Immunosuppression and Kidney Transplantation

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H. J. Eisen (ed.), *Pharmacology of Immunosuppression,*
Handbook of Experimental Pharmacology 272, https://doi.org/10.1007/164_2021_546

Abstract

Immunosuppression is complex, fraught with on-target and off-target adverse effects, and hard to get right but is the key to successful allotransplantation. Herein, we review the key immunosuppressive agent classes used for kidney transplant, highlighting mechanisms of action and typical clinical use.

Keywords

Antibody mediation rejection · Calcineurin inhibitors · Immunosuppression · Induction therapy · Kidney transplantation · Rejection · T-cell depleting agents · Transplant protocols

1 Introduction

The first successful kidney transplant was pioneered in 1954 by Joseph Murray at Harvard, a living donor transplantation between identical twins. Subsequently, it was the development and initiation of immunosuppressive medications that made organ transplantation between genetically dissimilar individuals possible. Multiple therapeutic options have emerged since, because of our better understanding of the immune response mechanisms that led to lower rejection rates, and better graft and patient survival in kidney transplantation.

1.1 Brief History

Among the first immunosuppressive strategies used was total body radiation. Along the same period, the anti-inflammatory properties of cortisone in patients with rheumatoid arthritis were discovered (Hench et al. 1949). Thereafter, prednisone was routinely combined with azathioprine which was introduced in early 1960s (Calne et al. 1962; Murray et al. 1963; Zukoski et al. 1960). In the 1970s, anti-thymocyte (ATG) globulin and antilymphocyte globulin (ALG), polyclonal antibody preparations were introduced and a typical kidney transplant immunosuppression protocol consisted of an induction regimen with ALG with prednisone and azathioprine being used for maintenance immunosuppression (Fig. 1).

Cyclosporine A, an extracted compound of the fungus *Tolypocladium inflatum (*Köhler and Milstein 1975*)*, was discovered in the early 1980s. This groundbreaking discovery revolutionized kidney transplant outcomes with a 30 to 40% reduction in rejection rates, and >80% graft survival at 1 year (Zand 2005). This dramatic benefit was easy to recognize considering the poor outcomes prior to its introduction. Cyclosporine was coupled with prednisone and oftentimes azathioprine was being added to constitute the "triple therapy." Major advancements to follow were the introduction of tacrolimus into liver transplantation and later to kidney transplant (Pirsch et al. 1997) as an alternative to cyclosporine and mycophenolate mofetil

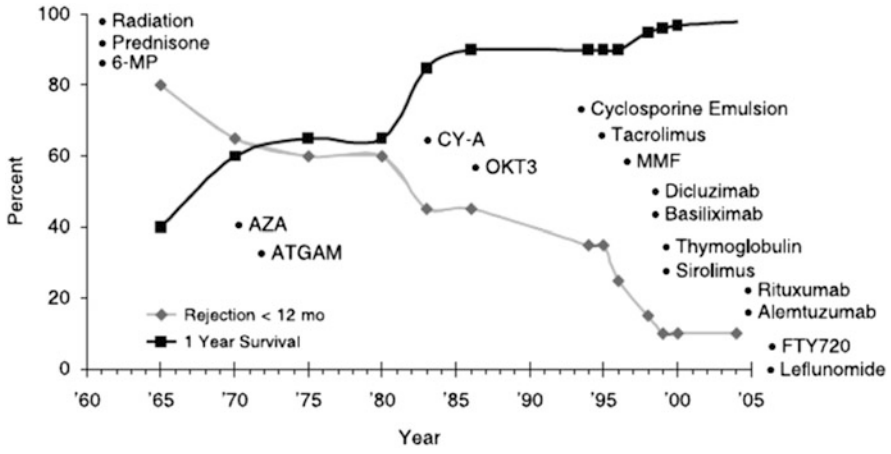

Fig. 1 The development of more potent immunosuppression medications over the years leads to lower rejection rates and subsequently better graft survival (Zand 2005)

which was superior to azathioprine with lower rejection episodes when used with cyclosporine and prednisone (Knight et al. 2009). Another key development was the introduction of the first monoclonal antibody (mAb) to be used in clinical medicine, OKT3 in 1985. It was being used for steroid-resistant rejections and occasionally as an induction agent. Of similar use, basiliximab and daclizumab two humanized monoclonal Il-2 inhibitors receptor antagonists (IL2-RA) were later introduced.

Sirolimus was introduced in 1999 as mammalian target of rapamycin (mTOR) pathway inhibitor, a new class of medications with antineoplastic properties (Shimobayashi and Hall 2014) in addition to immunosuppressive potential. The last major medication that gained FDA approval in 2011 was belatacept, which works through competitive costimulation blockade (Larsen et al. 2005).

1.2 Current Practices

With the multiplicity of immunosuppressive agents and the rapid advances in kidney transplant immunosuppression, a wide variety of treatment protocols and clinical practices are adopted at different transplant centers throughout the USA.

According to the organ procurement and transplantation network, scientific registry of transplant recipients data report, T-cell depleting agents remain the most common induction agent in 2018 used in 75% of cases. Twenty percent of kidney transplant used IL2-RA as induction agent and the remaining 5% didn't use any (Hart et al. 2020).

In regard to maintenance immunosuppression, tacrolimus and mycophenolate mofetil based regimen constitutes the major regimen used. Approximately 30% are steroid free regimens, a stable proportion over the past years. Ten percent of cases are non-calcineurin based regimens, mainly belatacept based. (Hart et al. 2020).

1.3 Alloimmune Reaction Targets

The understanding of the different mechanisms of the immune response, including B- and T-cell development, activation and proliferation, cytokine signaling, and complement activation contributed to the advancement of new therapeutics and vice versa. The target of the immunosuppressive agents can be divided based on the stage of the immune reaction. "Signal 1" is activated when an antigen (recipient HLA peptides) on the surface of antigen presenting cell (APC) (most commonly dendritic cell) triggers T cells via the CD3 complex. Costimulation or "signal 2" constitutes the interaction of CD80 and CD 86 (B7) on the surface of APC and CD28 on T cells. Both signal 1 and 2 are necessary to activate three signal transduction pathways: the calcium-calcineurin pathway, the RAS-mitogen activated protein (MAP) kinase pathway, and the nuclear factor-kb pathway (Wang et al. 2004). Transcription factors that trigger IL2, CD 25 (IL2 a subunit), and CD145 expression are then activated. IL 2 subsequently activates the target of mTOR pathway which constitutes "signal 3," the trigger for cell proliferation. Nucleotide synthesis, another target for immunosuppressive medications, is also required for lymphocyte proliferation and the mobilization of effector T cells. B cells are engaged when an alloantigen interacts with their antigen receptor in secondary lymphoid tissues, the lymphoid follicles or the red pulp of spleen, for example (MacLennan et al. 2003), or the kidney allograft itself (Sarwal et al. 2003) producing antibodies against the HLA antigens. The main agents of kidney allograft rejection are effector T cells and anti-HLA alloantibodies. In general, immunosuppression can be achieved by depleting lymphocytes, blocking their response pathways, slowing down the production and neutralizing the effect of alloantibodies.

This chapter will be divided into three sections according to the clinical use of each immunosuppressive medication. Immunosuppressive agents used for induction and maintenance immunosuppression, and rejection treatment (mainly antibody mediated rejection) with focus on those that are currently used in kidney transplantation will be reviewed.

2 Induction Therapy

Induction regimens are part of the immunosuppression protocols in over 80% of kidney transplant centers in the USA. The use of induction agents reduces the rate of acute rejection and subsequently improves short-term graft survival, however, there is no prospective data clearly demonstrating a superior outcome in long-term graft survival. Induction therapy seems to be clinically indicated in early steroid withdrawal protocols where maintenance immunosuppression is being minimized. Induction therapy is warranted in high immunologic risk individuals (high calculated panel of reactive antibody (cPRA), positive cross match transplants, positive donor specific anti-HLA antibodies (DSA), prior transplant recipients, recipient of black race) and those whom a delayed graft function is expected because of donor characteristics or high cold ischemia time.

Induction agents are divided into T-cell depleting agents – monoclonal and polyclonal anti-thymocyte globulins (ATG) and alemtuzumab and non-T-cell depleting – interleukin 2 receptor antagonist (IL2RA). In addition to their use as induction agents, T-cell depleting agents are used to treat T-cell mediated rejection.

IL2RA use is limited to kidney transplant recipients with low immunologic risk as ATG has been shown to be more effective in preventing acute rejection in the high-risk group (Brennan et al. 2006). Whereas, alemtuzumab had similar outcomes compared to ATG and was superior to IL2RA (Hanaway et al. 2011).

2.1 T-Cell Depleting Agents

2.1.1 Monoclonal Antibodies

Monoclonal antibody muromonab-CD3 (OKT3): OKT3 is the first mAbs approved by the FDA for use in humans in 1986 for prevention of rejection in kidney, heart, and liver transplant (OMTS Group 1985). It is an anti-T-cell receptor (TCR) antagonist that targets the CD3 subunit of the TCR complex inhibiting the first point of antigen presentation (targeting signal 1). It is a murine antibody, thus results in significant side effects related to its mitogenicity which are potentially fatal first-dose reactions. In efforts to minimize its mitogenicity, humanized forms of anti-TCR mAbs that target other subunits (Larsen et al. 2005; Hart et al. 2020; Wang et al. 2004) have been developed but their production has been on hold given ongoing safety and efficacy concerns.

2.1.2 Polyclonal Anti-thymocyte Globulin

Therapeutic antilymphocyte polyclonal antibodies are produced by immunizing with human thymocytes either horses (eATG (equine), ATGAM) or rabbits (Thymoglobulin-Genzyme), or immunizing rabbits with lymphocytes from a Jurkat cell leukemia line (Fresenius antithymocyte globulin [ATG]). Two forms of rabbit anti-thymocyte globulin (rATG) are available depending on the cell type used for rabbit immunization, thymoglobulin (Genzyme) which is available in the USA and anti-T-lymphocyte immune globulin (ATG-Fresenius) used in Europe. In small head-to-head trials, thymoglobulin was superior to ATG-Fresenius in regard to both efficacy and side effects (Gharekhani et al. 2013). rATG is the primarily used antilymphocyte in clinical practice whereas ATGAM, although available, is not widely used partly because it is less potent.

Rabbit Anti-Thymocyte Globulin

Specialized rabbits are immunized with thymocytes or activated human T cells and the resultant IgG fraction of the sera is purified to remove irrelevant antibody materials. These antibodies are polyclonal as directed against multiple thymocyte antigens. Its mode of action is not fully characterized, but rATG antibodies are predominantly anti-T lymphocytes and will cause T-cell depletion via complement-dependent cytotoxicity and T-cell activation-induced apoptosis (Zand et al. 2005) or can be cleared by the reticuloendothelial system. Since some antigens are shared

among T cells and other immune cells, rATG exhibits some activity against B cells, monocytes, and to a lesser neutrophils. Most importantly, rATG causes a sustained expansion of regulatory T cells which maintain immune balance and prevent acute rejection.

Dose and Administration: rATG is administered at 1.5 mg/kg doses with a cumulative dose ranging between 3–6 mg/kg depending on recipient characteristics and center practice. It is more effective when used in the operating room prior to anastomosis of the graft. Allergic reactions are prevented by administering premedication consisting of steroids and diphenhydramine. It is administered through a central vein over 4–8 h. When using a peripheral vein, it might be associated with vein thrombosis or thrombophlebitis which can be prevented by adding heparin and hydrocortisone to the infusion.

Adverse Reactions: The side effects associated with rATG administration are chills, fever, and arthralgia, commonly seen with polyclonal antibody preparations. Serum sickness is seen but rarely, because the continued immunosuppression reduces immune complex formation and deposition. Cytokine release syndrome (with pulmonary edema and hypotension) is the most worrisome. Anaphylaxis can be seen, especially with patients with prior history of rabbit sensitivity.

Leukopenia, a direct consequence of T-cell depleting therapy, and thrombocytopenia are seen. The subsequent dose is usually halved or held with a platelet count of 50,000 to 75,000 cells/mL or a white blood cell count of 2,000 to 3,000 cells/mL.

Cytomegalovirus (CMV) infection is a late manifestation of rATG use. This is usually prevented by the use of CMV prophylaxis with valganciclovir for 3–-9 months (depending on donor and recipient serostatus and rATG dose) after administration especially in high-risk populations (donor with CMV positive serostatus and recipients with negative serostatus). Post-transplant lymphoproliferative disorder, particularly EBV related lymphoma is an infrequent but grave consequence.

2.1.3 Alemtuzumab

Alemtuzumab (Campath 1H) is a humanized mAb, DNA-derived directed against CD52, a cell surface glycoprotein of unclear physiologic significance, present on both B- and T-cell lymphoid cell line. It was initially approved for the treatment of refractory chronic lymphocytic leukemia (Alinari et al. 2007) and reintroduced in 2012 as a treatment for multiple sclerosis (Freedman et al. 2013). The use of Alemtuzumab in kidney transplantation as an induction agent is “off-label.” It is administered as a single dose of 30 mg intraoperatively and has fewer infusion-related reactions as a humanized antibody. Its ease of administration and fewer side effects coupled with a comparable efficacy make it an attractive alternative to ATG. Alemtuzumab induces a significant, durable T-cell depletion up to 6–12 months after administration. The infectious and malignancy risks are similar to other T-cell depleting agents.

2.2 Interleukin 2 Receptor Antagonists

Once T lymphocytes become activated in response to signal 1 and signal 2, they express CD25, the α-subunit of the IL2 receptor. Subsequently, IL-2 will lead to the intracellular signaling and proliferation of T cells. Basiliximab (Simulect) and daclizumab (Zenapax) are anti-CD25 monoclonal antibodies targeted against the α-subunit that will prevent T-cell proliferation. Daclizumab is no longer in production for clinical kidney transplantation. Basiliximab reduces the risk of acute rejection in patients with lower immunologic risk. Although it originates as a murine monoclonal antibody, 75% of it has been replaced by human IgG, thus it is well tolerated and does not induce a first-dose reaction. Basiliximab half-life is prolonged (longer than 7 days) as it doesn't induce antimurine antibodies and is given as an intravenous dose of 20 mg twice. The first intraoperatively and the second 4 days after. IL2 R sites are usually saturated for 30–45 days.

3 Maintenance Therapy

Long-term immunosuppression regimens have changed significantly over the last decades and the number of agents available significantly increased. The aim of maintenance immunosuppression goes beyond the prevention of acute rejection, to the minimization of total immunosuppression and management of chronic allograft rejection and nephropathy. The results of the symphony trial where three major agents were compared cyclosporine, tacrolimus and sirolimus still govern our clinical practice to this day (Ekberg et al. 2007). Tacrolimus was shown to be superior to cyclosporine and sirolimus. Thus, it is the first-line agent in most transplant center protocols. It is generally coupled with mycophenolate which has substituted azathioprine given its superior outcome (Knight et al. 2009). Belatacept, a costimulatory blockade agent, is an alternative for calcineurin-inhibitor based regimens with promising outcomes (Vincenti et al. 2016).

3.1 Calcineurin Inhibitors

Calcineurin inhibitors (CNIs) remain the cornerstone of immunosuppression regimen used in most transplant centers for the past 30 years. The two main calcineurin inhibitors used are cyclosporine and tacrolimus. An investigational drug, voclosporin has been recently studied in lupus nephritis (Arriens et al. 2020). Cyclosporine and tacrolimus are similar not only in regard to their mechanism of action, but also in their clinical efficacy and adverse event profile. Nonetheless, they are biochemically distinct and have discrete differences.

They are both isolated from fungus species. Cyclosporine is an 11-amino acid cyclic polypeptide extracted from *Tolypocladium inflatum* (Köhler and Milstein 1975). Tacrolimus is a macrolide antibiotic compound isolated from *Streptomyces*

tsukubaensis. Its name is still oftentimes substituted by its laboratory designation FK506.

3.1.1 Mechanism of Action

CNIs inhibit the immune response by targeting signal 1. A calcineurin-dependent pathway is triggered after the initial binding of the APC to the TCR, that is necessary for initial gene transcription and subsequently additional T-cell activation. When CNIs are administered, cyclophilin in cyclosporine and tacrolimus-binding protein (FKBP) in tacrolimus bind to their cytoplasmic receptor proteins which in turn bind to calcineurin and inhibit its function. Calcineurin is a phosphatase which dephosphorylates nuclear regulatory proteins, particularly nuclear factor of activated T cells in the setting of immune response, facilitating their entry to the nucleus. CNIs thus inhibit calcineurin-dependent gene transcription including several critical cytokine genes (IL-2, IL-4, Interferon-γ, and tumor necrosis factor-α) and downstream lymphocyte proliferation.

They are unique when compared to their predecessors as they selectively inhibit the immune response. At a therapeutic level, the calcineurin activity is reduced by 50%; this allows for a degree of immune responsiveness to maintain appropriate host defense.

3.1.2 Dose and Administration

Cyclosporine: The original, non-modified form, oil-based Sandimmune has a great variability in absorption and has been substituted by the microemulsion, Neoral/Gengraf. Both are available in 25 mg and 100 mg capsules that are administered twice daily. Intravenous form is administered twice daily in a 4-h infusion. The conversion from po form is 3:1.

Initial dose is 9 mg/kg/day adjusted according to the target level which varies according to the different stages of transplant. A peak-level 2 h after dosing is the most accurate and consistent. It correlates better with drug exposure than a 12 h trough level, although the latter is more often used for convenience.

Tacrolimus: The immediate release (IR) preparation Prograf is available in 0.5 mg, 1 mg, and 5 mg capsules typically administered twice a day. IV formulations are available and the conversion is equal to one-third to one-fourth of the oral dose. It is less commonly used, as tacrolimus can be given sublingually when a po route is unavailable. Newer long-acting preparations are available – ER-tacrolimus (Astagraf) in 0.5, 1, and 5 mg capsules and LCP-tacrolimus (Envarsus) in 0.75, 1, and 4 mg tablets. These once-daily formulations improve medication compliance. LCP-tacrolimus requires 30% reduction from prograf dose as it has better bioavailability along with a decreased peak level (Budde et al. 2014; Tremblay et al. 2017). IR-tacrolimus is typically started at 0.05–0.1 mg/kg/day adjusted by 12 h trough level.

3.1.3 Metabolism and Drug–Drug Interaction

Cyclosporine and tacrolimus are both metabolized via cytochrome P450 (CYP) 3A4 and 3A5 in the liver, small intestine, and in the kidney to lesser extent.

P-glycoprotein (P-gp), an efflux pump that transports substances across the intracellular and extracellular membranes is also involved in CNIs metabolism. P-gp is found in hepatocytes, distal and proximal renal tubular cells, intestinal epithelium, and the luminal surface of capillary endothelial cells in the brain. In the gut, P-gp reduces the bioavailability of CNIs as they are repeatedly taken up and transported out of enterocytes. Polymorphisms in P-gp and CYP3A5 cause significant interpersonal drug level variability by affecting drug absorption, metabolism and distribution. This variability potentially influences drug efficacy and toxicity as it will affect its concentration at target sites. CYP3A5*1 allele (Kuehl et al. 2001) found predominantly in individuals of African descent encodes for a CYP3A5 enzyme that is associated with rapid metabolism of CNIs and subsequently lead to increased dose requirements as opposed to individuals who carry CYP3A5 *3/*3 alleles (Barbarino et al. 2013) that encode for a non-functional CYP3A5 protein and thus have reduced dose requirements.

Any drug that impacts CYPA3A4/5 or P-gp activity has a potential interaction with CNIs. Inducers of CYP3A activity will decrease CNIs concentration. These are anti-tuberculous drugs – rifampin and rifabutin; anticonvulsants – barbiturates, phenytoin, and carbamazepine; antibiotics – nafcillin; herbal preparation – St. John's wort; corticosteroids – tacrolimus level may increase by 25% after steroid discontinuation. CYP3A inhibitors increase CNI concentration. Drugs that raise CNIs levels are – non-dihydropyridine calcium channel blockers – diltiazem and verapamil; antifungals, all azole derivatives – ketoconazole, fluconazole, itraconazole, voriconazole, and isavuconazole; macrolide antibiotics – erythromycin and clarithromycin; antiretroviral therapy, mainly protease inhibitors – ritonavir; food – grapefruit juice. CYP3A inhibitors are occasionally added to boost CNI levels when a therapeutic level is not achieved despite using high CNI doses. Aside from medications, diarrhea and bowel inflammation significantly increase CNI levels due to decreased P-gp and CYP3A4 function in enterocytes.

3.1.4 Adverse Events

Kidney Related – Calcineurin Inhibitor Nephrotoxicity: CNI use may lead to significant nephrotoxicity. Acute CNI toxicity occurs early after kidney transplant and is often reversible with dose reduction (Thölking et al. 2017). There are three major acute nephrotoxicity manifestations: vascular vasoconstriction, tubulopathy, and thrombotic microangiopathy (TMA). CNIs cause endothelial cell injury and afferent arteriole vasoconstriction mediated by the production of vasoconstrictors such as endothelin, activation of renin-angiotensin II system, and inhibition of vasodilators such as nitric oxide and cyclooxygenase-2 (Naesens et al. 2009). This vascular effect is reversible and manifest as hypertension and decreased glomerular filtration rate. CNIs may lead to acute tubular damage, whose mechanism is not completely understood but could be related to direct toxicity affecting the endoplasmic reticulum and mitochondria (Pallet et al. 2008). A rare but more severe complication is thrombotic microangiopathy attributed to endothelial injury, causing platelet aggregation and activation of the coagulation cascade (Ponticelli 2007). Electrolytes disturbances are commonly encountered, similar to what is seen in Gordon

syndrome- pseudohypoaldosteronism with hypertension, metabolic acidosis and hyperkalemia even with normal kidney function. Chronic CNI nephrotoxicity occurs several months post-transplant due to cumulative and persistent vascular damage. Clinically, it manifests as hypertension, worsening kidney function and proteinuria and histologically by hyaline arteriolopathy, stripped tubulointerstitial scarring, and glomerulosclerosis (Nankivell et al. 2016).

Non-renal: Some manifestations differ among tacrolimus and cyclosporine particularly cosmetic complications. Cyclosporine is associated with hypertrichosis, and gingival hyperplasia whereas tacrolimus causes hair loss and alopecia. Metabolic complications include hyperlipidemia, more often seen with cyclosporine and post-transplant glucose intolerance and new-onset diabetes more so with tacrolimus which is toxic to the pancreatic islet cells. Neurotoxicity ranging from tremor, dysesthesias, headache is common and is level related.

3.2 Antimetabolites

3.2.1 Mycophenolic Acid

Mycophenolic acid (MPA) is a fermentation product of several *Penicillium* species. It is the active compound of the prodrug mycophenolate mofetil (MMF) (CellCept) that was introduced to kidney transplantation in 1995. It is available in 250 mg capsules and 500 mg tablets and the typical dose is 1 g twice daily. Myfortic is an enteric-coated form of MPA that became available in 2004 in two formulations 180 mg (equivalent to 250 mg of MMF) and 360 mg tablets. MPA is an inhibitor of the enzyme inosine monophosphate dehydrogenase (IMPDH), the rate limiting enzyme critical for de novo purine synthesis and thus DNA synthesis in T and B cells. Lymphocytes rely on de novo DNA synthesis more than other cell types that have a salvage pathway for guanosine nucleotide synthesis from guanine. It has been demonstrated that MPA blocks the proliferation of T and B lymphocytes and subsequently inhibits antibody formation and generation of cytotoxic T cells (Danovitch 2005). Primary side effects are gastrointestinal and hematopoietic. Diarrhea occurs in one-third of patients along with nausea, dyspepsia, and vomiting in up to 20% of patients. GI side effects are more frequently encountered with dosage >1 g twice daily. Hematopoietic side effects include leukopenia, anemia, and thrombocytopenia despite being specific to lymphocytes. These are seen at a similar rate to azathioprine. MPA is teratogenic and should be discontinued six weeks prior to planned pregnancy and substituted to azathioprine.

3.2.2 Azathioprine

Azathioprine (AZA) (Imuran) is an antimetabolite, an analog of the early immunosuppressant, 6-mercaptopurine. This metabolite acts as a purine analog that interferes with de novo purine and subsequently, DNA and RNA synthesis inhibiting gene replication and T-cell activation (Elion 1989). Its regular dose when used in conjunction with a CNI is 1–2 mg/kg. AZA is a bone marrow suppressant thus its hematologic side effects (anemia, thrombocytopenia, and leukopenia). Its

concomitant use with xanthine oxidase inhibitors (allopurinol and febuxostat) slows 6-mercaptopurine elimination and exacerbates these side effects (Berns et al. 1972). AZA is safe with pregnancy unlike MPA (Sifontis et al. 2006).

3.3 mTOR Inhibitors: Everolimus and Sirolimus

Clinically available mTOR inhibitors are sirolimus and everolimus. Sirolimus (Rapamune) is a macrolide antibiotic produced by *Streptomyces hygroscopicus* and is structurally related to tacrolimus, available in 0.5, 1, or 5 mg tablet. Everolimus (Zortress) is a derivative of sirolimus with different pharmacokinetics, available in 0.25, 0.5, and 0.75 mg tablets. The mammalian target of rapamycin (mTOR) pathway constitutes signal 3 of the immune response and will lead to cell cycle progression from G1 to S and proliferation in response to cytokine stimulation (mainly IL-2). mTOR inhibitors bind to FKBP (the same cytoplasm-binding protein that binds tacrolimus) and the complex engages with mTOR, a regulatory kinase, and inhibits its actions causing reduced cytokine-dependent cellular proliferation. mTOR signaling is ubiquitous, and not exclusive to lymphocytes and has been described in monocytes, dendritic cells, natural killer cells, as well as nonhematopoietic cells (endothelial cells, fibroblasts, hepatocytes, and smooth muscle cells) (Ferrer et al. 2011). In addition to its immunosuppressive effects, the inhibition of mTOR will lead to anti-proliferative, antiviral, anti-inflammatory, and antitumor effects (Peddi et al. 2013).

Similar to CNIs, mTOR inhibitors have nephrotoxic side effects. In addition to mTOR kinase, mTOR inhibitors also target the vascular endothelial growth factor (VEGF) (Guba et al. 2002; Knoll et al. 2014) inhibiting its activity, causing podocyte damage and eventually proteinuria and nephrotic syndrome (Diekmann et al. 2012). Other nephrotoxic effects include focal segmental glomerulosclerosis, TMA, acute tubular injury, and atypical casts (when combined with tacrolimus) (Smith et al. 2003). mTOR inhibitors prevent wound healing so should be avoided in fresh transplant recipients and be switched to CNIs 6 weeks prior to major surgery or immediately postoperatively for emergent surgery. Other side effects include edema, hypertension, gastrointestinal side effects – mouth ulcers, diarrhea, hyperlipidemia (hypercholesterolemia and hypertriglyceridemia), hyperglycemia, and cytopenia (mainly thrombocytopenia and anemia).

3.4 Corticosteroids

Corticosteroids, one of the first immunosuppression medications used, still play a central role in kidney transplantation. Steroid avoidance or withdrawal protocols have been developed, and when steroids are used, their dose is small, typically equivalent to prednisone 5 mg daily. Steroid receptor is expressed on most mammalian cells and modulates a multitude of cellular functions. Corticosteroids diffuse intracellularly and bind to their cytoplasmic receptor, the complex translocates to the

nucleus where it binds to DNA sequences – glucocorticoid response element (GRE), responsible for cytokine gene transcription, and blocking its action. It also inhibits other cytokine transcription factors such as nuclear factor-Kb (Rhen and Cidlowski 2005). As a result, the expression of IL-1, IL-2, IL-3, IL-6, TNF-a, and IFN-g is inhibited with the downstream result of T-cell depletion, inhibition of Th1 differentiation, induction of apoptosis, and macrophage dysfunction.

3.5 Belatacept

Belatacept (Nulojix) is a costimulatory blockade agent targeting signal 2 of the immune response. After TCR binding, optimal T-cell activation requires a costimulation signal conferred by the interaction of CD80/86 on APC and CD28 on T cell. After an effective T-cell response, cytotoxic T lymphocyte-associated protein 4 (CTLA4) competitively binds CD80/86 and downregulates the cell activity. Belatacept is a human fusion protein containing CTLA4 linked to Fc domain of human IgG1. Belatacept was demonstrated to be noninferior to cyclosporine in terms of patient and graft survival and has the potential to replace CNI-based immunosuppressive protocols. Belatacept is available as an intravenous formulation. When administered de novo at time of transplant, it is given at a dose of 10 mg/ kg on day 1, 5, 15, 28, 56 and then at 5 mg/kg q 28 days (Adams et al. 2017). Despite a higher risk of rejection, patients on belatacept have higher GFR, graft, and allograft outcomes and appear to develop fewer de novo DSA antibodies (Vincenti et al. 2016). It is well tolerated with few metabolic complications. EBV naïve patients are at risk for post-transplant lymphoproliferative disorder, thus its use is restricted to patients with positive EBV serology.

4 Antibody Mediated Rejection

Antibody mediated rejection (AMR) is a severe form of rejection resistant to standard treatment with immunosuppressant medications. Post-transplant AMR, chronic active AMR (CAAMR), and transplant glomerulopathy (TG) remain a significant problem in kidney transplantation leading to long-term graft failure. Will briefly discuss the therapeutic approaches used for the treatment of AMR.

1. *Intravenous Ig (IVIG)*: an IgG rich Ig extract pooled from thousand donors. IVIG immunosuppressive mechanisms are broad, including the direct binding to antibodies, superantigens and pathogens, inhibition of complement fixation, and stimulation of FcR-induced anti-inflammatory pathways.
2. *Rituximab*: a chimeric mAb against CD20 that is expressed on pre-B and mature B cells but not differentiated plasma cells leading to B-cell depletion via complement-dependent cytotoxicity, growth arrest, and apoptosis (Pescovitz 2006). Humanized (Ocrelizumab) and fully humanized (ofatumumab) anti-CD20 mAbs are available for clinical use.

3. *Anti-Plasma Cell Therapies*: Daratumumab is an anti-CD38 mAb, as CD38 is expressed on plasma cells. Bortezomib and Carfilzomib are proteasome inhibitors.
4. *Tocilizumab*: mAb directed at IL-6 receptor that induces a significant reduction of B-cell hyperreactivity with promising results in CAAMR.
5. *Eculizumab*: C5 inhibitor that prevents cleavage of C5 to C5a and C5ba and the formation of the membrane attack complex C5b-9.
6. *Newer Agents*: a number of agents that target different aspects of the B-cell and complement-mediated aspects of the immune response are coming online soon that have generated considerable excitement within the transplant community. It is felt that combinations of agents may prove more effective at managing acute and chronic antibody mediated alloimmune responses than currently available agents, which remain disappointing. IdeS-IgG-degrading enzyme derived from *Streptococcus pyogenes* (Imlifidase), an endopeptidase, cleaves human IgG into F $(ab')_2$ and Fc fragments inhibiting complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity permitted transplant between HLA-incompatible individuals by cleaving donor specific antibodies (DSA). Clazakizumab is an immunoglobulin G1 (IgG1) mAb aimed at the IL-6 ligand which is being currently studied for the use in CAAMR along with evidence of TG on kidney biopsy. Anti-C1s (BIVV009) is a novel investigational drug being examined to be used in the setting of C4d+ and C1q+ DSA. Similarly, C1 esterase inhibitor has been shown to prevent TG when used as an adjunct to AMR therapy when compared to placebo (Montgomery et al. 2016).

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