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Corneal transplantation and immunosuppressants

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ABSTRACT

Corneal graft is the most common and most transplanted tissue due to its immune privilege. After corneal transplantation, topical or systemic immunosuppression is applied depending on the patient's case and condition. Immunosuppressants are a group of drugs that suppress the body's immune system and prevent organ rejection by preventing the transplanted organs from being perceived as foreign by the body and stimulating the body's natural immunity. Immunosuppressants are subdivided into corticosteroids, calcineurin inhibitors, interleukin 2 receptor blockers, and mTOR inhibitors. Inflammation or corneal vascularization in the transplant recipient increases the risk of graft rejection and affects the success of transplantation. To prevent rejection, systemic immunosuppressants are used in high-risk transplantation patients, however, long-term immunosuppressants have low efficacy and severe side effects, making it difficult to manage this process. Patient-specific immunomodulation therapy is currently thought to be the most effective treatment for the high-risk transplantation group. In this review, the characteristics, effects, and efficacy of topical and systemic immunosuppressants used in the post-transplantation period are described in scope of the possibility of corneal graft rejection after transplantation and according to the immune privilege of cornea and risk factors for corneal transplantation. Novel immunoregulators and cellular therapies that may increase the success of corneal transplantation without side effects of immunosuppressants are also emphasized.

Keywords: Cornea transplant, graft rejection, immune privilege, immunomodulators, immunosuppressants.

CORNEA TRANSPLANT AND IMMUNE PRIVILEGE

Keratoplasty, more commonly known as "cornea transplant", is the replacement of the cornea layer of an eye that has lost its transparency due to various cornea degenerations or dystrophias with the cornea of a healthy donor. For this, usually under general anesthesia, a 6-9 mm wide circular region is removed from the patient's cornea and the healthy cornea from the donor is sewn into place.

The idea of cornea transplantation was first presented by Pellier De Quengsi in 1789.[1] The first successful keratoplasty was performed in 1905 by Eduard Konrad Zirm. Zirm successfully performed a penetrant keratoplasty on a 45-year-old patient with cornea defect caused by chemical burn.[2] In 1935, use of cadaver eyes for transplant was conceived, expediting developments in keratoplasty.[3]

The first keratoplasty in Turkey was conducted in 1937 at the Istanbul University Medical Faculty Department of Ophthalmology by Prof. Dr. Igersheimer.[4] Research and progress on techniques and preservation of donor cornea continues.[5]

A normal and healthy cornea is absent of blood and lymph vessels; this creates an "immune privilege" specific to the cornea.[6] Due to this privilege, corneal transplantations can be performed in patients without high risk with 90% success rate in the first year after transplant and 55% in the first 15 years.[7,8]

The microenvironment of the eye structurally possesses both immunosuppressant and anti-inflammatory properties. This immunosuppression

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formed by ocular cells and tissues in the eye is referred to as "immune privilege". Immune privilege helps prevent against the severe damage generated by infiltration of inflammatory cells.[9] To date, the immunosuppressive mechanism described in the eye consists of the microenvironment containing ocular fluids, the blood-retinal barrier, and ocular parenchymal cells. Ocular fluids such as aqueous humor and vitreous fluids have anti-inflammatory properties.[10,11] Production of regulatory T cells (Treg) also play a role in composing the immunotolerance of the eye.[12] Corneal epithelial cells and the retinal pigment epithelium (RPE) also contribute to the formation of an immune barrier with tight junctions. In addition, RPE cells structurally express immunosuppressive molecules and release immunomodulator factors which can provide mediation in an immunogenic environment.[13,16] Due to these unique immune circumstances in the cornea, cornea transplants are more successful and do not require systemic immunosuppression under normal circumstances, in contrast to other organ transplantations. It is true that the cornea is a tissue with "immune privilege" because of its structural properties and microenvironment, however, this should not be mistaken with "immune immunity" as the presence of lymphatic channels have been shown in vascularized corneas. In Changes in normal distribution of antigens and immune reactive cells have been shown in conditions such as inflammation, scarring, and vascularization in the cornea, leading to increase in number of type 2 antigen-bearing bone marrow-derived dendritic cells.[17-21]

CORNEAL GRAFT REJECTION

Loss of maintaining transparency after transplantation may occur due to two reasons: immune graft rejection (allograft rejection) and non-immune graft failure. The main risk factors for allograft rejection include: HLA incompatibility between the donor and receiver, use of grafts larger than 8 mm, and vascularization that develops in the donor cornea. Among these risk factors, corneal vascularization plays the greatest role. A study by Hill[22] revealed that the outcome of regraft in avascular graft with immune-induced rejection was no different from primary grafts, but that increased corneal vascularization significantly increased risk of rejection. Another study showed that using large grafts was associated with increased amount of HLA antigen which may increase the rate of rejection reactions.[23] According to studies by the CCTS (Collaborative Corneal Transplantation Studies), risk factors for graft rejection in corneal transplantations included: previous corneal transplantation surgery, formation and degree of stromal vascularization, glaucoma before operation, young age of the recipient, previous anterior segment operations, degree of anterior synechia, history of chemical burn, and incompatibility between donor and recipient blood groups. The study by Medawar and Bilingham[38] demonstrated the role of HLA antigen incompatibility between the donor and recipient in graft rejection. Previous corneal transplantation surgery earned antigenic sensitivity and increased the response to the newly transplanted tissue.[24] In children, immune reactions are more severe and rejection reactions are more frequent, therefore penetrant keratoplasty success is also affected by recipient age.[25,26] In addition, transplantation patients with known ocular allergies recorded during routine clinical examinations were found to have higher risk of corneal allograft rejection.[27,28] In a study conducted by Hau et al.,[29] corneal leukocyte density was examined *in vivo* after corneal transplantation and it was shown that increased leukocyte concentrations in the endothelium could be associated with severe graft rejection.

Corneal graft rejection was first described as "Malaide du Greffon" (Greffon's disease) in 1949 by Paufique, Sourdille and Offret.[30] Immunologic origin of this condition was first proposed by Edward Maumenee in the 1950s.[18] One of four graft recipients have experienced at least one rejection attack; 20% of these attacks are irreversible and cause transplantation failure.[31] Half of graft rejection cases occur in the first three months of the postoperative period, while 90% occur within the first year. Late graft rejection reactions are seen in 10% of cases and they develop 1-15 years after surgery.[32]

Studies by researchers including Khoda-Doust and Silverian indicated that since the cornea is an avascular tissue, immune response may be suppressed, however, graft rejection may occur especially in the endothelial layer in patients with vascularization.[30] Therefore, the appearance of a line formed by the accumulation of inflammatory

cells on the endothelial surface of the cornea is called the "Khodadoust line" (rejection line).[33] Corneal graft rejection is characterized by rejection lines, reactions of the anterior and vitreous infiltrations in the subepithelial space or keratic precipitates in the endothelium, and stromal edema.[34,35]

Development of neovascularization and inflammation during placement of the healthy cornea is considered "high risk" for corneal transplantation rejection. Because it causes chronic inflammation, eyes with herpetic, interstitial, or traumatic keratitis sequalae have increased vascularization, increasing the incidence of graft rejection.[23] There are three main factors that contribute to corneal allograft vitality: prevention of the induction of immune reaction against allograft antigens, production of regulatory T cells (Treg) that can suppress destructive alloimmune reaction, and activation of apoptosis of inflammatory cells in the graft/recipient interface.[36] Strategies used to prevent graft rejection include: antigen alteration, determining HLA compatibility, use of UV light, hyperbaric oxygen application, and immunosuppressive drug therapies.

IMMUNOSUPPRESSANTS TO SUPPRESS IMMUNE RESPONSES

One of the strategies used to prevent graft rejection is the use of immunosuppressive agents such as corticosteroids, cyclosporine A, tacrolimus, mycophenolate mycotil, and rapamycin. In addition, new immunomodulatory approaches are currently being developed for high-risk corneal transplantations. One of these approaches is the inhibition of corneal angiogenesis by suppressing VEGF.[37] Studies have shown that topical treatment with specific antibodies, trap proteins, or receptor antagonists prevents graft rejection.[38,39] Also using gene therapies, inhibition of potential immunoreactivity in the recipient by reducing antigen presenting cells (APCs) in the donor cornea and subsequently the number of antigens transferred to the recipient is also an objective.[40]

Corneal transplantations that are not vascularized and do not develop inflammation are considered "low risk" for graft rejection and these cases do not require any systemic immunosuppression or HLA-compatibility. High-risk corneal transplantations, however, have less than 35% survival in the first five years after transplantation, despite the use of immunosuppressives.[41-43] This data is known to be even worse than numbers from kidney, liver, or heart transplantations.[44]

In recipients that carry high risk for graft rejection, neovascularization causes invasion of blood and lymph vessels into the corneal graft. This allows transport of immune effector cells to the graft through new corneal vessels, inducing immune reaction and graft rejection.[45] Many studies have demonstrated that as neovascularization advances, immune alloantigenic response increases.[46,47] Khodadoust and Silverstein[48] reported that 65% of heavily vascularized corneas developed graft rejection and transplantation resulted in failure despite intense immunosuppression.

Prophylactic immunosuppressive treatments following transplantation are determined according to the patient's degree of risk. Use of topical steroids in the low risk group has been proven to increase transplant success.[49] However, there are different protocols in the length of using topical corticosteroids.[50] The most commonly preferred method is the topical use of 1% prednisolone acetate preparations initially for four times daily and after three months once daily for a period of 12 months. In case of signs of rejection reactions frequency of application must be switched to once hourly.[51] New surgical techniques have been used to reduce the amount of allogeneic tissue in the transplanted graft and to prevent the transfer of endothelial cells to the recipient.[52] Nevertheless, such approaches have not achieved the desired degree of positive impact on the high-risk group.[41] Interestingly enough, according to the American Cornea Society, endothelial keratoplasty patients encountered less graft rejection compared to penetrant keratoplasty patients.[50,53]

According to the results of the Collaborative Corneal Transplantation Studies (CCTS), aside from these approaches, immunosuppressants, especially topical corticosteroids, have always played a key role in inhibiting immunological pathways to suppress graft rejection.[54]

As in low risk corneal transplantations, corticosteroids are also used as the first-line for prophylactic treatment in high-risk groups. 1% prednisolone or 0.1% dexamethasone drops are

applied 6-8 times daily. Although length of use varies depending on the surgeon, according to studies by the Cornea Society, treatment continues for an average of eight months.[50]

Systemic immunosuppressants are used as the basis for prophylactic treatment in high-risk patient groups; however, there is no definite margin or provision on this matter as it usually depends on the surgeon's evaluation and judgement. This makes it difficult to compare studies and results. Along with their effects in preventing graft rejection, systemic immunosuppressants may also cause ocular side effects such as glaucoma, infection, and potentially life-threatening systemic side effects.[55-57] The main purpose of immunosuppression is to prevent rejection in the recipient by pharmacologically developing a specific tolerance towards the graft.

Corticosteroids play a key role in the approach to the corneal transplantation process. They prevent proliferation, chemotaxis, and neovascularization of T cells. Studies on animal models have demonstrated that in the case of high risk of graft vascularization, topical steroid use delayed passage of vessels through graft margins, increasing graft vitality.[51] They can be used before, during, or after transplantation. They can be used alone, or in combination with other immunosuppressive agents in the case of acute graft rejection.[58,59] According to the study by the American Cornea Society, oral prednisolone (40-80 mg/day and for 2-7 days) in addition to topical steroids is almost always used as a prophylactic treatment by 22% of surgeons in high-risk patients; apart from this, it is applied as prophylactic treatment when corneal graft rejection occurs.[60,61]

In animal model studies by Kim et al.,[62] corticosteroid use was initiated two weeks before corneal transplantation, and they succeeded in reducing corneal neovascularization. Supportive of this finding, there are studies that show that initiation of preoperative corticosteroids reduces angiogenesis in both low- and high-risk patient groups.[57]

Surgeons have leaned towards use of single-dose intravenous corticosteroid use in addition to topical steroids in 14% of high-risk cases. In this sense, single-dose 125 mg intravenous methylprednisolone in addition to hourly application of topical steroid treatment was shown to significantly prevent severe graft rejection in the high-risk patient group.[63] Another study indicated that single-dose 500 mg intravenous methylprednisolone treatment was as effective as oral corticosteroid treatment in preventing endothelial rejection and emphasized that it also provided the advantage of preserving the patient against the systemic side effects caused by long-term oral corticosteroid use.[64,65] A study by Hill et al.[65] reported that intravenous administration achieved 79% and oral therapy achieved 63% success in terms of corneal graft survival, while corneal graft rejection was observed in 25% of intravenously treated patients, and 67% in recipients receiving oral therapy. Crouzet et al.[66] experiment on rabbits that underwent penetrant keratoplasty, in which corneal vascularization and graft rejection rate was significantly reduced when dexamethasone implants were placed in the subconjunctiva compared to the placebo group; this rate was found to be significantly similar to subjects receiving Dexamethasone in drop form as an immunosuppressant after corneal transplantation.

According to results published by the American Cornea Society in 2011, use of postoperative subconjunctival methylprednisolone injection, oral prednisolone use, and intravenous methylprednisolone and corticosteroid applications was reported to be increasing in corneal transplantations; when these treatment regimens were compared, subconjunctival steroid applications were found to be more effective in low-risk corneal transplantation groups with 76% success rate. In the high-risk group, the success rate of these applications remained at 57%.[50] The main factors limiting the application of corticosteroid treatment are its systemic toxic effects and increased ocular pressure in long-term use, formation of subcapsular cataracts, risks of infection development, delayed wound healing, and systemic pathologies such as Cushing's syndrome.[67,68]

Azathioprine: Although it is commonly used as a systemic immunosuppressant in solid organ transplantations and bone marrow transplantation, its use in cornea transplantation is limited. It prevents proliferation by changing DNA and RNA structure in cells with rapid proliferation. They are mostly used together with corticosteroids; in

some cases, administration together with both corticosteroids and cyclosporine is available.[51]

Cyclosporin A (CsA) is a strong immunosuppressant effective against T cell function. Cyclosporin A is an 11 amino acid peptide isolated from the Tolypocladium inflatum fungus. It inhibits nuclear factor activation by playing a role in the production of intracellular protein of cyclophilin, which inhibits calcineurin enzyme activation.[67] By inhibiting the IL-2 pathway, it prevents the synthesis and release of proinflammatory cytokines including IL-2, IL-4, interferon gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α). Therefore, it blocks differentiation of cytotoxic and helper T cells.[69] On the other hand, it is thought that it does not affect B lymphocyte functions. It prevents lymphocyte migration to the graft and is relatively effective in preventing rejection. While it is used in autoimmune diseases and to prevent immune rejection after many solid organ transplantations, high doses or long-term oral or intravenous use is known to cause side effects such as hypertension, nephrotoxicity, hepatotoxicity, gastrointestinal toxicity, gingival hyperplasia, fainting, paresthesia, hypersensitivity to heat, elevated blood urea and creatinine values and rarely lymphoma.[56,70,71]

Cyclosporin A has been used in both oral and topical treatment in ophthalmology. Its topical treatment is especially preferred in order to avoid systemic side effects and to administer greater amount of drug to the eye and has been used by ophthalmologists for the treatment of various immune system diseases such as dry eye disease, atopic keratoconjunctivitis, Behçet's disease and ocular graft-versus-host disease.[95,96] Both topical and oral forms are used to prevent graft rejection in cornea transplantations. Although outcomes of its oral use seem to have reached a definite consensus, a study by Hill[55] showed that graft rejection occurred in 49% of the group who received CsA treatment after cornea transplantation compared to 73% in the group who was not administered CsA. Researchers have also proposed that longer periods of CsA use such as 12 months would be more effective rather than short periods such as 4-6 months and that length of use was positively associated with success rates in preventing graft rejection.[74] Gürelik et al.[75] conducted a study on CsA use in high-risk penetrant keratoplasty cases and reported that the use of CsA at the latest one week after the operation had a positive effect on the prognosis and that the use of CsA did not change the prognosis more than one month later. In contrast, other studies containing high-risk corneal transplantation groups revealed the effect of CsA was limited or even unsuccessful.[56,76] Shimazaki et al.[77] suggested that there was no significant difference between the treatment group and the control group in terms of the success of preventing graft rejection (rates 30-16%), even in the systemic use of CsA, and that graft rejection was also seen in patients who were initially successful with discontinuation of treatment. Another prospective randomized study by Reinhardt et al.[78] did not find a significant difference between oral CsA and oral mycophenolate mofetil after corneal transplantation. Inadequate success of oral CsA following corneal transplantation in the high-risk group was associated with inability of CsA to adequately reach aqueous humor despite high levels in serum and therefore lack of ability to maintain immune privilege.[77]

Tacrolimus (FK506) is an antibiotic isolated from *Streptomyces tsukubaensis*. It is a calcineurin inhibitor with effect similar to CsA. Due to its mechanism of action, it inhibits T cell activation and therefore T-lymphocute signal transmission and IL-2 transcription. In addition, it may also suppress release of TNF-α, IFN-γ, and other cytokines.[79] Although 25-100 times more effective than CsA, it has been reported to cause less side effects in areas such as inducing hypertension or causing dysfunction in lipid metabolism.[80]

Tacrolimus is used for treatment of immune ophthalmologic diseases such as keratoconjunctivitis, posterior uveitis, and chronic graft-versus-host disease and also as an immunosuppressant in high-risk corneal transplantation cases. Daily (2-12 mg) use of systemic Tacrolimus in the high-risk group was shown to significantly decrease graft rejection and increase graft vitality by 65% in high corneal transplantation.[81] Although daily dosage was found as 1-12 mg, there is no definite information about its ideal length of use.[82] Its most common side effect is hypertension (23%) and other side effects include headache, fatigue, and gastrointestinal disturbances.[81]

Yamazoe et al.[83] reported significantly less corneal rejection and longer graft vitality in patients administered 10-20 ng/mL dose Tacrolimus compared to patients undergoing CsA treatment following transplantation. In addition, patients treated with Tacrolimus had better tolerance compared to those treated with CsA.

Mycophenolate mofetil (MMF) works by inhibiting *de novo* synthesis of guanosine nucleotides and as a result, inhibiting T and B lymphocyte proliferation.[84] Its most common side effects include infections, anemia, leukopenia, and gastrointestinal disorders. In ophthalmology, it is used in the treatment of uveitis and high-risk corneal transplantations.[67,78] Reinhard et al.[85] conducted a prospective study on 86 transplant patients and reported that immune reaction did not develop in 89% of the group that was administered MMF compared to 67% in the control group. Both patient groups were applied topical corticosteroids for five months postoperatively. Mycophenolate mofetil is a relatively well tolerated immunosuppressant.

In terms of effectiveness and side effects, there are several studies that compare MMF and CsA. Reis et al.[86] conducted a prospective study on 41 patients and reported that there was no significant difference between MMF an CsA in terms of success in preventing tissue rejection (10% vs. 9.5%) and that side effects were observed in both groups at the end of the 10 month period. The authors also reported similar success levels between MMF and CsA used in combination with oral corticosteroids following acute tissue rejection in high-risk corneal transplantation cases.

In the study by Reinhard et al.,[78] cases were investigated postoperatively for three years and found no significant difference between MMF and CsA in terms of preventing graft rejection (74% vs. 69%). In a retrospective study of 417 high-risk transplantation patients by Birnbaum et al.,[67] MMF was significantly more successful in preventing graft rejection compared to CsA with a rate of 72 to 60%, but no significant difference was found in terms of graft vitality (87% vs. 77%). The same study reported that there were fewer side effects in patients treated with MMF compared to those treated with CsA.

Rapamycin (Sirolimus) is a bacterial macrolide isolated from *Streptomyces hygroscopicus* with antifungal and immunosuppressive properties. It consists of FK-binding protein complex (FKBP-12) and inhibits mTOR.[87] Despite its similar structure to tacrolimus, since it is not a calcineurin inhibitor, it has no nephrotoxic effect. It reduces T-lymphocyte activation induced by IL-2.[88] Rapamycin is also used in solid organ transplantations since it inhibits proliferation of growth factor originated fibroblasts, endothelial cells, and smooth muscles cells.

A prospective study conducted by Birnbaum et al.[89] compared the outcomes of Rapamycin or MMF treatment in the postoperative period of corneal transplantation. At the end of six months, it was reported that there were no immune reactions in either group, however, in the second postoperative year, only two patients developed reversible immune reaction in the Rapamycin group. Chatel and Larkin[90] reported various side effects including arterial thrombosis associated with rapamycin treatment following high risk corneal transplantation and they suggested that its use should be limited to “safe doses”.

NEW APPROACHES IN IMMUNE THERAPIES

These studies aim to develop new strategies to increase corneal vitality success by conducting experiments on animal models. One rat keratoplasty model succeeded in suppressing APCs with malononitrilamide (FK778) maturation.[91] Various experimental animal models have also shown that antibody-based treatment agents decreased or delayed tissue rejection in vascularized organ allografts.[92] There are ongoing studies on polyclonal, monoclonal, and recombinant antibodies or their combined versions to target IL-1 blockage, leukocyte function antigen-1 (LFA-1), VLA-1 (very late antigen-1), VLA-4, CD40-CD154 pathway, and immune cells such as CD28 and CD3, as well as the suppression of molecules effecting these targets. Experimental studies have shown Cytotoxic T Lymphocyte Antigen 4 (CTLA4-Ig) protein inhibits T cell activation and increased corneal allograft success.[93] Nevertheless, use of antibody therapies as immunosuppressants is limited in humans due to systemic side effects and risks of anti-idiotypic/anti-isotypic antibody development

in the transplant recipient.[94] In addition, due to the structure of the eye, injected antibodies have high likelihood of being transferred into the eye, which is why only a fraction of these studies have passed into their clinical phase.

Studies on cellular treatments for immunosuppression have demonstrated significantly high levels of Foxp3 levels in corneal allograft recipients who developed rejection, suggesting that Treg dysfunction plays a major role in allograft rejection.[95] Another study stated that administration of systemic IL-2 treatment in mice with high risk of rejection increased effectiveness and suppression level of Treg cells, reduced leukocyte infiltration in the graft, and improved corneal allograft vitality. It is hoped that new studies on this subject will contribute to further understanding of the function and mechanisms of Tregs to apply Treg-based treatments to increase success of corneal transplantation, even in corneas with vascularization or inflammation.[96]

Today, there are ongoing studies on new approaches to keep alloimmunity under control, focusing on morpholine oligonucleotides, cell-specific gene therapy, RNA interaction, anti-VEGF therapy, thrombogenic APC and IL-2 therapy.[97]

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