

Kidney Transplantation

Chapter 1

Immunological Challenges in Kidney Transplantation

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1. Matching Donor and Recipient

End stage renal disease (ESRD) is an epidemiological notion that starts from the idea that persistent kidney damage may be the consequence of many etiological factors. ESRD consists of the progressive nephron loss and renal function damage.

When the glomerular filtration rate (GFR) reaches a critical level below 60 ml/minute/1.73m², adaptive renal and systemic mechanisms become harmful resulting in a reduction in the number of nephrons which leads to chronic kidney disease (CKD).

There are many etiologies for CKD but the most common cause is glomerulonephritis (60%), followed by chronic pyelonephritis (30%), vesicoureteral reflux and polycystic kidney disease and the remaining 10% is represented by diabetic nephropathies, collagen diseases and Henoch–Schönlein purpura [1]. In pediatric patients, is more likely to have hereditary nephropathy and congenital kidney hypoplasia. Focal sclerosing glomerulonephritis is the most common form of glomerulonephritis leading to end stage renal disease in childhood.

ESRD may benefit from two treatment methods: hemodialysis and kidney transplant. Hemodialysis improves survival rates until transplantation and is also useful after transplant in the event of failure. However, long-term dialysis treatment for more than one year conferred a higher risk of graft rejection. For children who are dependent on dialysis, there are well documented adverse effects on cognitive function, growth, anemia, osteodystrophy and many other effects due to the lack of endocrine function. The first line of treatment in a patient with

end stage renal disease is kidney transplantation. It is the most effective, accepted and required treatment method (**Figure 1**).

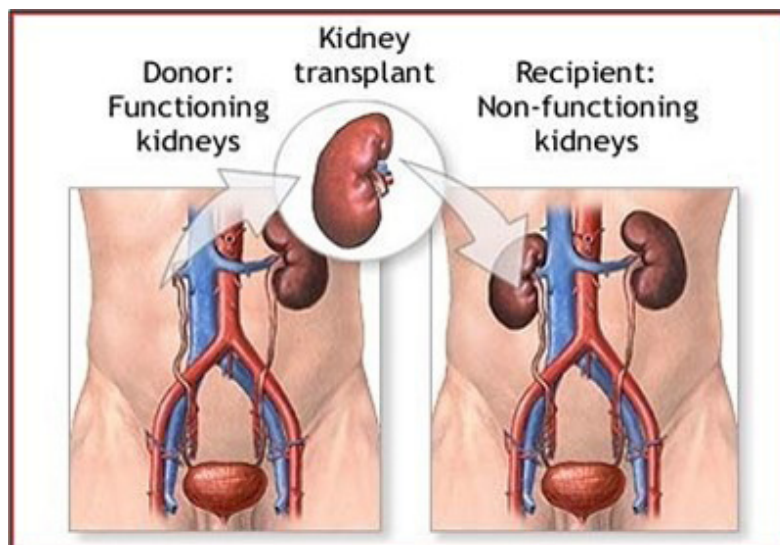


Figure 1: Kidney transplant

Kidney transplantation represents the optimal treatment for patients with ESRD and offers more substantial benefits in comparison with dialysis. These benefits include replacement of the regulatory, excretory and endocrine functions of the kidney. It corrects problems such as renal anemia, improves social adjustment and quality of life. For these advantages, preemptive transplantation, defined as transplantation prior to the initiation of dialysis, is the preferred treatment for pediatric patients with kidney disease.

Recent advances in pre and post-transplantation management, immunosuppressive medications, surgical techniques and donor selection have contributed to improved patients and graft survival among kidney transplant recipients.

2. Tissue Typing

Clinical engraftment of histocompatibility mismatched organs between two genetically different individuals of the same species produces an immune response by the host immune system. The allorecognition is determined by alloantigens of the host which are encoded within the major histocompatibility complex (MHC) [2].

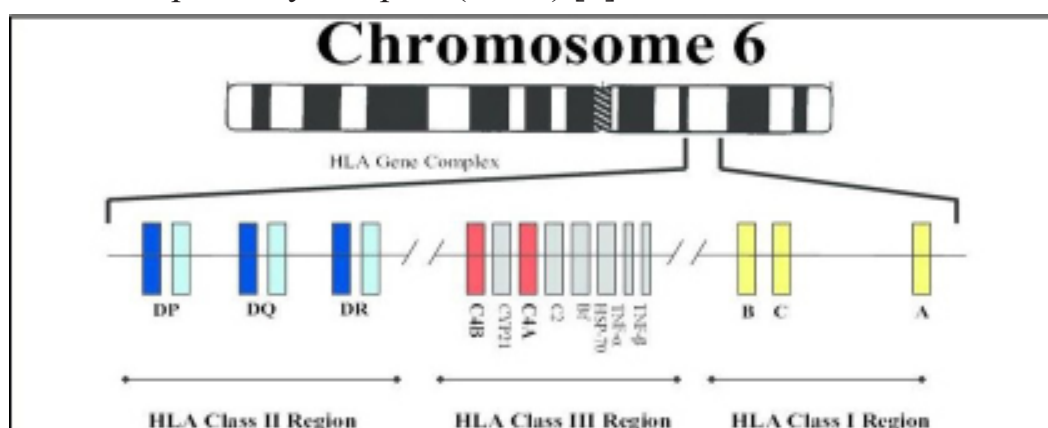


Figure 2: Chromosome 6

The major histocompatibility complex represents a cluster of genes and is located on the short arm of chromosome 6 (**Figure 2**). This is the most polymorphic and the most studied region in the human genome because variants at this loci are associated with transplant compatibility, autoimmune diseases, infectious and inflammatory diseases [3]. The MHC encodes the human leukocyte antigens (HLA) genes, which is divided into four regions A, B, C and D. Class I is represented by A, B, C regions and they code for class I molecules (HLA-A, -B, -C). Class II is represented by D region and code for class II molecules (HLA-DR, -DP, -DQ). The MHC also contains class III region, the most gene-dense region in the genome, that code for molecules such as complement proteins, C2, complement factor B (CFB), C4, TNF, heat shock protein cluster, growth proteins, that are called class III MHC molecules.

Class I HLA molecules are expressed in all nucleated cells and platelets while class II HLA molecules are expressed on antigen presenting cells (APC) like dendritic cells, B lymphocytes, macrophages.

The fundamental role of class I and class II molecules is to bind to their self and non-self peptides which then transports to the plasma membrane of the cells for T cell antigen receptor recognition [4].

Class I HLA molecules bind peptides made of 8-10 amino acids and present these peptides to CD8 cytotoxic T lymphocytes. Class I proteins are highly polymorphic. HLA-B is the most polymorphic gene known in the human genome. Class II HLA molecules bind peptides made of 13-25 amino acids and present peptides to CD4 helper T lymphocytes.

Activation of CD4 and CD8 T cells by these two pathways leads to cell division and differentiation resulting in a cellular and humoral immune response.

An allele represents a variant of a given gene which is found at the same place on a chromosome. The nomenclature of HLA alleles is composed of the locus and the gene name which are separated by a hyphen and an asterisk, followed by two digits which define the allele of the gene and this number frequently but

Not always matches the serological type [5]. The third and fourth digits are used to list the subtypes (**Figure 3**).

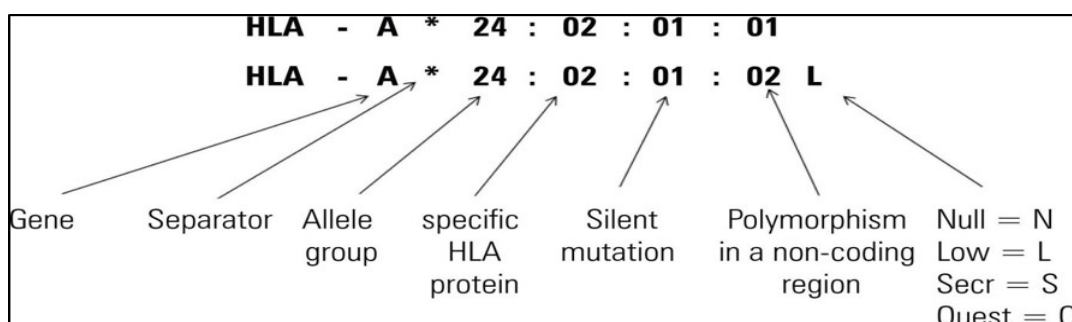


Figure 3: HLA nomenclature

Each individual inherits from parents one set of HLA genes in a Mendelian transmission (**Figure 4**).

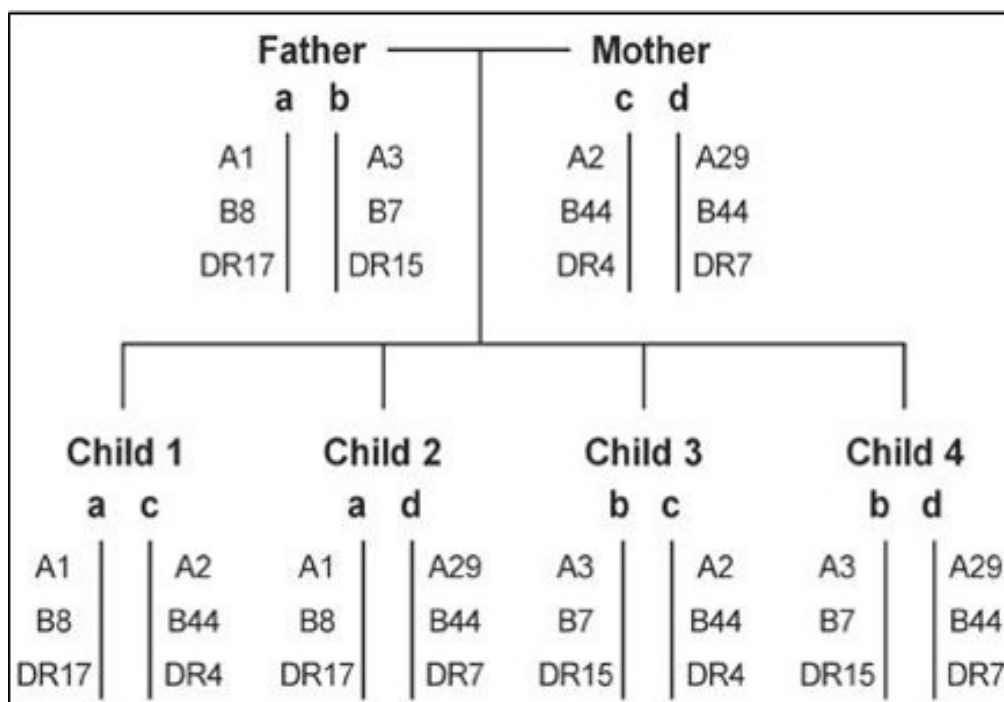


Figure 4: Mendelian Transmission

HLA genes are normally inherited “en bloc” from parents because of their close physical linkage.

Despite the immense complexity of these genes clusters, there are normally four genotypes transmitted. All children inherit a haplotype from their mother and a haplotype from their father. The chance to find between siblings a 100% HLA compatible donor is 25%. There is a 50% chance of two siblings sharing 1 haplotype (50% HLA compatible donor) and a 25% chance of two siblings not sharing a haplotype (0% HLA compatible donor).

In the case of consanguineous marriages, there is a chance that parents and children can be 100% compatible.

In the case of heart/liver/kidney organ failure, the best therapy is an organ transplant. The kidney graft is perceived by the recipient immune system as non-self so the allograft is infiltrated with host cells within seven to ten days resulting in graft loss.

HLA has an important role in long-term graft preservation.

In kidney allografts, the most important determinants of early transplantation success or failure are:

- method of organ preservation,
- cold ischemia time,

- number of blood transfusions
- recipient's age
- the primary cause for kidney failure,
- prior organ transplant,
- immunosuppressive therapy.

The role of a histocompatibility test in organ transplant is to select the best donor for a recipient. HLA laboratories perform various tests to support transplant programs like HLA typing, detection of HLA antibodies and the cross-matching test.

HLA matching improves long-term graft survival rates (GSRs). This is an important fact because the treatment of organ failure with a transplant is limited by the number of available donors. To increase the chances for a retransplant, when necessary, we need to increase the duration of graft survival.

The highest survival rate occurs when all six antigens are matched (6/6 match). Kidney transplants may be done with an HLA compatibility below 50% but the DR locus must match between donor and recipient.

Before a transplant, all patients who are on the waiting list are tested for HLA antibodies. This offers valuable information for donor selection.

Sera are collected and screened for the presence of HLA antibodies on a regular basis. HLA antibodies develop during blood transfusions, pregnancy or previous organ transplants. All immunization can occur prior to kidney transplant thus continuous patient monitoring is required.

Antibody determination involves two steps. In the first step, the serum is put in contact with purified lymphocytes from a donor or HLA antigens of a donor to see the reactivity expressed in percent of the antibodies. In the second step, we need to identify the specificity of HLA antibodies present in the serum.

HLA antibodies are involved in graft rejection, so their periodic determination is very important (**Figure 5**).

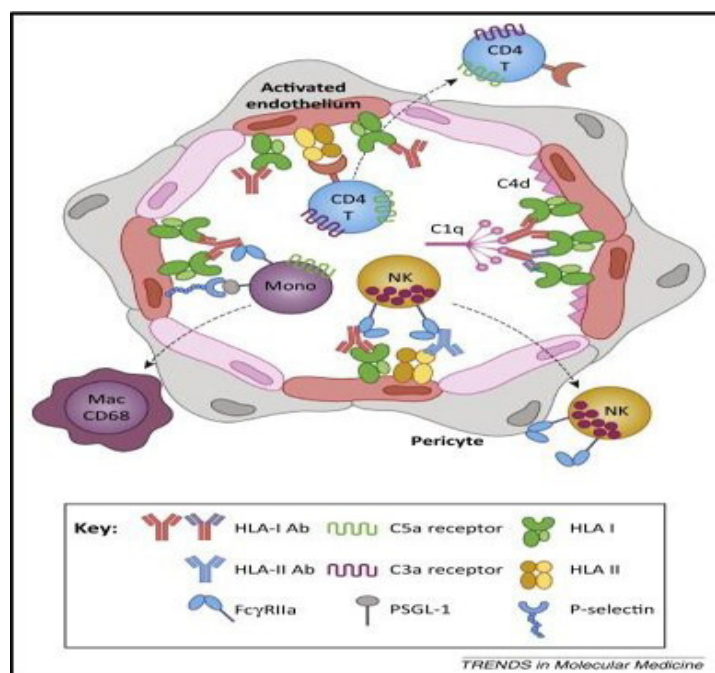


Figure 5: HLA antibodies cause graft injury in the donor vasculature

We may consider a possible antibody-mediated rejection when we have a lower renal function.

Kidney allograft rejection is categorized into 4 categories according to the time of post-transplantation, clinical and anatomic-pathological manifestations, response to anti-rejection treatment (**Figure 6**).

1. Hyper acute rejection: Occurs within the first few hours or even the first few minutes after renal transplantation. The graft stops functioning and must be removed. Anti-HLA class I antibodies attack the donor cells thinking of it as a non-self or foreign antigen. Antigen-antibody interaction within glomerular capillaries results in the development neutrophil-infiltrated complement deposits, platelet aggregation, and intracapillary thrombus formation. At the anatomic-pathological exam the kidney is congested and edematous, with blue-violet or cyanotic blue color changes. Microscopically, extensive cortical infarction is reported in glomeruli and the renal tubules. Studies in immunofluorescence show excess fibrin and immunoglobulins (IgG) as well as complement deposits in glomerular capillaries.

Clinically, the patient has anuria, hypertension, hyperkalemia, metabolic acidosis and pulmonary edema. The thrombotic process initiated at the kidney can expand and progress to Disseminated Intravascular Coagulation (DIC). DIC is a condition which is caused by small thrombi which block the small blood vessels. It causes thrombocytopenia and depletes clotting factors which in turn leads to internal hemorrhages.

Renal artery thrombosis or renal artery embolism have the same appearance on kidney radiography.

Hyper acute rejection should be distinguished from other anuria causes such as urinary tract obstruction, ureter necrosis with urinary fistula, acute ischemic tubular necrosis or severe intravenous volume depletion.

2. Accelerated acute rejection: Appears days or weeks after transplantation. In most cases, patients have good urine volume and normal kidney function at first and then develop acute kidney failure with oliguria due to immunological rejection. The mechanism behind this is due to graft lesions caused by donor-specific antibodies that developed after the transplant. Clinically and anatomic-pathologically acute accelerated rejection and hyper acute rejection are hard to distinguish, the differentiation criteria are as follows:

- The time span of post-transplant rejection;
- The presence or absence of anti-donor antibodies at the time of transplantation.

3. Acute rejection: This is a cell-mediated rejection. The key players are mainly T lymphocytes, B lymphocytes, macrophages and the rest of the cell population composed of neutrophils and natural killer cells.

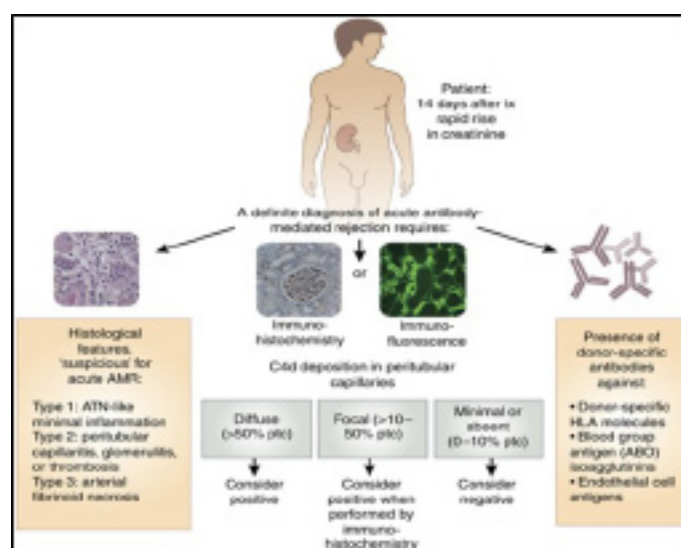


Figure 6: HLA antibodies cause graft injury in the donor vasculature

4. Chronic rejection: The triad of hypertension, proteinuria, and progressive deterioration of renal function is commonly attributed to chronic renal allograft rejection. This complication may occur at the earliest 6 months post-transplant and is the most common cause of graft loss more than 1 year after transplant. The exact pathogenesis of chronic rejection is unknown but immunofluorescence shows the presence of immunoglobulins (IgG, IgM) and complement deposits in glomerular capillaries.

3. Blood Group Matching

Blood group compatibility is essential for successful kidney transplantation (Figure 7). Kidneys transplant with ABO incompatible blood groups are largely unsuccessful due to the

hyper acute rejection phenomenon caused by anti-A and/or anti-B antibodies. Antibodies bind to A and/or B antigens from the endothelium of the graft, activating the complement cascade, inducing platelet aggregation and intravascular thrombosis. The most important blood antigens are A, B and O group antigens. These antigens are found in many cells including erythrocytes, platelets and endothelial cells of all vascular vessels. Blood group antigens are polysaccharides and they do not need T cell sensitization for antibody induction.

Blood group matching in ABO system at the recipient and donor is the first condition to perform a kidney transplant. Patients who are candidates for renal graft will be determined erythrocyte antigens in ABO, Rh, Lewis, P, Ss, Ii systems and will be investigated the presence of irregular antibodies.

Recipient	Blood donor			
	O	A	B	AB
O	✓	✗	✗	✗
A	✓	✓	✗	✗
B	✓	✗	✓	✗
AB	✓	✓	✓	✓

Figure 7: ABO matching.

When ABO compatible donors are not available, crossing the ABO blood type barrier is the only chance for some patients to receive a renal graft [6]. Kidney transplant from ABO incompatible blood group (ABOi) donors became the only option for the patients who are on the waiting list. Although ABOi renal transplant is now feasible, antibody-mediated rejection of the graft remains an important issue. Because the number of patients with ESRD is increasing and there is a lack of kidney donors, researchers in Japan developed new therapeutic strategies.

Current strategies of ABOi renal transplant consist in pretransplant removal of isoagglutinin titers to prevent antibody mediated rejection. Desensitization protocols are based on plasmapheresis or plasma exchange preoperatively or the use of anti – CD20 monoclonal antibody (Rituximab) reduced the high titers of isoagglutinin.

Splenectomy is another procedure that can be used to reduce the high titers of isoagglutinin which are responsible for hyperacute rejection in ABOi blood group patients. But splenectomy also increases the risk of sepsis in patients with aggressive immunosuppression therapy and plasma exchange [7].

The number of ABOi kidney transplants has increased worldwide because of the good results and improved outcomes obtained by Japanese surgeons.

4. Cross Matching

The purpose of cross-matching test is to detect in the patient's serum, the presence of antibodies directed against donor HLA antigens. Cross-matching is performed on T cell lymphocytes which express HLA class I molecules and on B cells which express class I and class II molecules [8].

If antibodies are present, it shows that the recipient's immune system has been sensitized to the donor antigens and is susceptible to allograft hyperacute rejection and/or early graft loss (**Figure 8**).

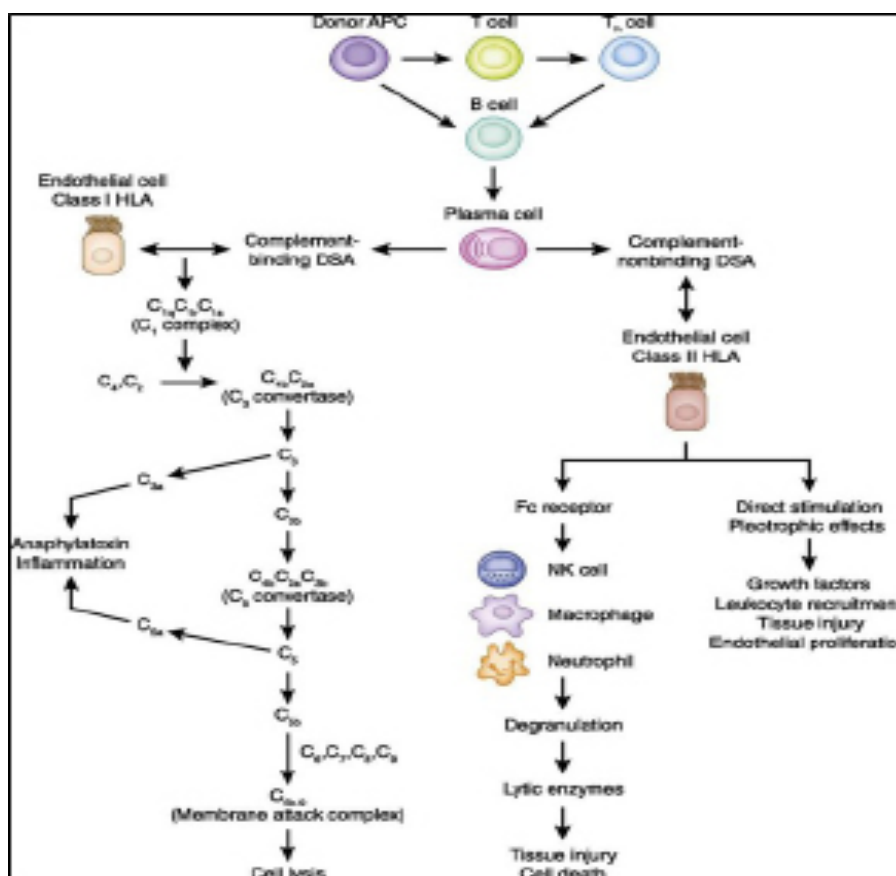


Figure 8: Antibody mediated rejection.

The sera of some patients with an autoimmune disease like systematic lupus erythematosus and patients on blood pressure medication (hydralazine and procainamide), can often contain autoantibodies.

These autoantibodies do not affect the graft and are important because they can mimic donor specific antibodies and subsequently deprive a recipient of a graft donor. The donor specific antibodies and the autoantibodies can be removed with dithiothreitol (DTT) treatment. The crossmatch test should never be interpreted alone, without any information about the patient's history.

The virtual crossmatch (v-XM) decrease the workload in HLA-laboratories and facilitates the organ allocation even in sensitized recipients [9]. In a study published in September 2014

Piazza et al. showed that V-XM protocol had a good sensitivity in predicting donor-recipient immunologic compatibility [10].

5. Highly Sensitized and Regraft Recipients

Sensitization is a process where the patient is exposed to nonself HLA antigens. Sensitization can occur during blood transfusions, Pregnancy or a prior organ transplant [11].

The number of patients waiting for a kidney retransplants after a failed kidney graft has increased over time. The approach to the retransplant recipients with high sensitization, represent a challenge for organ transplantation.

Recipients of kidney retransplant are at high immunological risk for graft rejection. Causes include prior surgery, side effects from chronic immunosuppression therapy – nephrotoxic drugs, the risk of sensitization with an elaborated panel of reactive antibody levels and other comorbidities.

Outcomes in retransplant patients include many factors like the source of the donor (living or deceased donor), functional duration of the first graft and elapsed time between first and second graft.

The rate of kidney graft failure is higher in patients who receive a graft from a deceased donor compared with patients with a graft from a living donor [12]. One of the reasons is prolonged cold ischemia time (CIT) which leads to delayed graft function (DGF). DGF after primary transplants is associated with an increased probability of recurrence.

Long-term graft survival decreases with subsequent retransplants. Graft survival is affected by factors such as repeated surgery, acute rejection, a non-functional primary graft, the immunosuppressive regimen, the number of HLA mismatches and surgical complications.

Failed graft nephrectomy may be indicated before kidney retransplant if it is associated with refractory hypertension, urinary tract infections, urinary tuberculosis, and proteinuria. It is not yet clear if the presence of a failed graft can stimulate the production of antibodies, therefore nephrectomy decision should be based on clinical indications. Between kidney retransplant and hemodialysis, retransplantation remains the treatment of choice.

6. Networks and Tissue Type Matching

Many patients with end-stage kidney failure have difficulties in finding a donor in their own country. To fix this problem, international organ procurement and transplantation networks appeared.

Worldwide transplantation networks play an important role in the allocation and distri-

bution of donor organs for transplantation based upon medical, ethical criteria and in compliance with the national legislation of the members' countries.

In the world are many transplantation networks such as:

- Euro transplant (Austria, Germany, Belgium, Croatia, Hungary, Luxembourg, the Netherlands and Slovenia).
- Scandia transplant (Iceland, Norway, Finland, Denmark and Sweden),
- Bal transplant (Estonia, Latvia and Lithuania)
- NHS Blood and Transplant in the UK
- United Network for Organ Sharing (UNOS) in the USA

They serve as a common organ exchange organization and allocation resource for their member hospitals including kidney, liver, heart and lung.

7. Immunosuppressive Agents

The integrity of humoral and cellular immune response is essential for preventing infections. In some situations, such as autoimmune diseases and solid organ transplantation, the activity of humoral and cellular immune system has to be suppressed.

Post-transplant status involves cellular immunity. Both for graft-versus-host disease (GvHD) and host-versus-graft reactions, CD4 + T lymphocytes are activated when a foreign antigen binds to HLA class II antigens on the surface of macrophages (antigen presenting cells).

Specific T-cell clones will bind to the antigen via the T cell receptor (TCR). TCR activation results in a signal transduction cascade that will eventually lead to endocytosis of the antigen in macrophages and its destruction in lysosomes. In this cascade, calcium ions are activated to activate calcineurin, a phosphatase that forms a calmodulin complex.

Activated calcineurin dephosphorylates T-cell cytosolic factor (NF-AT) C and thus activates it. The activated T-cell cytosolic factor (NF-AT) C migrates to the nucleus and binds to its counterpart (NF-AT) N. The resulting transcriptionally active complex will induce the synthesis of interleukin-2 (IL-2) that is secreted as an extracellular mitogen.

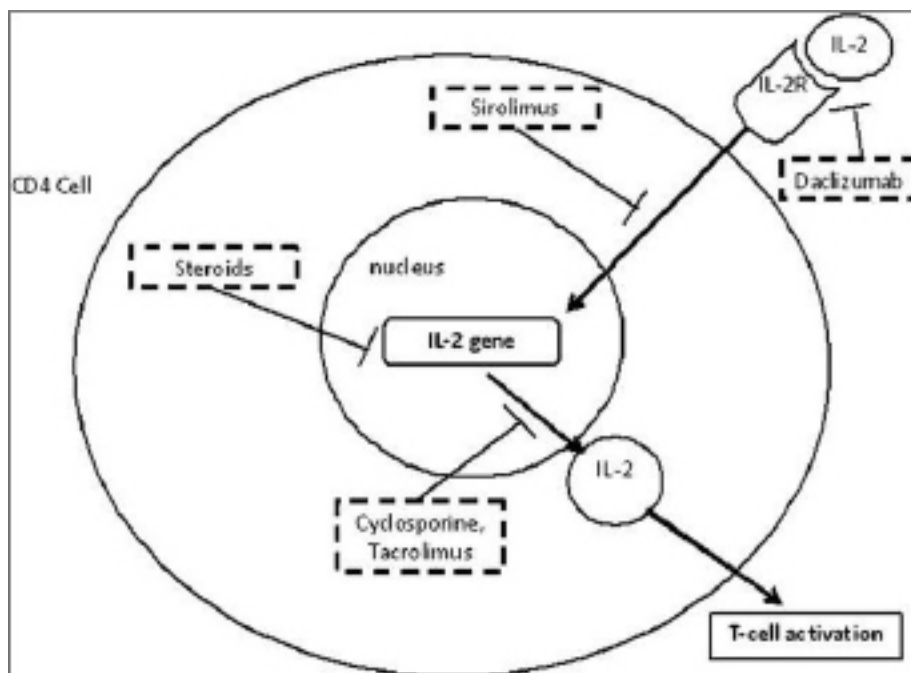


Figure 9: Immunosuppressive drugs affecting IL-2

IL-2 binds to its T lymphocyte receptor, which it activates by binding a protein, termed rapamycin target (TOR). TOR activates cyclin kinases that facilitate cell cycle progression from G1 to S and stimulate nucleotide synthesis. The process results in the differentiation and proliferation of T cells and will eventually lead to the destruction of the antigen.

Specific drugs have been developed. They block one or more of the above-mentioned steps, thus inhibiting the destruction of the antigen.

Cyclosporine and tacrolimus are cyclic polypeptides that bind to intracellular proteins called immunophilins (while tacrolimus binds to the immunophilin called FKBP12), resulting in complexes that subsequently block the activation of calcineurin-induced NF-AT and consequently the synthesis of IL-2.

Cyclosporine

Cyclosporine A is recommended to prevent graft rejection in allogeneic transplantation of kidney, liver, heart, and lung-heart.

it is also used as a medicine of the first or second line in the treatment of acute graft-versus-host disease (acute GvHD) for bone marrow arising after the transplantation, severe rheumatoid arthritis, and in some severe forms of psoriasis.

The maximum blood level is reached approximately 3.5 hours after administration; about 20-40% of the administered dose is absorbed intestinally and it is metabolized at the first passage of the liver. The isoenzyme CYP3A4 of cytochrome P450 is responsible for the metabolism of cyclosporine in at least 30 metabolites. Because a large number of drugs can induce the enzyme or may be metabolized by CYP3A4 (antibiotic, antifungal or other immu-

nosuppressant's) the combination could alter the levels of cyclosporine in the blood and thus complicate treatment.

Cyclosporine has a narrow therapeutic interval with frequent side effects, which makes monitoring of its blood concentration essential.

Since 80% of cyclosporine is sequestered in erythrocytes, it is recommended that drug levels were determined in whole blood samples. In the first 2 months post-transplant, the doses are adjusted to maintain concentrations between 150 ug/L and 400 ug/L. Trough concentrations obtained samples can be varied according to the clinical protocol, such as allograft rejection risk, concomitant use of other immunosuppressant's and toxicity. After the first 2 months, the therapeutic target is generally lower (between 75 and 300 ug/L).

The side effects of cyclosporine may occur in any organ; maximum concentration values (trough samples) > 500 mg/L. The most common adverse reaction is nephrotoxicity followed by hyperkalemia, hyperuricemia, hypertension and gingival hyperplasia.

Tacrolimus

It is a macrolide class antibiotic with a mechanism of action similar to cyclosporine but with a more potent inhibitory effect than it. It is used to prevent organ rejection.

Tacrolimus is metabolized in the liver by CYP3A4, so its blood levels are affected both by drugs that inhibit this enzyme (calcium channel blockers, antifungals, some antibiotics) and those that induce enzyme activity (anticonvulsants, rifampicin).

Since 90% of tacrolimus is found in blood cell components, it is recommended that drug levels were determined in whole blood samples. Concentrations in trough samples may vary depending on the clinical protocol, allograft type, risk of rejection, concomitant administration of other immunosuppressant's and toxicity (mainly nephrotoxicity) but should not exceed 20 ug/L.

The toxic potential is similar to that of cyclosporine: nephrotoxicity, neurotoxicity (tremor, headache), gastrointestinal disorders (nausea, diarrhea), HAT, glucose metabolism disorder, hyperkalemia, and infectious diseases. However, there is no gingival hypertrophy or hirsutism. Anaphylaxis is described only for intravenous administration [13].

Rapamycin (sirolimus)

Another immunosuppressive agent - rapamycin (sirolimus) - does not exert any effect on T cells.

Rapamycin is a similar antibiotic to tacrolimus that suppresses the proliferation of B and

T lymphocytes; also has anti-neoplastic and antifungal activity.

It inhibits TOR protein kinase and blocks the cell cycle. It has no effect on calcineurin and can, therefore, be used with cyclosporine and tacrolimus.

Sirolimus is metabolized by CYP3A4, so its blood levels are affected both by drugs that inhibit this enzyme and those that induce enzyme activity.

The pharmacokinetic interaction between sirolimus and cyclosporine or tacrolimus increases both therapeutic immunosuppression and the toxicity of these agents; as a result of combined use, lower doses are needed.

Adverse reactions are generally dose-dependent, so monitoring blood levels of the drug are important. These include gastrointestinal disorders and thrombocytopenia; the medicine does not appear to be nephrotoxic.

Mycophenolate mofetil (MMF)

Mycophenolate mofetil (MMF) is an antibiotic that is hydrolyzed in cells in free mycophenolic acid. This agent is a potent inhibitor of inosine monophosphate dehydrogenase and guanosine monophosphate synthase, ultimately inhibiting DNA synthesis [14].

Corticosteroids also exert immunosuppressive effects on cell-mediated immunity, but these are much less specific and accompanied by numerous adverse reactions.

8. Immunosuppressant Antibodies

Anti-thymocyte globulin

This is a polyclonal IgG antibody from horses or rabbits immunized with human thymocytes. Infusions of anti-thymocyte globulin cause profound T-cell depletion and the lymphopenia typically persists beyond one year. An unwanted effect is the release of cytokines. This is associated with the 'cytokine release syndrome' characterized by fever, rigors, and hypotension.

Antibodies against CD25

Basiliximab and daclizumab are monoclonal antibodies against CD25. CD25 is a receptor on the surface of T-lymphocytes.

These antibodies are well tolerated and hypersensitivity reactions are uncommon. So, they don't need monitoring [15].

Muromonab-CD3

This is a mouse-derived monoclonal antibody which binds to the CD3 component of the T-cell receptor complex leading to T-cell depletion. Muromonab is also associated with the cytokine release syndrome.

A longer-term concern is the increased incidence of lymphoma [15].

9. Prophylactic Regimens for Renal Transplantation

In any solid organ transplantation, prevention of post-transplant infection is vital.

Bacterial infections usually come from surgical wounds and the urinary tract in kidney transplant. Hence, it is essential to give perioperative antibacterial prophylaxis to prevent wound infections in kidney transplant recipients [16].

Many studies showed that prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX) or ciprofloxacin after kidney transplantation are effective in preventing UTIs and bacteremia [17]. TMP-SMX prophylaxis also protects against *P. carinii*, *L. monocytogenes*, *N. asteroides*, and *T. gondii* infections [17].

In case of Purified Protein Derivative (PPD)-positive recipients, isoniazid (INH) prophylaxis is recommended by the American Lung Association but with some concerns [18]. Despite being proven effective in preventing tuberculosis, INH can cause hepatotoxicity and alter the metabolism of cyclosporine and tacrolimus [19].

Prophylaxis with ganciclovir, valganciclovir or foscarnet is effective for preventing Cytomegalovirus (CMV) infection in solid organ transplant recipients with latent CMV [20].

10. Induction Therapy in Adults

The goal is to prevent acute rejection, optimize the function of the transplanted organ and minimize the risk of infections and complications. Induction therapy can be used for up to two weeks following transplantation.

The Immunosuppressive protocol includes induction therapy and long-term maintenance therapy.

Antibody induction is recommended in patients with immunological risk. In the US, approximately 60% of renal transplant patients in 2011 received an induction of T-cell antibodies, predominantly anti-thymocyte (ATG). Another 40% of patients received either antibodies of IL-2 receptor (IL-2R AB) or no antibody induction [21].

11. Induction Therapy in Children

Many of the induction immunosuppressive agents currently used in the UK are biological agents including monoclonal antibodies (such as basiliximab) and polyclonal antibodies (such as rabbit anti-human thymocyte immunoglobulin).

12. Maintenance Immunosuppressive Therapy in Kidney Transplantation in Adults

Maintenance immunosuppression is necessary as long as allograft is well-functioning to prevent rejection of the transplanted kidney [21].

There are several factors to take in consideration when choosing a maintenance immunosuppressive regimen for a particular patient such as clinical characteristics, immunologic risk, comorbidities and the medications side effects [21].

The risk of acute rejection is highest in the first months after transplantation. Infection and other side effects correlate with a high level of immunosuppression. Malignancy is a late complication of immunosuppressive drugs.

When there is no episode of graft rejection, immunosuppression is usually decreased slowly to a maintenance level by 6 to 12 months following transplantation [22].

In order to find the best regimens associated with less adverse drugs events, researchers studied a few regimens with and without steroids, CNIs and sirolimus.

The *FREEDOM* trial included three groups: steroid-free, steroid-withdrawal and standard-steroids. The results of Freedom trial and other studies showed that steroids should be part of the maintenance protocol in sensitized patients [23, 24].

The *CAESAR* study proved that cyclosporine withdrawal later after transplant was associated with higher incidence of acute rejection than the group with continued cyclosporine [25].

The *SYMPHONY* study concluded that the group treated with daclizumab/ MMF/ steroid/ low-dose tacrolimus had the lowest rate of rejection, higher graft function and better graft survival than other groups treated with standard-dose cyclosporine/MMF/steroid, daclizumab/ low-dose cyclosporine/ MMF or daclizumab/ MMF/ steroid/ sirolimus [26,27].

In other long-term study, patients with tacrolimus/MMF had a significantly lower incidence of acute rejection and better renal function compared to other combinations of immunosuppressive drugs [28].

In the USA, combination of mycophenolic acid (MFA), corticosteroid and tacrolimus is

the most used maintenance both at beginning of transplant and later.

13. Maintenance Immunosuppressive Therapy in Kidney Transplantation in Children

Calcineurin inhibitor (CNI; usually tacrolimus) in combination with an antiproliferative agent (e.g., mycophenolate mofetil or azathioprine) and a steroid (e.g., prednisolone), is used in this population.

Long-term maintenance therapy is in a lower dose than the initial dose as transplanted kidney becomes more stable [29].

This therapy is continued throughout the graft survival [30].

14. Barriers to Transplantation Tolerance

There are some barriers to transplantation tolerance. The optimal outcome for patients after transplantation would be induction of specific immunological unresponsiveness or tolerance to the kidney allograft.

By this transplanted patients could avoid the adverse side effects associated with current immunosuppressive regimens. To achieve tolerance is really problematic in the case of mismatched kidney allografts. The less mismatched HLA alleles the more acceptance of the kidney with less immunosuppressive therapy combinations.

Since the first renal transplant, more than 55 years ago, only sporadic cases of clinical operational tolerance (COT) have been documented after renal transplantation in the absence genetic identity between a donor and recipient.

Memory T cells are less sensitive to T-cell depleting antibodies and costimulatory blockade and thus may be more resistant to some tolerance induction strategies.

Kidney recipients Treated with immunodepletion antibodies could have an increase in the naive B-cell population.

There is a significant development of alloantibodies in kidney recipients treated with depleting antibodies therapy.

Lack of tolerance means episodes of acute rejection that can severely affect the kidney allograft survival. There is a lack of validated biomarkers of tolerance or predictors of rejection. On the horizon there are some promising initiatives: -introduction of antigen-specific regulatory T cell therapy;

-identification of predictor of regimen and tolerance signatures;

-use of immunomodulatory stem cell population (i.e., mesenchymal stromal cells).

Despite providing the best quality of life and the most cost effective treatment option for treatment of end stage renal disease, renal transplantation remains at the challenging and exciting interface between clinical research and laboratory science. Its development has also been the catalyst for transplantation of other solid organs, the practice of immunosuppression and much of clinical immunology.

Despite all progresses more work needs to be done to achieve long term immunotolerance in kidney transplantation.

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