IMMUNOSUPPRESSION IN KIDNEY TRANSPLANTATION

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Abstract

For most patients with chronic kidney failure, kidney transplantation has the greatest potential for restoring a healthy and productive life. The risk of acute rejection is the highest in the first months after transplantation (induction phase) and diminishes afterwards (maintenance phase). Immunosuppression should be at the highest level in the early period and reduced for long-term therapy. At present, conventional immunosuppressive protocols consist of the triple therapy: a calcineurin inhibitor, an adjunctive agent, corticosteroids. The development of new immunosuppressives drugs is aimed not only at improving short-term outcomes, but also achieving better safety, less nephrotoxicity and minimal side effects.

Keywords: immunosuppression, kidney transplantation, induction therapy, maintenance therapy.

A short history of immunosuppression in kidney transplantation

The first attempts of immunosuppression used total body irradiation. *Azathioprine* was introduced in the 1960s, soon accompanied by *prednisolone*. The polyclonal antibody preparations: *antithymocyte globulin (ATG)* and *antilymphocyte globulin (ALG)* became available in the 1970s. With ATG or ALG used for induction or for the treatment of steroid-resistant rejection and azathioprine and prednisolone as the baseline regimen, the success rate of kidney transplantation was 50% at 1 year and the mortality rate was 10% to 20%. In the 1980s, the introduction of *cyclosporine* by Borell produced statistically significant improvement in graft survival rates to greater than 80% at 1 year. The standard immunosuppressive regimen consisted of cyclosporine, prednisone and azathioprine The rate of mortality decreased but the major detriment of cyclosporine is that it causes acute and chronic nephrotoxicity [1]. *OKT3* was the first monoclonal antibody introduced in 1985, initially for treating the first acute rejection, although the toxicity of the drug restricted its use as induction agent or to treat acute rejection resistant to high-dose steroids. In the 1990s, with limited types of medication and antibody preparations, the graft survival was up to 90% at 1 year and minimal mortality [2].

Tacrolimus has been introduced in liver transplantation and as an alternative to cyclosporine since 1994. *Mycophenolate mofetil (MMF)*, introduced in 1995, was found to be more effective than azathioprine, reducing the incidence of acute rejection when used with cyclosporine (or tacrolimus) and corticosteroids [3]. Two humanized monoclonal antibodies*: Basiliximab* and *Daclizumab* reduced the incidence of acute rejection, and *Thymoglobulin*, a polyclonal antibody were approved for use because of its capacity to treat acute rejection [2].

A major new drug were available since 1999*: sirolimus* and *everolimus,* and also new agents with novel immunological targets such as anti-CD40 ligand, FTY720, anti-CD20 *(rituximab)* and anti-CH52 *(alemtuzumab)*, thus transplant immunosuppression became more diverse and complex [4].

Biological immunosuppressive agents in induction therapy

The polyclonal antibodies are: *ALG* is an anti-lymphocyte globulin*, ATG* - anti-T-lymphocytes globulin, *Thymoglobulin (TG)* - anti-thymocytes globulin obtained by immunization of rabbits and *Lymphoglobulin (LG)*- anti-thymocytes globulin obtained by immunization of horses. The mechanism of polyclonal antibodies is the cytotoxicity of antibodies directed against a variety of T-cell markers and depletion of the lymphocyties from the peripheral blood. Thymoglobulin causes sustained and rapid expansion of CD4, CD25 and other regulatory T cells that play an important role in maintaining the immune response and limiting antigraft immunity. The prolonged immunosuppressive effect may account for the relative infrequency of rejection recurrences. Polyclonal antibodies can produce leukopenia and thrombocytopenia. Infection, most commonly with cytomegalovirus, varies with the number of courses and the overall amount of

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immunosuppression given. The development of lymphoma is a consequence of effective immunosuppression [5].

Muromonab (OKT3) is a lymphocyte-depleting monoclonal antibody produced by the hybridization of murine antibody-secreting B lymphocytes with a nonsecreting myeloma cell line. It reacts with the human T-cell by binding the CD3 complex and T-cells became ineffectual and removed from the circulation into the reticuloendothelial system. After a few days, T-cells reappear in the circulation but are modulated cells, with CD4, CD8 and CD11 markers, but are devoid of CD3. In the first and second day of treatment with OKT3, potentially life-threatening adverse reactions may occur and this is the reason why it is now used only when Thymoglobulin is contraindicated because of leukopenia or thrombocytopenia. Episodes of rejection may occur up to 60% of courses of OKT3, but the episodes are mild and usually well controlled with prednisone pulse.

Alemtuzumab (Campath 1H) is a recombinant DNA-derived humanized monoclonal antibody directed against the cell surface glycoprotein CD52 and induces a profound, rapid and effective depletion of peripheral and central lymphoid cells. Its use may facilitate minimization of maintenance immunosuppressive protocols. The hematological, infective and lymphoma risks are similar to other depletion-inducing agents.

Humanized anti-CD25 monoclonal antibodies are *Basiliximab (Simulect)* and *Daclizumab (Zenapax)*. These antibodies are targeted against the alpha chain of the IL-2 receptor and the IL-2 mediated responses are blocked. They are designed to prevent, but not to treat the acute rejection. These antibodies complement the effect of calcineurin inhibitors and have no significant side effects.

Rituximab (Rituxan, Mabthera) is a monoclonal anti-CD20 antibody, targeted against the CD20-antigen on B lymphocytes. In transplantation it is used to suppress antibody formation, such as to treat acute humoral rejection, to treat recurrent post-transplantation focal and segmental glomerulosclerosis, or to treat post-transplantation lymphoproliferative diseases (PTLD) [6].

Other monoclonal antibodies: *Efalizumab (Raptiva)* is a humanized CD11a-specific IgG1, targeted against lymphocyte-associated function-1 (LFA-1) molecule; *Alefacept (Amevive)* is a humanized LFA-3-IgG1 fusion protein that binds to CD2 in the T lymphocyte and interferes with T-Cell activation; *Janus Kinase* and *Protein Kinase inhibitors* are a family of cytoplasmic tyrosine kinases involved in cell surface signaling [7]; *Bortezomib (Velcade)* is a proteosomal inhibitor and suppresses the T-cell function, it may be used for the prevention and treatment of antibody-mediated and cell-mediated rejection and reduces the level of donor-specific antibodies [8].

Intravenous immune globulins (IVIG) are pooled human gammaglobulin preparations which inhibit anti-HLA antibodies and produce long-term suppression of anti-HLA reactive T cells and B cells. They are used in transplantation to reduce high levels of preformed anti-HLA antibodies in sensitized patients, to treat acute humoral rejection and to treat certain post-transplantation viral infection [9].

Immunosuppressive agents in maintenance therapy

Calcineurin inhibitors (CNI): *Cyclosporine* (CsA) and *Tacrolimus* (Tac)

Cyclosporine is a cyclic polypeptide of fungal origin. *Tacrolimus* or FK506 is a macrolide antibiotic compound isolated from Streptomyces tsukubaensis. The calcineurin inhibitors impair the expression of several critical cytokine genes that promote T-cell activation, including those for IL-2, IL-4, interferon-γ (ITF-γ) and tumor necrosis factor-α (TNF-α). CsA enhances the expression of transforming growth factor-β (TGF-β) and may be responsible for the development of interstitial fibrosis, an important feature of CNI nephrotoxicity. The original formulation of cyclosporine is the oil-based Sandimun, while the microemulsion formulation-Neoral. Tacrolimus (Prograf) is primarly absorbed from the small intestine, with large interpatient and intrapatient variability, particularly in cases with gastro-intestinal diseases. A long acting once-daily formula- Advagraf is available.

CNI are nephrotoxic: enhancement of early post-transplant graft dysfunction, a dose related reversible renal vasoconstriction, chronic interstitial fibrosis, acute microvascular disease, hypertension and electrolyte abnormalities and non-renal toxicity: -gastrointestinal: hepatic dysfunction, mild, self-limited, dose-depending, increasing serum aminotransferase levels, mild hyperbilirubinemia, cholelithiasis are associated most frequently with CsA therapy. Varying degrees of anorexia, nausea, vomiting, diarrhea and abdominal discomfort occur in up to 75% of patients receiving tacrolimus; -cosmetic complications like hypertrichosis, gingival hyperplasia, gynecomastia, more frequently in patients receiving CsA, often enhanced by concomitant use of corticosteroids. Tacrolimus is responsible for hair loss or frank alopecia. This complications have to be considered seriously, particularly in women and adolescents because it can be a major cause of non-compliance to the treatment; -hyperlipidemia is more marked with CsA, glucose intolerance and new-onset diabetes mellitus is dose-related, increased by concomitant use of corticosteroids, more marked with Tac; hyperuricemia and gout particularly when diuretics are also used, more common in patients receiving CsA than Tac; -neurotoxicity is more marked with Tac: coarse tremor, dysesthesias, headache and insomnia, uncommon complications like seizures, leukoencephalopathy, or bone pain, infection and malignancy, thrombembolism [10].

The measurement of CsA and Tac levels is an intrinsec part of the management of transplanted patients,

there is a relationship between the levels of the drug and the episodes of rejection and toxicity.

Mycophenolate mofetil (MMF) and *Mycophenolic Acid* (MPA)

MMF is a prodrug, a fermentation product of several Penicillinum species, having MPA as active compound. MPA is a reversible inhibitor of the enzyme inosine monophosphate dehydrogenase and a selective antimetabolite. It blocks the proliferation of T and B cells, inhibits antibody formation and the generation of cytotoxic T cells, down-regulates the expression of adhesion molecules on lymphocytes and impairs their binding to vascular endothelial cells. MMF have the capacity to treat ongoing rejection and to prevent development and progression of proliferative arteriolopathy, a critical lesion in chronic rejection

MMF (CellCept) and enteric-coated MPA (Myfortic) have gastro-intestinal adverse effects more frequently, such as diarrhea (30%), varying degrees of nausea, bloating, dyspepsia, vomiting (20%), frank esophagitis, gastritis. Most of these symptoms respond to the reduction of drug dosage. The gastro-intestinal effect of Myfortic is not statistically significantly different from CellCept. Hematological side effect like leucopenia, anemia or thrombocytopenia may require dose adjustment. The incidence of lymphoproliferative disorders and infections are similar to other immunosuppressive drugs, and rare cases of progressive multifocal leukoencephalopathy (PML) have been described. Nephrotoxicity, neurotoxicity and hepatotoxicity have not been reported with MMF [11].

The mTOR inhibitors: *sirolimus (Rapamune)* and *everolimus (Certican)*

Sirolimus is a macrolide antibiotic compound and everolimus is a similar compound with a short half-life. They inhibit mTOR, a key regulatory kinase in the process of cell division. Both hematopoietic and non-hematopoietic cells are affected. The mTOR inhibitors do not produce acute or chronic reductions in glomerular filtration rate, unless administered with a standard dose of CNI, when it appears to have increased nephrotoxicity. Thus, the dose of CNI should be lower in combination with sirolimus. The sirolimus may be tubulotoxic and may produce hypo-kaliemia and hypomagnesemia, proteinuria or nephritic syndrome, de novo or enhancing preexisting proteinuria. Sirolimus may delay the recovery of the renal function after acute tubular necrosis. Sirolimus may replace MMF or be used in combination with MMF, but as a primary agent in less than 10% of cases, because of the side effects and the failure to show its superiority over MMF. MTOR inhibitors may increase the incidence of lymphoceles, poorly granulating wounds, particularly in obese patients, painful mouth ulcers. Hyperlipidemia may occur in more than 50% of patients, but this elevation may be controlled by statines. A noninfectious interstitial pneumonia has been described as a bilateral lower-lobe pneumonia, or several cases of fatal Pneumocystis pneumonia in patients who did not receive prophylactic Sumetrolim. Anemia or thrombocytopenia are more severe with MMF or azathioprine; thrombotic microangiopathy occurs more frequently when CNI are used in combination with sirolimus. The incidence of malignancy and post-transplant PTLD is small, which is why it should be used in patients with high risk to develop post-transplant malignancy, or those who have already developed malignancy [12].

Azathioprine (Imuran) is an imidazole derivative of 6-mercaptopurina, an antimetabolite which inhibits gene replication and consequently T-cell activation. It was introduced as an adjunctive agent of CsA and it has been discontinued in many programs when the MMF was introduced. It prevents the acute rejection but it is ineffective in the treatment of rejections. The most important side effects are leucopenia and thrombocytopenia, occasionally hepatitis and cholestasis.

Corticosteroids (Prednison and *Methylprednisolon*)

They were first used to treat rejection in the 1960s. The new immunosuppressive protocols allow for avoidance or withdrawal in many patients, even though the dosage is small. Corticosteroids have a specific action, they inhibit the dendritic cells, inhibit the transcription of cytokines genes and all the stages of the T-cell activation. The nonspecific immunosuppressive effects are lymphopenia because of the redistribution of lymphocytes from the vascular compartment back to lymphoid tissue and the inhibition of the migration of monocytes to the sites of inflammation. Corticosteroids are used as high doses intravenous or oral pulses, or as a steady low-dose daily or every other day maintenance regimen, or tapering oral dose over days or weeks. The most important complications are cosmetic changes, growth impairment, osteonecrosis, osteoporosis, impaired wound healing, cataracts, hyperlipidemia, glucose intolerance, psychological effects.

The development of generics of immunosuppressive agents have financial implications, these drugs being cheaper, and experience has not demonstrated them to be inferior to the original drug. However, it is better to use them consistently, without switching formulations and to monitor closely the drug levels and the renal function.

The protocols should be individualized for each patient. Patients with high levels of preformed antibodies, delayed graft function, previously transplanted, or young patients may require higher doses of immunosuppression than older patients. Kidneys from older donors may be less tolerant to immunological assault and other aggression. Patients transplanted from well-matched donors may require less immunosuppression [13].

The first kidney transplantation were performed in our institution in October 1992, and since then the number of patients has permanently increased to over 1500 patients over twenty years of sustained activity. In present, we use Methylprednisolon, Thymoglobulin and Basiliximab for

induction therapy and Azathioprine, CsA, Tac, sirolimus, MMF, MPA, Prednisone and generic drugs like Tacrolimus Sandoz and Tacni (tacrolimus) for maintaining therapy.

Owing to our experience, which we wanted to complete and improve, we took part in European studies regarding immunosuppressive protocols: OSAKA study- "Both Advagraf and Prograf give results similar to kidney transplant" and TWIST study - "A randomized trial to assess the impact of early steroid withdrawal on growth in pediatric renal transplantation" which allow us to adapt the medication to every patient, in accordance with their needs and characteristic features.

References

1. Ponticelli C, Tarantino A, Vegeto A. Renal transplantation, past, present and future. J Nephrol, 1999; 12 Suppl 2:S105-110.
2. Mahmud N, Klipa D, Ahsan N. Antibody immunosuppressive therapy in solid-organ transplant: Part I. MAbs, 2010; 2(2):148-156.
3. Knight SR, Russell NK, Barcena L, Morris PJ. Mycophenolate mofetil decreases acute rejection and may improve graft survival in renal transplant recipients when compared with azathioprine: a systematic review. Transplantation, 2009; 87(6):785-794.
4. Gourishankar S, Turner P, Halloran P. New developments in immunosuppressive therapy in renal transplantation. Expert Opin Biol Ther, 2002; 2(5):483-501.
5. Meier-Kriesche HU, Li S, Gruessner RW, et al. Immunosuppression: evolution in practice and trends, 1994-2004. Am J Transplant, 2006; 6(5 Pt 2):1111-1131.
6. Halloran PF. Immunosuppressive drugs for kidney transplantation. N Engl J Med, 2004; 351(26):2715-2729.
7. Vincenti F, Kirk AD. What's next in the pipeline. Am J Transplant, 2008; 8(10):1972-1981.
8. Trivedi HL, Terasaki PI**,** Feroz A, et al. Abrogation of anti-HLA antibodies via proteasome inhibition. Transplantation, 2009; 87(10):1555-1561.
9. Glotz D, Antoine C, Julia P, et al. Intravenous immunoglobulins and transplantation for patients with anti-HLA antibodies.Transpl Int, 2004; 17(1):1-8.
10. Leichtman AB. Balancing efficacy and toxicity in kidney-transplant immunosuppression. N Engl J Med, 2007; 357(25):2625-2627.
11. Dudley C, Pohanka E, Riad H, et al. Mycophenolate mofetil substitution for cyclosporine a in renal transplant recipients with chronic progressive allograft dysfunction: the "creeping creatinine" study. Transplantation, 2005; 79(4):466-475.
12. Flechner SM. Sirolimus in kidney transplantation indications and practical guidelines: de novo sirolimus-based therapy without calcineurin inhibitors. Transplantation, 2009; 87(8 Suppl):1-6.
13. Danovitch GM. Immunosuppressive medications for renal transplantation: a multiple choice question. Kidney Int, 2001; 59(1):388-402.