented an important step forward in this field.[1]

However, despite the progress in immunosuppressive therapy, allowing modulation of recipient immunologic responses to prevent or treat graft rejection, the activities of these drugs remain nonspecific with regard to the different phases of the immune response. The result is an overall decrease in immune responses, including those directed against infectious agents and tumor antigens.[2] For this reason, some of the most significant long-term complications in patients who have undergone organ transplantation are the higher incidence of

infections and malignancies. Despite these significant long-term complications from chronic immunosuppressive therapy, the phenomenon of chronic rejection is a limitation in this field.

Beginning with basic immunologic concepts, this article analyzes the mechanisms involved in rejection following heart transplantation, with particular emphasis on chronic rejection manifested as cardiac allograft vasculopathy (CAV).

Immunity and Transplantation

The immune system, in carrying out its protective function against external agents, uses its unique ability to discriminate between autologous constituents (self), to which it is tolerant, and foreign antigens (nonself), to which it becomes activated by generating a specific response,[3] thereby affording protection from any foreign body, whether micro-organisms, cancer cells, or transplanted organs. The success of organ transplantation is related to the ability to modulate the immunologic response of the allograft recipient with the aim of avoiding a rejection reaction.

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There are essentially two defense mechanisms contributing to nonself rejection: cellular immunity, employing mainly T cells, and humoral immunity, mediated by antibodies produced by B lymphocytes. The immune system cells primarily involved in allograft rejection are the monocytes-macrophages and other cells of the macrophage line, such as dendritic cells and Langerhans cells, and the T and B lymphocytes. These cells are localized in the spleen, Peyer's patches, tonsils, thymus, and bone marrow. There is also a pool of lymphocytes and monocytes circulating in the peripheral blood.[4] Allograft rejection is a complex event characterized by three stages: the cognitive phase, the recruitment phase, and the effector phase.

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Cognitive Phase

The first stage of the rejection reaction is the recognition of foreign antigens of the transplanted organ by immune system components. The antigen presenting cells, macrophages, or dendritic cells phagocytose the antigen, degrade it, and present it on their membrane in association with class II major histocompatibility system (MHC) antigens. This phenomenon, associated with the secretion of interleukins (ILs), leads to the activation of T helper/inducer $CD4^+$ cells. The majority of studies on the immune response during transplantation have focused on this process.[5] $CD8^+$ lymphocytes also recognize MHC class I antigens expressed by the allograft cells, triggering the complex series of events leading to rejection.[6] $CD8^+$ differentiation, just as all the other effector cells acting in rejection, depends largely on stimulation by $CD4^+$ cells.

Recruitment Phase

The recruitment phase involves the proliferation of activated cells, and the recruitment and activation of effector cells, both in peripheral lymphoid organs and in the transplanted tissue, under the stimulus of several cytokines produced by lymphocytes and macrophages, mainly IL-1, IL-2, IL-4, IL-6, interferon-γ and tumor necrosis factor (TNF)-β.

Effector Phase

The activation of the effector phase of the response to transplantation eventually leads to graft rejection by different mechanisms:

1. Killing of allogeneic cells by cytotoxic T lymphocytes (CTLs), featuring the classic $CD8^+$ and $CD4^+$ phenotype.
2. Killing of allogeneic cells by monocytes and macrophages, able to lyse allogeneic cells through release of cytokines (TNF), H_2O_2, O_2 radicals, and nitric oxide.
3. Killing of allogeneic cells by $CD16^+$ cells capable of killing the target using a bridge constituted of specific IgG for the graft alloantigens, through a mechanism of cell-mediated antibody-dependent cytotoxicity.
4. Platelet activation and subsequent complement activation (C3a, C5a) with damage to endothelial cells, resulting in coagulation system activation and formation of microthrombi in the vascular bed of the graft.
5. Activation of the coagulation system.

Types of Rejection

Immunosuppressive drugs can effectively intervene at several stages of the immune response to limit the process of rejection. Graft rejection is commonly classified according to chronological stages that correspond to a histopathological pattern: hyperacute rejection, acute rejection, and chronic rejection.

Hyperacute Rejection
Hyperacute rejection is the earliest and most severe form of rejection, and occurs within a few hours or even minutes after completion of vascular anastomosis between the organ donor and recipient. The pathogenesis is usually related to the presence in the serum of the recipient of preformed IgM antibodies, directed toward the antigens of the ABO system.

Histologically the organ reveals hemorrhage with massive thrombosis of large and small intraparenchymal vessels. Fortunately, hyperacute rejection is now an historical remnant. With the current matching techniques between donor and recipient, such histoincompatibility is very unlikely.

Acute Rejection
Acute rejection, which usually occurs in a period of 1 to 3 months after transplantation, can be divided into acute vascular rejection and acute cellular rejection, depending on the immunopathological mechanism involved.

Acute vascular rejection is supported mainly by the presence of IgG directed to antigens (either human leukocyte antigen [HLA] or not) present on the vascular endothelial cells of the graft. These antigens are able to activate the complement cascade and trigger vessel wall inflammation, characterized by intimal proliferation and microthrombosis, accompanied by a perivascular parenchyma infiltration of variable degree.

Acute cellular rejection is characterized by intense infiltration of the graft parenchyma by lymphomonocytes, with extensive necrosis of parenchymal cells and a lesser degree of inflammatory reaction of endothelial cells against the microvasculature. The mechanism supporting this type of rejection, linked to the lysis of target cells, is mediated by CTLs, activated macrophages, and CD16 cells.

Chronic Rejection
Chronic rejection is the most insidious form of rejection, occurring months after heart transplantation, and is responsible for long-term morbidity and mortality after transplantation. Although there is still inadequate knowledge of the influence of different etiological mechanisms that lead to chronic rejection, we know its pathologic expression well.

Chronic rejection and CAV. Chronic rejection often manifests as coronary heart disease in the transplanted heart (CAV), characterized by accelerated coronary atherosclerosis with predominantly peripheral (but occasionally proximal) distribution. Upon microscopic examination these lesions appear diffuse and concentric, and rarely include calcified lesions.[7] The disease involves the entire cardiac allograft vascular tree, including portions of the aorta and pulmonary artery from the donor.

The histopathological appearance is characterized by initial endothelial damage, platelet aggregation, subintimal myofibroblast hyperplastic proliferation, deposition of lipid material, thickening of the tunica media and elastic membrane abnormalities, with inflammatory infiltrates.[8]

Chronic rejection and myocardial fibrosis. Another manifestation of chronic rejection failure, although rare, is diffuse myocardial fibrosis, sometimes without coronary lesions. This occurs as a result of repeated and never completely resolved rejection episodes, or as a result of coronary microcirculation alteration not identified by coronary angiography.

Etiopathogenesis of CAV. Despite the fact that the exact pathogenesis of CAV has yet to be defined, and that different factors may predispose certain individuals to CAV, there are data suggesting it is primarily an immune-mediated disease. CAV is limited to the allograft arterial and venous tree, with a diffuse pattern of involvement, but spares the recipient's other native organs. Experimental studies suggest an immunologic mechanism acting on a setting of predisposing nonimmunologic risk factors. The initial event is probably a coronary endothelial injury with subclinical manifestation. Endothelial damage would alter its functions, causing arterial inflammation, vasoconstriction, thrombosis, and vascular smooth muscle cell growth with progressive myointimal hyperplasia.[9] Following heart transplantation, endothelial damage is thought to occur from humoral or more significant cellular responses to HLA antigens and endothelial cell antigens.[10] However, the role of MHC donor–recipient differences and HLA class I or class II mismatching in patients presenting with CAV is not yet completely clear.[11] Further investigations would help to discover other different allograft-specific antigens that may prove to play a role in the development of CAV.[12]

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Other associated conditions may predispose individuals to CAV, such as cytomegalovirus infection, age, sex, obesity, dyslipidemia, diabetes mellitus, hypertension, smoking, and hyperhomocysteinemia.[13,14] Ischemia-reperfusion injury plays an early but important role in endothelial damage soon after heart transplantation.[15,16] A variety of oxidative molecules and aggressive mediators play a role in this initial injury.

Diagnosis of Cardiac Allograft Rejection

Endomyocardial biopsy still represents the gold standard in the diagnosis of rejection; however, it is an invasive procedure with risk of sampling error and a morbidity of 0.5% to 1.5%.[17] A major objective would be a noninvasive diagnostic technique for the management of cardiac allograft recipients. An early detection in the preclinical stage of rejection would help to better modulate immunosuppressive treatment, with the aim to reduce clinical complications due to acute or chronic rejection.

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Clinical Manifestations of CAV

With regard to the clinical manifestations of CAV, cardiac-transplanted patients usually have nontypical angina due to the enervation of the transplanted heart. Therefore, they may undergo silent myocardial infarction (MI), presenting later with heart failure symptoms or MI complications.

Imaging Studies for CAV Detection

Several imaging methodologies have been used for the diagnosis of CAV: multidetector computed tomographic coronary angiography, intravascular ultrasonography, and cardiac magnetic resonance (CMR) seem promising techniques for the early diagnosis of CAV.[18,19] Percutaneous coronary angioplasty has been used to treat lesions in CAV patients.[20] Ultrasonic myocardial backscatter, radionuclide imaging, and intramyocardial electrogram have been investigated as well.[21-23] Gene expression profile analysis has been used to detect cardiac allograft rejection, and both the Invasive Monitoring Attenuation Through Gene Expression (IMAGE) and Cardiac Allograft Rejection Gene Expression Observational (CARGO) studies were designed for this purpose.[24,25] However, gene expression analysis seems to have limited applicability, mainly due to high cost and weakness in detecting mild rejection. Recently, strain rate (SR) based on tissue Doppler imaging (TDI) has been proposed as an imaging methodology potentially able to detect mild rejection not yet associated with hemodynamic changes.[26] TDI, with its quantitative assessment of regional myocardial wall motion, reflects both systolic and diastolic ventricular functions.[27] Cardiac allograft rejection, histologically characterized by inflammatory cell infiltration and edema, will reflect on left ventricular myocardial contraction and filling abnormalities due to stiffness and abnormal relaxation.[28] SR imaging may have clinical value in monitoring subclinical rejection.[26] CMR may represent a promising technique for early detection of graft vasculopathy. CMR using delayed contrast enhancement adds a precise tissue characterization that permits the identification of both ischemic and nonischemic scar tissue and viable tissue. CMR may prove useful in the preclinical detection of CAV, calling for coronary treatment, with the aim of preventing further cardiac allograft complications.[29]

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Immunosuppression Targets

According to the above-described immunologic steps, following organ transplantation, the recognition of allogeneic cells by the recipient's lymphocytes would inexorably lead to rejection. The transplantation outcome is based on the prevention of this seemingly inevitable event. Immunosuppressive therapy, acting on both cognitive and effector steps, has improved the results of cardiac allograft recipients.

The ultimate goal of immunosuppressive treatment is not only to modulate a selective interaction between allograft and recipient, trying to minimize the side effects secondary to the use of these immunosuppressive agents, but also to preserve the immune system against infection and the development of malignancies.[30] The intention is always to prevent the patients who are submitted to

immunosuppressive therapy from paying a higher price than what was won through transplantation.

Immunosuppression protocols have evolved over the years by comparing the experiences of different institutions and the study of new pharmacological agents. If we exclude the historical stages when total body irradiation and azathioprine in combination with steroids were mainly used, it was the introduction of cyclosporin A into clinical practice that changed the face of immunosuppressive therapy. Therefore, since the beginning of the 1980s, there has been a progressive improvement in immunosuppressive therapy, with a gradual reduction of cyclosporine doses, the transition from dual therapy with cyclosporine and corticosteroids to triple therapy with the addition of azathioprine, the use of monoclonal antibodies or antimitotic globulins for the first 3 or 4 days after transplantation, the use of mycophenolate instead of the azathioprine, and tacrolimus instead of cyclosporine.[31] In general, immunosuppressive protocols in use today provide an early stage of induction, followed by chronic maintenance therapy. Although this strategy is shared internationally, the choice of immunosuppressive agent, dosage, and combination of drugs varies among different institutions.[32-34] Therefore, the immunosuppressive protocols used for heart transplantation require a combination of drugs acting on different stages of the immune system (Table 1), with the aim to enhance the immunosuppressive effect while reducing the dosage of each pharmacological agent to reduce their respective side effects.

Immunosuppression in Clinical Practice

A so-called triple therapy is currently used, involving the use of a calcineurin inhibitor such as a cyclosporine or tacrolimus, an antimetabolite agent such as azathioprine or mycophenolate mofetil (MMF), and a corticosteroid such as prednisone or prednisolone.

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Results of clinical trials comparing cyclosporine and tacrolimus have shown similar survival and freedom from acute rejection.[35-37] The choice between those two calcineurin inhibitors is mainly based on institutional preference and on side effects related to each individual patient. Instead, comparative studies between the antimetabolites (azathioprine and MMF) in cardiac allograft recipients have shown a reduced acute rejection rate and improved 1-year survival in the MMF group.[38] Therefore, MMF is currently preferred in immunosuppressive protocols.[39]

Many institutions use an initial quadruple therapy in the induction phase by adding a fourth component, such as polyclonal antilymphocyte (eg, antilymphocyte serum), antilymphocyte globulin, or antithymocyte globulin, or a monoclonal antibody such as muronomab-CD3, directed against the CD3 receptor, or basiliximab or daclizumab, directed against the IL-2 receptor.[40] In recent years, two other pharmacological agents have been introduced. Everolimus and sirolimus belong to a new family of immunosuppressives called mTOR inhibitors (mammalian target of rapamycin). They have very interesting features both from the point of view of immunomodulation, acting at different stages of the immunologic process, and because they promise an anticancer effect. Figure 1 represents an example of immunosuppressive therapy in relation to the timing after heart transplantation.

Many new-generation immunosuppressive drugs are currently under investigation, both preclinically and clinically. They are usually directed against new immunologic targets.[41] Among these, the most promising appear to be FTY720 (S1P receptor blockade),[42-44] FK778 (active metabolite of the leflunomide inhibitor of tyrosine kinase),[45-47] LEA29Y (inhibitor of activation of T lymphocytes by blocking the receptor CD80 and CD86),[48] and alemtuzumab (anti-CD52 monoclonal antibody).[49,50] However, the true research goal would be the induction of complete immunologic tolerance in the recipient.

Although heart transplantation is now a reality, with the use of immunosuppressive therapy, toxic effects and failure to prevent forms of chronic rejection (including the development of long-term complications such as cancer) have led researchers to consider alternative strategies.

The possibility of effective tolerance of an allograft was first highlighted by Billingham in 1953.[51] Experimental models have shown how the induction of tolerance would represent the best way to prevent chronic rejection.[52]

Future prospective trials should have the endpoint of the absence of CAV to demonstrate the efficacy of

Table 1
Immunosuppressive Agents With Action Mechanism and Collateral Effects

Drug	Mechanism of Action	Collateral Effects
Corticosteroids	Broad-spectrum anti-inflammatory and immunomodulatory action, lymphocyte depletion, inhibition of monocyte-macrophage production of cytokines	Cushing's facies and weight gain, hypertension, hypokalemia, hyperglycemia, diabetes, hyperlipidemia, peptic ulcer, GI bleeding, glaucoma, osteoporosis, psychosis, delay of cicatrization, skin fragility
Cyclosporin	Calcineurin inhibitor, blockage of IL-2 production, inhibition of T lymphocyte proliferation and differentiation	Nephrotoxicity, hypertension, neurotoxicity, gingival hyperplasia, hypertrichosis, hyperuricemia, hepatoxicity, hyperlipidemia, hyperglycemia
Tacrolimus (FK506)	Calcineurin inhibitor, blockage of IL-2 production, inhibition of T lymphocyte proliferation and differentiation	Collateral effects similar to cyclosporin: nephrotoxicity, hypertension, neurotoxicity, gingival hyperplasia, hypertrichosis, hyperuricemia, hepatoxicity, hyperlipidemia, hyperglycemia
Azathioprine	Antimetabolite agent: inhibition of purine metabolism, inhibition of T lymphocyte actions, inhibition of antibody synthesis, reduction of circulating monocyte and granulocyte	Bone marrow suppression, pancreatitis, hepatitis, cholestatic icterus, GI complications (nausea, vomiting, abdominal pain, diarrhea)
Mycophenolate mofetil	Antimetabolite agent: selective inhibition of purine synthesis, stronger and more specific action on B and T lymphocyte proliferation	GI disorders, leucopenia, thrombocytopenia, pancytopenia
Sirolimus (rapamycin) and everolimus	Mammalian target of rapamycin (mTOR) inhibition, inhibition of T lymphocyte proliferation and differentiation from G_1 to S phase	Hyperlipidemia, thrombocytopenia, leucopenia, hypertension, peripheral edema, delay of cicatrization
Antilymphocyte antibodies (ALS, ALG, ATG)	Polyclonal antibodies, reduction of circulating T lymphocyte (antibody-mediated cell destruction)	Fever, polyarthralgia, anemia, leucopenia, thrombocytopenia, GI disorders, skin rashes, alopecia
Muronomab-CD3	Monoclonal antibody, inhibition of T and CTL lymphocytes	Fever, chills, malaise, bronchospasm, hypotension from peripheral vasodilation, GI disorders, pulmonary edema, infections, lymphatic neoplastic disorders, rebound effects
Daclizumab and basiliximab	Monoclonal antibody, IL-2 receptor inhibition, inhibition of IL-2–dependent T lymphocytes	Usually better clinical tolerability

ALG, antilymphocyte globulin; ALS, antilymphocyte serum; ATG, antithymocyte globulin; GI, gastrointestinal; IL, interleukin.

immunologic tolerance in the cardiac allograft recipient.[53] However, despite the many protocols on the induction of tolerance in small animals, very few models have been developed in pigs or primates. This problem applies to all organs, but especially to thoracic organs. In fact, it is generally more difficult to induce tolerance to the heart and lungs, than for example to the liver or kidney.[54] Today we know three models that allow for prolonged survival of heart transplants in large animals (none of the lung): stimulation of cell chimerism inducing tolerance at the central level,[55-58] the use of costimulatory blockers provoking a state of peripheral anergy,[59] and the combined transplantation of multiple organs, involving a variable degree of immunomodulation of patients receiving different tissues simultaneously, according to mechanisms not yet known.[60]

Conclusions

Despite the progress in immunosuppressive therapy, cardiac transplant

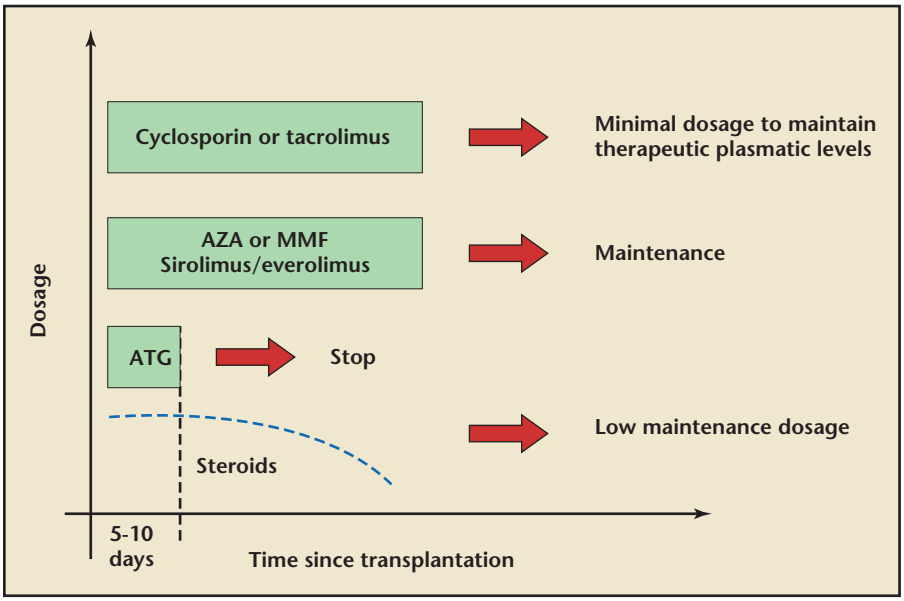

Figure 1. *Example of quadruple immunosuppressive therapy. Induction is by ATG for the first days after transplantation. ATG, antithymocyte globulin; AZA, azathioprine; MMF, mycophenolate mofetil.*

recipients still face problems related to rejection. CAV represents a major complication in long-term survivors. This type of coronary involvement, affecting the cardiac allograft, primarily has an immunologic cause. Imaging studies may help to reach an early diagnosis of this disease to prompt specific treatment. However, the best imaging methodology is still a matter of debate. An early diagnosis would help to better modulate specific immunologic treatments. Future research on new and more specific immunosuppressive agents and prompt diagnosis of mild rejection with new methodologies would help patients after cardiac transplantation. ■

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Main Points

- Immunosuppressive therapy following heart transplantation has improved over the years; however, despite these advances, this therapy remains nonspecific with regard to the different phases of the immune response. This results in an overall decrease in immune responses, including those directed against infectious agents and tumor antigens. Consequently, the most significant long-term complications are represented by the higher incidence of infections and malignancies. Nevertheless, despite these complications due to immunosuppression, the phenomenon of chronic rejection is still a limitation.
- The success of organ transplantation is related to the ability to modulate the immunological response of the allograft recipient with the aim of avoiding a rejection reaction. Essentially, two defense mechanisms contribute to non-self rejection: cellular immunity, employing mainly T cells, and humoral immunity, mediated by antibodies produced by B lymphocytes. Allograft rejection is a complex event characterized by three stages: the cognitive phase, the recruitment phase, and the effector phase.
- Graft rejection is usually classified as hyperacute, acute, or chronic. Hyperacute rejection occurs within a few hours after completion of vascular anastomosis between the organ donor and recipient. It is usually related to ABO system incompatibility but is now an historical remnant with the current "matching" techniques between donor and recipient. Acute rejection usually occurs in a period of 1 to 3 months after transplantation, and can be divided into acute vascular rejection and acute cellular rejection. Chronic rejection is the most insidious form, occurring months after heart transplantation. It often manifests as coronary heart disease in the transplanted heart (cardiac allograft vasculopathy [CAV]). This manifests as accelerated coronary atherosclerosis with predominantly peripheral distribution.
- Early diagnosis of cardiac allograft rejection is important to better modulate immunosuppressive treatment. Endomyocardial biopsy still represents the gold standard in the diagnosis of rejection; however, this is an invasive procedure with risk of sampling error and with a morbidity of 0.5% to 1.5%. One major objective is a noninvasive diagnostic technique for the management of cardiac allograft recipients.
- Heart-transplanted patients often have subtle clinical manifestations of CAV. Usually, they do not have typical angina due to the enervation of the transplanted heart. Therefore, they may undergo silent myocardial infarction (MI), presenting later with heart failure symptoms or MI complications.
- Several imaging methodologies have been used for the diagnosis of CAV; multidetector computed tomographic coronary angiography, intravascular ultrasonography, and cardiac magnetic resonance seem promising techniques for early diagnosis of CAV. Percutaneous coronary angioplasty has been used to treat lesions in CAV patients.
- Immunosuppression protocols have evolved over the years. They require a combination of drugs acting on different stages of the immune system. The aim is to enhance the immunosuppressive effect while reducing the dosage of each pharmacological agent in order to reduce their specific side effects. The goal is not only to modulate a selective interaction between allograft and recipient, but also to preserve the immune system against infection and the development of malignancies. A so-called triple therapy is currently used in cardiac allograft recipients; however, many institutions employ an initial quadruple therapy in the induction phase by adding a fourth component. New-generation immunosuppressive drugs, usually directed against new immunological targets, are currently under investigation.

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