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# IMMUNOSUPPRESSIVE TREATMENT FOR KIDNEY TRANSPLANTATION

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A b s t r a c t: Immunosuppressive treatment minimizes unwanted immune reactivity, but it also leads to complications such as metabolic disorders, cardiovascular diseases and malignant tumours. In this paper we summarise the recent developments in action mechanisms of available immunosuppressive drugs and their usage for renal transplantation. These drugs act at various levels of lymphocytic activation and proliferation, and they may have additive or synergic effects when combined. In the majority of patients, the immunosuppressive protocol includes a calcineurin inhibitor (tacrolimus or cyclosporin), an antimetabolite (mycophenolate mofetil or mycophenolic acid) and a corticosteroid. Most patients also receive induction with monoclonal or polyclonal antilymphocytic antibodies. These immunosuppressive drugs allow a one-year survival of renal allografts in over 90% of cases and an incidence of acute rejection episodes below 15%. In most cases, acute cell-mediated rejection can be reversed with pulse doses of methylprednisolone; less often antilymphocytic antibodies must be applied. Acute humoral rejection can be suppressed with high doses of intravenous immunoglobulines or low doses of cytomegalovirus hyperimmune globuline, in combination with plasmapheresis, to obtain a satisfactory reduction of anti-donor antibodies. This treatment also allows renal transplantation for sensitised recipients, or transplantation against a positive cross match or AB0 incompatibility. Less often, immunoadsorption, alemtuzumab, rituximab or splenectomy are applied. New immunosuppressive drugs and protocols are currently under investigation. Immunosuppressive agents and methods targeting the induction of immune tolerance to the donor organ are especially promising.

**Key words:** immunosuppression, rejection, renal transplantation.

*Introduction*

Renal transplantation has been significantly improved with the development of new immunosuppressive drugs, which can be used in multiple combinations through various immunosuppressive protocols. The main goal of immunosuppressive treatment is to minimise unwanted immune reactivity, but complications often arise, such as infections, metabolic disturbances, arterial hypertension, tumours and other unwanted side-effects. Immunosuppressive agents target different levels of lymphocytic activation and proliferation (Figure 1).

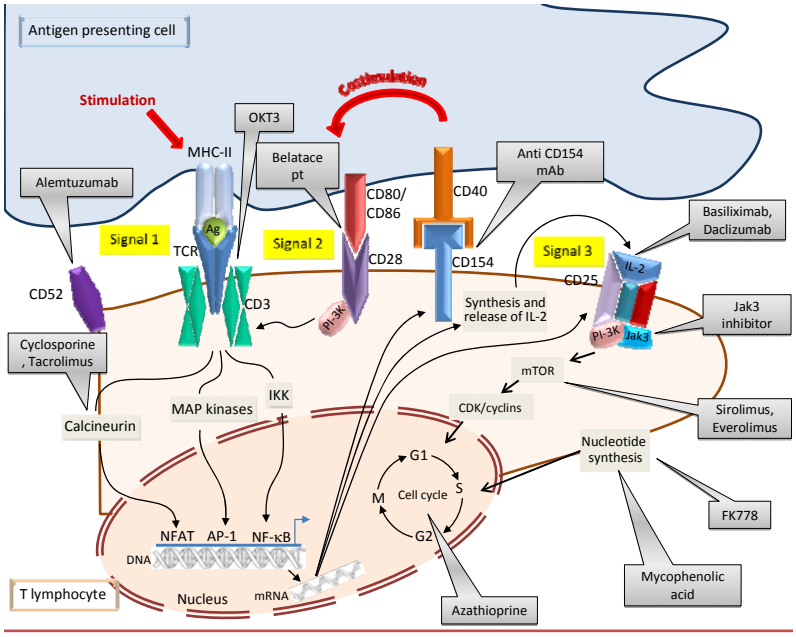


*Figure 1 – Targets of immunosuppressive drugs, modified according to Halloran PF[8]*
*Legend: MHC: major histocompatibility complex; TCR: T cell receptor; Ag: antigen; mAb: monoclonal antibody; IL-2: interleukin-2; PI-3K: phosphatidylinositol-3 kinase; Jak: Janus kinase; MAP kinase: mitogen activated protein kinase; IKK: inhibitor of κB kinase; CDK: cyclin-dependent kinases; DNA: deoxyribonucleic acid; mRNK: messenger ribonucleic acid; NFAT: nuclear factor of activated T cells; AP-1: activator protein-1; NF: nuclear factor*

They can inhibit transmembranous signalling induced by T-cell receptor interaction with antigen-presenting cells. This inhibitory effect includes blockage of the CD3 complex-mediated signal transduction (signal 1), as well as blockage of the non antigen-specific costimulatory signal (signal 2), which is induced by the interaction of the CD80/86 (B7) molecule on the antigen-presenting cell

with the CD28 molecule on the T-lymphocyte [1]. The summarised effects of these two signals are needed to overcome the activation threshold and to induce interleukin-2 receptor (IL-2R) expression and cytokine release. Interleukin-2 (IL-2) plays a major role in lymphocytic proliferation. Its ligation to the IL-2R induces the activation of mTOR (mammalian target of rapamycin), providing the signal 3, which is necessary for cellular proliferation [2]. All stages of lymphocyte reactivity can be blocked by immunosuppressive drugs with different mechanisms of action, hence these drugs are applied in combination.

## *Immunosupressive drugs*

### *Calcineurin inhibitors*

Cyclosporin (cyclosporin A) and tacrolimus (FK506) diminish lymphocytic activation by inhibiting the calcineurin pathway of intracellular signal transmission. Antigen binding to the T-cell receptor induces inositol 1, 4, 5-triphosphate ($IP_3$) and diacylglycerol (DAG) production. $IP_3$ increases the intracellular concentration of calcium which binds to calmodulin. This complex activates several enzymes, including the phosphatase calcineurin. Cyclosporin and tacrolimus form a complex with their cytoplasmatic receptors cyclophilin and FKBP (FK binding protein) which binds to calcineurin and blocks its activity. This affects the function of the regulatory protein NFAT (nuclear factor of activated T-cells) by blocking its translocation into the nucleus [3, 4]. As a result, the expression of several cytokine genes, important for T-lymphocytic activation, is inhibited, including IL-2, IL-4, IFN-γ, TNF-α (tumor necrosis factor α), CD154 (CD40L), H-ras and c-myc. In addition, cyclosporin enhances the expression of TGF-β (transforming growth factor-β) which further inhibits the secretion of IL-2 and the recruitment of cytotoxic T-lymphocytes. But, TGF-β has also been associated with the development of interstitial fibrosis in the renal allograft (nephrotoxic effect of calcineurin inhibitors) and the increased growth of tumour cells [5].

Calcineurin inhibitors are metabolized in the liver into numerous metabolits, which can exert pharmacologic and/or nephrotoxic effects. These metabolites are mostly excreted by bile, less by urine. At therapeutic levels cyclosporin and tacrolimus decrease the activity of calcineurin to less than 50%. This allows strong signals to overcome the inhibition and to stimulate a desired immune response for the protection of the transplant recipient. Calcineurin inhibitors interact with many drugs which influence the activity of P450 enzymes in the liver and gut. Moreover, they inhibit the protein MDR (multidrug resistance protein) leading to interactions with other drugs. Adequate dosing of calcineurin inhibitors is based on the regular determination of their whole blood levels. Usually, the trough level (twelve hours after calcineurin administration) is mea-

sured. In the case of cyclosporin, C2-monitoring (two hours after administration) which approximates the maximum level, and abbreviated AUC (area under the curve during the first four hours after dosing) is also recommended. The most specific and referent method for the determination of unmetabolized cyclosporin is HPLC (high performance liquid chromatography), but it is expensive and complicated to perform. Therefore immune tests with monoclonal antibodies against cyclosporin as FPIA (fluorescence polarization immunoassay) and EMIT (enzyme-multiplied immunoassay technique) are used. However, in these tests reagents cross-react with degradation products of cyclosporin, giving false results by overestimating drug levels for 45% (FPIA) and 15% (EMIT) [6]. Tacrolimus levels are usually determined by the monoclonal antibody test MEIA (microparticle enzyme immunoassay) [7].

Cyclosporin and tacrolimus can have many unwanted effects, primarily cardiovascular and nephrotoxic, which are the main limiting factors for their prolonged usage. They induce a reversible vasoconstriction which is dose-dependent and affects primarily the renal afferent arterioles. Hence, the perioperative administration of these drugs may increase the incidence and severity of delayed allograft function (acute tubular necrosis), especially in the case of a prolonged ischaemia time or higher dosages of calcineurin inhibitors. Long-term administration may cause a focal or striped chronic interstitial fibrosis, related to arteriolar damage, which may lead to a progressive loss of allograft function (CAN = chronic allograft nephropathy, i.e. IFTA = interstitial fibrosis and tubular atrophy). Sometimes thrombotic microangiopathy can develop, localized to the allograft or as a systemic disorder, and is similar to thrombotic thrombocytopenic purpura (TPP). Renal vasoconstriction may lead to diminished sodium excretion, development of arterial hypertension and oedema. Calcineurin inhibitors often cause hypercalaemia, linked with a mild hyperchloremic acidosis. Hypomagnesaemia or hypocalcaemia are usually the result of their elevated urine exretion; hyperuricaemia and gout are caused by a lowered urinary uric acid excretion. Among other important side-effects are alterations in liver function, hyperlipidaemia, diabetes, neurotoxicity, cardiotoxicity, gastrointestinal disorders, thromboembolism, cosmetic problems (hypertrichosis or alopecia, gingival hyperplasia, gynecomasty), infections and the development of malignant tumours [8, 9].

*Antimetabolics*

*Mycophenolate mofetil (MMF).* In combination with cyclosporin and prednisone, MMF is more efficient than azathioprine for the prevention of acute renal allograft rejection. The active compound of MMF is mycophenolic acid (MPA, ERL-80, myfortic) that, through a reversible inhibition of inosine monophosphate dehydrogenase (IMPDH), blocks purine synthesis and guanosine nucleotide formation in activated lymphocytes. This inhibits the $G_1$ cell cycle phase of T-lymphocyte proliferation in the IL-2 dependent pathway. Contrary to

other cells, lymphocytes do not have an alternative metabolic pathway for the transformation of guanine into guanosine nucleotides [9–11].

Unwanted side-effects develop mainly in the gastrointestinal and haematopoietic systems and may require dose reduction. Therefore it is useful to determine MPA blood levels, which can also reveal patient non-compliance. Cyclosporin lowers the blood MPA levels by a reduction of enterohepatic recirculation, whereas drugs like antacids, cholestyramine or peroral iron-sulphate decrease apsorption of MPA in the gut [8, 9].

*Azathioprine.* This imidazol derivative of 6-mercaptopurine has been mostly replaced by MMF or MPA. Azathioprine blocks purine nucleotide synthesis by incorporation into DNA, which interferes wtih RNA synthesis and degradation. This inhibits gene replication and T-lymphocyte activation. Azathioprine has a broad suppressive effect on the myeloid lineage through the inhibition of promyelocyte proliferation in the bone marrow, which reduces the percentage of circulating monocytes capable for maturation into macrophages. Azathioprine strongly blocks the primary immune response, but it is not effective for the treatment of allograft rejection. Unwanted side-effects impact the gastrointestinal and haiematopoetic systems. Inhibition of xanthine oxidase by allopurinol interferes with the degradation of azathioprine, which may cause a severe leucopenia and thrombocytopenia [8, 9].

*Corticosteroids*

These drugs act on the specific immunity by blocking the expression of genes for cytokines and cytokine receptors, which mediate functions of antigen presenting cells and T-lymphocytes. What is more, they exert antiinflammatory effects and reduce the unspecific immune response. Corticosteroids are liposoluble substances that can easily diffuse through cellular membranes and bind to cytoplasmatic receptors linked to heat shock proteins (hsp90, hsp70) and FK506-binding proteins. After the ligation of corticosteroids, these proteins are released and the receptor complex translocates to the nucleus where it binds to DNA sequences called glucocorticoid response elements (GREs). As such, corticosteroids inhibit the nuclear translocation of the transcriptional factors nuclear factor κ-B (NFκ-B) and activator protein-1 (AP-1), and their binding to specific DNA sequences [12]. This suppresses cytokine gene expression, which consequently reduces the secretion of IL-1, IL-2, IL-3, IL-6, TNF-α and INF-γ, while also inhibiting all stages of T-lymphocyte activation and proliferation. Through the synthesis blockage, release and action of numerous chemokines and vasodilatory factors, corticosteroids inhibit monocyte migration and diapedesis into the inflamed tissue [13]. Fever, which is a consequence of cytokine release during acute rejection, quickly resolves after the administration of pulse doses of corticosteroids. Lymphopenia may develop as a consequence of lymphocyte-homing from the circulatory system back to the lymphoid tissue, and of a direct

antilymphocytic apoptotic effect. However, the total number of PBL may increase several times during treatment with high doses of corticosteroids [8].

Prednisolone, a metabolite of prednisone, is the most often used corticosteroid for the treatment of renal transplant recipients. Corticosteroids are metabolized in the liver by microsomal enzymatic systems. Therefore, drugs which stimulate these enzymes, like barbiturates, reduce plasma levels of corticosteroids, while oral contraceptives and ketoconazole increase their levels.

Corticosteroids have a strong immunosuppressive, antiinflammatory and hormonal effect. Significant individual differences in the distribution of glucocorticoid receptors in tissues and in the degradation rate of corticosteroids cause varying responses to application. Unwanted side-effects can develop on many organ systems, as most cells have cytoplasmatic glucocorticoid receptors. Among the important side-effects are: a weaker resistance to infections, arterial hypertension, hyperlipidaemia, diabetes, delayed wound healing, growth retardation, osteonecrosis, osteoporosis, cataract, cosmetic and psychological alterations. The incidence and severity of unwanted effects depends on the applied dose of corticosteroids. Newer immunosuppressive protocols, where corticosteroid administration is reduced or avoided, are being investigated [8, 9].

*m-TOR (mammalian target of rapamycin) inhibitors*

Sirolimus (rapamycin) and everolimus (rapamycine-derivative, RAD) bind to the FKBP, but, instead of inhibiting calcineurin, this complex suppresses the action of the protein m-TOR, a key regulatory kinase in the process of cellular division [4]. m-TOR inhibitors suppress the cytokine-dependent proliferation of haematopoietic and non-haematopoietic cells in the $G_1$ and S phase of the cell cycle. These drugs can be used together with tacrolimus as there is no competitive inhibition between them due to an abundant amount of cellular FKBP. Contrary to calcineurin inhibitors, m-TOR inhibitors can delay the further deterioration of a modestly damaged allograft function. Everolimus is more hydrophylic and has a shorter half-life, but a higher bioavailability than sirolimus [8, 9].

m-TOR inhibitors are mainly metabolized in the liver by CYP3A and p-glycoproteins whilst their renal elimination is minimal. The intake of sirolimus should be four hours after the morning dose of cyclosporin inhibitors, to cirumvent a saturation of enzymatic systems in the liver and a consecutive excessive elevation of the sirolimus blood levels with the development of unwanted side-effects. Sirolimus has important interactions with other drugs, which necessitates their dose adjustment. If combined with sirolimus, the dose of calcineurin inhibitors has to be lowered to reduce their nephrotoxicity. m-TOR inhibitors can delay the recovery from acute tubular necrosis and restoration of allograft function. Due to a tubular damage, similar to that in multiple myeloma, the combination of sirolimus and tacrolimus can cause acute renal failure. m-TOR

inhibitors can also lead to hypokalaemia and hypomagnesaemia through toxic effects on renal tubules and their higher urinary excretion. Among other important side-effects are: delayed wound healing, development of lymphoceles, oral ulcers and oligospermia. More than half of the patients develop hyperlipidaemia, especially if m-TOR inhibitors are given in combination with cyclosporin. Prophylaxis with cotrimoxazole is recommended during the first posttransplant year because of a higher incidence of pneumonia during treatment with m-TOR inhibitors. These drugs can also cause a reversible decrease in the blood cell count, especially of platelets. An antitumorous effect of m-TOR inhibitors has been described due to angiogenesis inhibition and growth inhibition of malignant cells [8, 9].

It is recommended to include induction therapy with a biological agent, monoclonal or polyclonal antilymphocytic antibodies, as part of the initial immunosuppressive treatment [14]:

*Humanized anti-CD25 monoclonal antibodies*

Daclizumab and basiliximab inhibit cellular division by binding to the α chain (CD25, Tac) of the IL-2R, which is highly expressed on activated T-lymphocytes. Basiliximab is a chimeric antibody consisting of 75% human and 25% murine, and daclizumab of 90% human and 10% murine origin. This genetic modification has lowered their immunogenicity and prolongs their half-life to over one week. Binding of daclizumab or basiliximab inhibits the IL-2 induced response, which enhances the suppressive effect of calcineurin inhibitors on the production of IL-2. Anti-CD25 monoclonal antibodies block signal transmission through the IL-2R and engagement of the Jak/Stat (Janus kinases/signal transducers and activators of transcription) pathway of transmission in the cells, leading to cell division blockage [15, 16]. It has been described that, immediately after the administration of daclizumab, the proportion of $CD3^+CD25^+$ lymphocytes decreases from 15–30% to less than 3%. A significantly stronger effect has been achieved with basiliximab during 6–8 weeks, due to its stronger affinity for the IL-2R [17, 18]. The mechanism of action of anti-CD25 blockade is not related only to the inhibition of proliferation, but also to the inhbition of the IL-15 and IL-7 mediated activation pathway because these drugs down-regulate the common IL2/IL15 receptor beta chain [19]. Among the mechanisms of their immunosuppressive effect, the direct inhibition of the CD156 (CD40L) expression on the membrane of T-helper lymphocytes is very important [20].

In combination with calcineurin inhibitors and corticosteroids, basiliximab and daclizumab lower the incidence of acute rejection episodes. It is known that the administration of basiliximab, at a dose of 20 mg intravenously before the transplantation procedure and on the fourth postoperative day, saturates IL-2R for thirty to forty-five days. It is recommended to apply dacli-

zumab in five doses of 1 mg/kg BW. The first dose should be before the transplantation procedure and the following doses at two-week intervals. However, a satisfactory immunosuppressive effect can also be achieved with two doses of daclizumab of 1–2 mg/kg BW. A serum level of 1 μg/ml is sufficient for the saturation of IL-2R [21]. Anti-CD25 monoclonal antibodies do not induce significant unwanted side-effects, but an anaphylactic reaction may occur.

*Antilymphocytic antibodies*

*Antilymphocytic globulins (ALG)* are polyclonal antilymphocytic antibodies obtained by the immunisation of rabbits or horses with human thymocytes, or by the immunisation of rabbits wirh a Jurkat cell line (T-lymphocytic leukaemia). ALG contain cytotoxic antibodies against a large number of surface antigens of T-lymphocytes, NK cells, B-lymphocytes, adhesion molecules and chemokine receptors [22]. Their primary effect is a consequence of T-lymphocyte depletion ($CD3^+$ over 50%) in blood and lymphoid organs, but the exact mechanism of action is unknown. Lymphopenia develops within twenty-four hours and lasts for several years and $CD8^+$ T-lymphocytes recover earlier than $CD4^+$ T-lymphocytes. The degree of T-lymphocyte depletion in peripheral tissues is influenced to a greater extent by the maximal drug concentration than the applied cumulative dose. Through their immunomodulatory effect, ALG also induce changes in T-lymphocyte function and the development of $CD4^+CD25^{high}$ $Foxp3^+$ (forkhead box protein 3) T-regulatory cells [23]. They can also lower cellular infiltration during organ reperfusion and during acute rejection episodes. The intraoperative administration of ALG, before reperfusion, can lower the incidence of delayed graft function (tubular necrosis), but it is not known whether cytokine release caused by ALG may worsen the reperfusion damage.

In combination with conventional triple immunosuppression, which includes a calcineurin inhibitor, MMF (or MPA) and corticosteroids, ALG administration lowers the incidence of acute allograft rejection and allows the delayed introduction of calcineurin inhibitors [24]. It allows a reduction of the dose of other immunosuppressants or the avoidance of corticosteroids. ALG induction is indicated for recipients with a high risk for rejection or for delayed allograft function [14]. Dosing and duration of ALG treatment depend on the mode of administration and combination with other immunosuppressive agents. The elimination half-life of ALG is very variable, and monitoring is based on the determination of the T lymphocyte count in peripheral blood. ALG are diluted in saline or 5% glucose solution and administered very slowly (over several hours) through a large vein. The daily dose of corticosteroids, an intravenous antihystamine and antipyretic should be given before infusion to reduce the severity of the cytokine release syndrome which often develops during ALG administration. This syndrome is characterized by chills, fever, low blood pres-

sure, tachycardia, vomiting and dyspnea. During treatment with ALG, prophylaxis of phlebothrombosis and opportunistic infections is important. Signs of thrombophlebitis may develop at the infusion site and, rarely, allergic reactions such as serum illness or anaphylaxis. In the first two days, but also at the end of treatment, neutropenia and/or thrombocytopenia may develop, which may require a dose reduction or cessation of treatment with ALG. Without antiviral prophylaxis, there is a higher incidence of cytomegaloviral and BK polyomaviral infection, cancer and posttransplant lymphoproliferative disease. Therefore, in recipients with low risk for acute rejection, administration of ALG has no advantages over conventional triple immunosuppressive treatment [14].

*Intravenous immunoglobulins (IVIG)* are antilymphocytic antibodies obtained by the collection of plasma from several thousand blood donors. Their application in the field of organ transplantation is constantly growing, and in addition to unspecific IVIG, specific cytomegalovirus hyperimmune globulins (CMVIG) are also applied [14]. IVIG have an immunomodulatory effect. They inhibit anti-HLA antibodies and cause longterm suppression and elimination of anti-HLA reactive T and B-lymphocytes. IVIG also inhibit cytokine signal transmission and aloimmunisation by T-cell receptor blockade. They are mostly used in combination with plasmapheresis, but also with splenectomy or rituximab. In sensitized patients, these protocols reduce the high titre of preexisting anti-HLA antibodies. They also allow renal transplantation from living donors in the case of a positive cross-match or ABO incompatibility [25]. IVIG are used for the reversal of humoral rejection and for the treatment of posttransplant viral infections [26, 27]. During their application, mild unwanted effects may arise (redness, chills, headache, nausea, myalgia, arthralgia) as well as a transient aseptic meningitis or reversible acute renal failure due to an osmotic damage of proximal tubules.

*OKT3 or muromonab-CD3* is a murine monoclonal depletional antibody against the CD3 complex of T-lymphocytes. OKT3 is highly efficient as an induction agent for the prevention of allograft rejection, as well as for the reversal of early acute rejection episodes [28]. Over the last decade the use of OKT3 was abandoned because it can cause severe side-effects, including the cytokine release syndrom and complement activation (fever, chills, pulmonary oedema, worsening of graft function), graft thrombosis, thrombotic microangiopathy, opportunistic infections and posttransplant lymphoproliferative disease [29]. Currently, humanized CD3-specific antibodies that do not bind Fc receptors are under investigation in order to reduce the incidence of side-effects, especially the cytokine release syndrome [30].

*Alemtuzumab* is a humanized anti-CD52 monoclonal antibody against B and T-lymphocytes which is approved for the treatment of chronic lymphocytic leukaemia. It induces lymphopenia which lasts for several months [31, 32]. In organ transplantation, alemtuzumab is used as an induction agent and for the

treatment of acute rejection episodes, but large controlled trials are still missing. Alemtuzumab is useful for the treatment of acute cellular and humoral rejection and for the reversal of steroid- resistant rejection episodes with a documented infiltration of $CD20^+$ B-lymphocytes. The dose of other immunosuppressants should be reduced and prophylaxis against infections should be given to lower the rate of infections and posttransplant lymphoproliferative disease [14, 33, 34].

*Rituximab* is an anti-CD20 monoclonal antibody against B-lymphocytes, approved for the treatment of some forms of non-Hodgkin lymphoma. It leads to a rapid depletion of B-lymphocytes in the circulation and tissues that lasts for months [35]. Rituximab is used in organ transplantation to reduce the high titre of anti-HLA antibodies in sensitized patients, for the transplantation of organs from living donors in the case of a positive cross-match or AB0-incompatibility, for the reversal of acute humoral rejection, and for the treatment of posttransplant lymphoproliferative disease characterized by $CD20^+$ cell infiltration [36–38]. Rituximab is applied after a premedication with antipyretic drugs and antihistamines.

### *Immunosuppressive protocol*

The conventional immunosuppressive protocol, which is used in most patients, includes a calcineurin inhibitor, MMF (or MPA) and a corticosteroid [9]. In the early period after transplantation a stronger immunosuppression is needed to prevent rejection [39, 40]. Higher doses of immunosuppressive drugs are used or induction agents are added, such as polyclonal or monoclonal antibodies. These drugs allow a one-year graft survival over 90%, and an incidence of rejection episodes below 15% [41]. As immunosuppressive drugs act at different sites of the immune response, they are applied together in various combinations. In addition, some agents have additive or synergic effects, what allows their dose reduction and, consequently, a lower incidence of side-effects.

Immunosuppressive induction with antilymphocytic antibodies is especially important for recipients who have a high risk for rejection or delayed allograft function. Contrary to anti-CD25 monoclonal antibodies, depletional antilymphocytic antibodies (ALG or OKT3) allow the delayed introduction of calcineurin inhibitors. In addition to the immunosuppressive treatment, it is necessary to apply prophylaxis against peptic ulcer disease, infections and thrombosis. During the early posttransplant period, the procoagulatory effect of immunosuppressive drugs may cause allograft thrombosis. Calcium antagonists, especially verapamil and diltiazem, reduce the vasoconstrictory effect of calcineurin inhibitors, which can protect the graft against ischaemic damage and nephrotoxicity. Because of a competition with the enzyme sytem P450, calcium antagonists increase the blood levels of calcineurin inhibitors and allow a reduction of their dose.

In our clinic, we routinely apply the following immunosuppressive protocol:

– MMF 2 g/day; the first dose preoperatively and, in case of living donors, three days before transplantation;

– methylprednisolone 500 mg intraoperatively (before releasing the clamps of the vascular anastomoses); the dosage is tapered down to a maintenance dose of 0,3 mg/kg, slower in patients with a higher risk for rejection; in low-risk patients the dose is further reduced by 4 mg monthly and, eventually, corticosteroids are withdrawn after six months; in the case of renal transplantation from living donors, corticosteroids are applied only intraoperatively, but if a rejection episode arises, corticosteroid therapy is maintained for the whole period of graft function;

– tacrolimus has almost completely replaced cyclosporine, which had been used in our clinic since 1984 [42, 43]; tacrolimus is introduced on the first postoperative day, but can be delayed if the patient is receiving ALG; the dosage is increased until target blood levels are achieved (10–12 ng/l; lower target level in case of older donors or recipients, longer ischaemia times or otherwise damaged renal allograft);

– we routinely apply induction therapy; IL-2 receptor antibodies (1 mg/kg BW for two to five doses) are given to patients with a low immunologic risk, and ALG (1 mg/kg BW 3–9 days) to patients with a high risk for delayed graft function or rejection.

Several months or years after transplantation the recipient and graft adapt to each other and the dose of immunosuppressive drugs has to be reduced, but the drugs must be taken without interruption for the whole time of graft function. Non-compliance, as inappropriate intake or cessation of immunosuppressive drug intake, leads to worsening of graft function and graft loss in approximately one-fourth of the patients [44]. Treatment has to be adjusted to the individual needs of the patient to achieve a balance between drug effectiveness and tolerance of side-effects. In older transplant recipients and patients with abnormal liver function, which is most often caused by drug toxicity or earlier hepatitis, there is a lower incidence and severity of rejection episodes, but a higher incidence of infections [45]. A more intensive immunosuppression is needed for recipients with a higher immunologic risk, as in case of lower HLA compatibility with the donor, senzitation against tissue antigens, retransplantation or combined organ transplantation, delayed graft function, young and/or black recipients. Protocols with newer immunosuppressive drugs and a lower dose, or avoidance, of corticosteroids or calcineurin inhibitors are studied in order to reduce the incidence and severity of unwanted side-effects. The long half-life of graft and patient survival implicates that the introduction of new immunosuppressive drugs and protocols must follow a long-term study of a large number of transplant recipients in controlled clinical trials.

*Treatment of acute allograft rejection*

Acute cell-mediated rejection episodes are primarily treated with the intravenous administration of pulse doses of methylprednisolone for three to five days. In our clinic, methylprednisolone is given at a dosage of 10 mg/kg BW, which is, over five days, tapered down to a new maintenance dose. The dose of other immunosupressants is adjusted, if needed. Prophylaxis of peptic ulcer disease, infection and thrombosis is also applied. If there is no improvement during the first three days, ALG is administered. ALG obtained from immunized rabbits are more effective than those obtained from horses. Although ALG are more effective in reversing a first rejection episode, corticosteroids are used as first-line treatment because of their lower price and lower incidence and severity of side-effects. ALG are recommended for the treatment of severe cellular and humoral rejection episodes, recurrent or corticosteroid-resistant rejection episodes, and if corticosteroid treatment is contraindicated.

Acute humoral rejection, which is characterized by typical pathohistological findings and detection of donor-specific antibodies in the recipient's serum, can be reversed with high doses of IVIG or low doses of CMVIG, which are administered in combination with plasmapheresis until a satisfactory reduction of antidonor antibodies is achieved. Less often, immunoadsorption, rituximab, alemtuzumab or splenectomy are applied. In our clinic, humoral rejection is usually treated with IVIG, most often in combination with plasmapheresis. Humoral rejection episodes may recur and they may be accompanied or followed by cellular rejection episodes.

Rejection episodes that occur late, after the third posttransplant month, are usually treated with pulse doses of corticosteroids. In most cases, the underlying pathological finding is interstitial fibrosis and tubular atrophy (IFTA), which impends a further worsening of graft function and graft loss. An intensification of immunosuppression may cause additional complications and lead to a worsening of the patient's condition [46].

Investigations of immunosuppressive treatment for renal transplantation have led to the discovery of a large number of potential immunosuppressive drugs and new methods for the suppression of the immune reaction. Most promising are methods which induce immune tolerance to the donor organ, as the infusion of donor-specific bone marrow in combination with a short unspecific immunosuppression, or combined transplantation of a renal allograft and vascularized thymic tissue which establishes a mixed haematopoetic chymerism. Furthermore, methods of genetic engineering, incapsulation, and usage of stem cells as well as xenotransplantation are intensively investigated [47, 48].

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Р е з и м е

## ИМУНОСУПРЕСИВЕН ТРЕТМАН ЗА БУБРЕЖНА ТРАНСПЛАНТАЦИЈА

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А п с т р а к т: Имуносупресивниот третман ја минимизира несаканата имунолошка реактивност, но, исто така, доведува до компликации како што се метаболички нарушувања, кардиоваскуларни болести и малигни тумори. Во овој труд ќе се сумираат последните случувања во акциските механизми на расположливите имуносупресивни лекови и нивната употреба при трансплантација на бубрези. Овие лекови дејствуваат на различни нивоа на лимфоцитно активирање и пролиферирање, и тие може да имаат адитивни или синергиски ефекти кога се комбинираат. Кај поголемиот дел од пациентите, имуносупресивниот протокол вклучува калцинеурински инхибитор (такролимус или циклоспорин), антиметаболит (микофенолат мофетил или микофенолична киселина) и кортикостероид. Повеќето пациенти, исто така, добиваат индукција со моноклонални или поликлонални антилимфоцитни антитела. Овие имуносупресивни лекови овозможуваат една година преживување на реналните алографти во повеќе од 90% од случаите и инциденца на епизоди на акутно отфрлање под 15%. Во повеќето случаи, акут-

ното отфрлање со посредство на клетките може да се промени со ударни дози на метилпреднисолон; поретко, мора да се применуваат антилимфоцитни антитела. Акутното хуморално отфрлање може да биде потиснато со високи дози на интравенски имуноглобулини или ниски дози на цитомегаловирусни хиперимунски глобулин, во комбинација со плазмафереза, за да се добие задоволително намалување на антителата од анти-донаторот. Овој третман, исто така, им овозможува ренална трансплантација кај сензибилизирани приматели, или трансплантација наспрема позитивно накрсно совпаѓање или АВ0 некомпатибилност. Не толку често се применуваат и имуноадсорпцијата, алемтузумаб, ритуксимаб или спленектомијата. Во моментов се испитуваат нови имуносупресивни лекови и протоколи. Особено се ветувачки имуносупресивните агенси и методи насочени кон поттикнување на имунолошката толеранција кон органот од донаторот.

**Клучни зборови:** имуносупресија, отфрлање, ренална трансплантација.

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