Immunosuppression therapy in retinal pigmented epithelial cell transplantation: A targeted literature review

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Background

Geographic atrophy (GA) and transplantation of retinal pigment epithelium (RPE) cells

- GA secondary to dry age-related macular degeneration (AMD) is characterized by atrophic lesions in the outer retina, RPE degeneration, and photoreceptor loss[1,2]
- GA is a progressive disease associated with irreversible loss of vision[1]. There is currently only one approved pharmacological treatment to prevent or slow down the progression[3]
- The replacement of lost/damaged cells by subretinal transplantation of RPE cells derived from human embryonic stem cells (hESCs) or human-induced pluripotent stem cells (hiPSCs) has the potential to rescue photoreceptor function and restore vision[4]

Ocular immune privilege and RPE transplantation

- The ocular microenvironment, delineated by the blood–retinal barrier (BRB) and the lack of direct lymphatic drainage, is considered an immune-privileged site under homeostatic conditions (**Figure 1A**)[1,2,4–8]
- The immune privilege of the eye can be compromised in dry AMD. The features implicated in compromising immune privilege due to inflammation are shown in **Figure 1B**[1,2,8,9]
- Similarly, physical damage to the outer BRB caused by surgical RPE transplantation (RPEt) also disrupts homeostasis and activates a local retinal immune response
- Consequently, immunosuppression may be needed to prevent the rejection of subretinally transplanted RPE cells[4]

Objectives

- A targeted literature review was conducted to summarize the recent literature on mechanisms of retinal immune reactions in AMD and immunosuppression use in RPEt based on *in vivo* studies and clinical studies

Methods

- Using Embase, 303 preclinical and clinical papers on RPEt published between 1999 and 2020 were identified
- Of these, 22 publications (11 preclinical and 11 clinical) were considered relevant for this review that included: (a) history of RPEt, (b) evidence of localized immune reactions post-RPEt, and (c) data on immunosuppressive agents used with RPEt

Author disclosures

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Figure 1. Retinal immune system. (A) Immune privilege during homeostasis in normal retina[1, 2, 6–8] and (B) immune privilege compromised in retinas with dry AMD[1, 2, 8, 9]

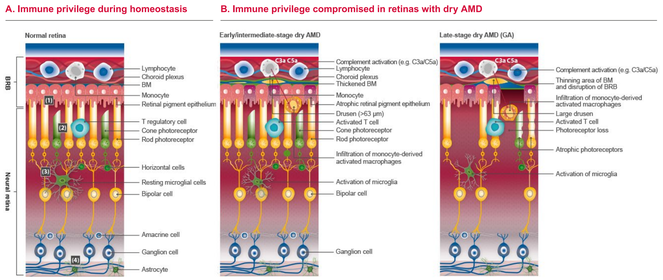
Mechanisms conferring immune privilege include:

(1) **Physical blood–retinal barrier** – which is partly composed of Bruch's membrane and tight junctions between RPE cells, acts as a barrier and entry of circulating immune cells inside the retina and prevents antigens from escaping the retina[1,2,8]

(2) **Immunosuppressive nature of the RPE and retinal neurons** – reduces the activation of T cells, further inhibiting the production of cytokines and recruitment of other immune cells, such as macrophages and microglial cells[6–8]

(3) **Microglial cells** – are retinal immune cells found in a resting state in the healthy retina[2,8]

(4) **Retinal microenvironment** – composed of Müller glial cells, astrocytes, or RPE cells promotes cell survival[8]



AMD, age-related macular degeneration; BM, Bruch's membrane; BRB, blood–retinal barrier; C, complement; GA, geographic atrophy; RPE, retinal pigmented epithelium

Results

Mechanisms of retinal immune reactions following RPEt

- Under transplantation conditions, *in vivo* studies[10–15] show that the immune privilege of the subretinal space (SRS) is compromised, with both activated microglia and macrophages prevalent in inflammatory infiltrate around rejected RPE transplants (**Table 1**)

Table 1. Evidence of localized immune reactions following RPEt in non-immunosuppressed animal models (*in vivo* studies)

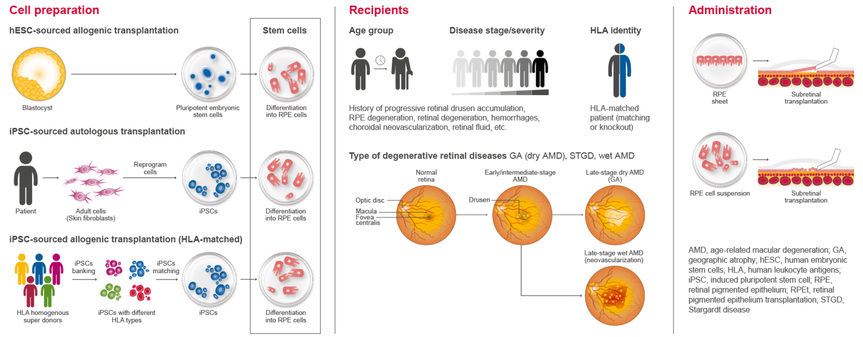
First author, year	Type and number of cells transplanted	Animal models	Follow-up period	Evidence of immune reaction
Kamao, 2014[10]	Autogenic and allogeneic miPSC-RPE: 50k cells per eye	Cynomolgus monkeys	1 year	Formation of whitish fibrous tissues, leakage of fluorescein on FA, and hyperreflective material under the retina or retinal edema on OCT
Sohn, 2015[11]	Allogeneic iPSC-GFP-RPE: 250k cells per eye	WT Yucatan miniature swine	3 weeks	Elevated IL-12 levels and presence of CD45+ leukocytes in the vitreous. Activation of macrophages and T cells in the subretinal space
Kennelly, 2017[12]	Allogeneic DH01 RPE: 100k cells per eye	Male C57BL/6 mice	28 days	Infiltrating cells expressing markers of innate immunity Macrophages and neutrophils co-label with and engulf graft cells
Zhu, 2017[13]	Allogeneic hESC-RPE: 500–750k cells per eye	IL2rγ-/- and WT mice	3 months	CD11c-expressing dendritic cells, CD49b-expressing NK cells, mature T cells (CD4+ and CD8+), and activated macrophages and microglia were absent in the immunocompromised model but present in the WT non-immunosuppressed host
McGill, 2018[14]	Allogeneic iPSC-GFP-RPE: 500k cells per eye	Adult female rhesus macaques (Macaca mulatta)	7 weeks	A strong inflammatory response in a highly localized and aggressive manner possibly mediated by microglia/macrophages and T cells
Xian, 2019[15]	Retinal organoid sheet made of two hiPSC lines	Male rhesus macaques	8 weeks	Presence of activated microglia (CD68-positive), Müller cells, and fibrous nodule-like structures

FA, fluorescein angiography; hESC-RPE, human embryonic stem cell-derived RPE; hiPSC, human-induced pluripotent stem cells; IL-12, Interleukin 12; iPSC-GFP-RPE, induced pluripotent stem cells-retinal pigment epithelium-green fluorescent protein; NK, natural killer; miPSC-RPE, cynomolgus monkey (Macaca fascicularis) somatic cells induced pluripotent stem cells-retinal pigment epithelium; OCT, optical coherence tomography; RPEt, retinal pigment epithelium transplantation; WT, wild type.

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Figure 2. Key considerations for determining RPEt immunosuppression requirements



AMD, age-related macular degeneration; GA, geographic atrophy; hESC, human embryonic stem cells; HLA, human leukocyte antigens; iPSC, induced pluripotent stem cell; RPE, retinal pigmented epithelium; RPEt, retinal pigmented epithelium transplantation; STGD, Stargardt disease

Conclusions

This literature review discusses the immune reaction and breach of SRS immune privilege following RPE transplantation and highlights the need for systematic immunosuppression to ensure transplant survival

Until further clinical research identifies how RPE transplantation triggers an immune response, how RPE immune rejection is mediated, and which patients are likely to be affected, low-dose perioperative immunosuppression is likely warranted to optimize clinical outcomes

Determining RPEt immunosuppression requirements

- Key considerations for determining RPEt immunosuppression requirements are shown in **Figure 2**
- Autologous RPEt does not need the use of immunosuppressants, but requires time to prepare cell suspension or graft[20]
- Also, autologous cells derived from hiPSCs may carry patients' own genetic vulnerabilities contributing to disease processes[21]
- A review of 13 preclinical animal studies in which recipients were administered immunosuppressive drugs reported no immune rejection, suggesting that immunosuppressants are effective for the prevention of immune rejection of grafts within the SRS[4]

Immunosuppression used for RPEt in clinical trials

- Eight of 11 clinical studies reported delivery of allogenic RPE cells derived from stem cells as suspensions or monolayers along with immunosuppression in patients with GA (dry AMD), Stargardt disease, and wet AMD (**Table 2**)
- In all eight clinical trials, the persistence of transplanted cells in the SRS was observed when perioperative immunosuppression was employed[20,22–34]
- Low-dose tacrolimus was the most used immunosuppressant, often in combination with mycophenolate mofetil
- Immunosuppression was often prescribed up to 1 week prior to the transplant and typically continued up to 6–13 weeks (depending on agent used), and up to 1 year in two trials[25, 32]

Table 2. Clinical trials of RPEt in patients with GA (dry AMD), STGD, and wet AMD

First author, year	Trial phase (country); trial identifier	Type and number of cells transplanted and transplant delivery forms (location)	Targeted disease and patient characteristics	Follow-up period	Immunosuppression regimen	Structural changes in the eye following RPEt	Functional improvement following RPEt	Safety (adverse events [AEs])
Banin, 2018[22] and Banin, 2019[23]	Phase 1/2a (USA and Israel); NCT02286089	OpRegen: Allogenic clinical xeno-free GMP-grade hESCs: 50k, 100k or 200k cells per eye Cell suspension (SRS)	GA (dry AMD) N=24 Age: ≥50 years	2 years	Systemic immunosuppression (not specified) administered from 1 week prior to transplantation until 1 year after.	FAF, CFP, and OCT imaging indicated potential signs of long-term engraftment and survival of transplanted cells. Subretinal pigmentation was observed in majority of patients, often accompanied by hypo-hyper-fluorescent spots on FAF imaging and irregular reflectance areas on OCT scans. Signs of immune rejection were limited or not present.	BCVA improved in one patient and remained the same in other treated eyes.	No unexpected ocular treatment emergent AEs/SAEs. Observed surgery-related AEs included: conjunctival hemorrhage, worsening of cataract and ERM formation. ERM did not require surgical intervention in any of the affected patients.
Kashani, 2018,[24] Kashani, 2021[25] and Kashani, 2022[26]	Phase 1/2a (USA); NCT02590692	Allogenic CPCB-RPE1 implant (GMP): 100k cells Monolayer of hESC-RPE on an ultrathin, synthetic parylene substrate (SRS)	GA (dry AMD) N=5 Age: 69–85 years	1- and 2-year follow-up	Tacrolimus (0.075 mg/kg per day to a target trough range of 3–10 ng/mL) was administered. Tacrolimus dosing was tapered by successive 50% increments starting on day 42 until it was stopped on day 60.	No signs of graft rejection in any patient. Postoperative OCT confirmed the subretinal location of the implant in all patients up to and including day 365 within and outside the area of GA. Functional RPE cells survived within the area of GA in one patient at least 2 years after implantation.	BCVA improved by >5 letters in 27% of implanted patients.	No unexpected SAEs related to the implant, surgical procedure, or immunosuppression through 1-year post-implantation. One patient was diagnosed with pneumonia requiring hospitalization during the period of immunosuppression taper postoperatively. There was no evidence that the pneumonia was associated with the surgery or implant. One patient passed away from pneumonia after ~2 years of implantation.
Song, 2015[27]	Phase 1/2 (South Korea); NCT01625559 (STGD; N=3), NCT01674829 (GA; N=12)	Allogenic MA09-hESC-RPE: 50k cells per eye Cell suspension (SRS)	STGD and GA (dry AMD) N=4 (STGD, n=2; and GA, n=2) Age: 40–79 years	1 year	Low-dose tacrolimus (target trough level of 3–7 ng/mL) and mycophenolate mofetil (1.0–1.5 g per day orally) were administered. Both started 1 week prior surgery and continued for 7 weeks until post-operative 6 weeks. Mycophenolate mofetil continued for an additional 7 weeks after tacrolimus discontinuation.	No signs of clinically significant intraocular inflammation and immune rejection including fluid collection, edema, fibrous membrane formation, persistent leakage on fluorescein angiography, or graying or loss of pigmentation of the graft.	VA improved by 9 to 19 letters in three patients and remained stable in one patient.	No serious systemic or ocular adverse events occurred. Moderate AEs related to immunosuppression–creatinine and potassium level elevation (n=1, dry AMD), pneumonia (n=1, dry AMD). These AEs subsided after stopping immunosuppression 4 weeks after the transplantation.
Schwartz, 2012[28] and Schwartz, 2015[29]	Phase 1/2 (USA); NCT01345006 STGD), NCT01344993 (GA)	Allogenic MA09-hESC-RPE: 50k, 100k, or 150k cells per eye Cell suspension (SRS)	STGD and GA (dry AMD) N=18 (STGD, n=9; GA, n=9) Age: 20–71 (STGD) and 70–88 (GA) years	Median 22 months (n=4); 12-36 months (n=12); >36 months (n=2)	Tacrolimus (target blood concentrations 3–7 ng/mL) and mycophenolate mofetil (0.25–2.00 g/day) were administered 1 week before surgery and continued for 6 weeks. Tacrolimus discontinued at week 6; mycophenolate mofetil discontinued at week 12.	No signs of immune rejection or acute graft rejection were observed. The fundus photograph and OCT showed patches of increasing subretinal pigmentation in 72% of patients consistent with survival of transplanted cells.	BCVA improved in ten patients, remained stable in seven patients and decreased by >10 letters in one eye.	No serious adverse safety concerns related to the transplanted cells. Complications were associated with systemic immunosuppression in some of the older patients with AMD. In two patients (n=1, AMD; n=1 STGD) immunosuppression was discontinued after 12–15 days due to AEs.
Mehat, 2018[30] and Mehat, 2019[31]	Phase 1/2 (UK); NCT01469832	Allogenic MA09-hESC-RPE: 50k, 100k or 200k cells per eye Cell suspension (SRS)	STGD N=12 Age: 34–53 years	1- and 5-year follow-up	Low-dose tacrolimus (0.1 mg/kg twice/day) was given from 1 week before transplantation until 6 weeks after transplantation (titrated to achieve trough serum levels of 3–7 ng/ml). Mycophenolate mofetil started on the day of surgery in an escalating fashion (0.25–1.00 g twice/day) until it was discontinued after 12 weeks.	No signs of vitreitis, retinitis, retinal exudates, retinal edema, or vascular hyperpermeability indicating graft rejection. Intraretinal fluid was not evident on OCT, and no change of clinical concern was apparent on fundus fluorescein angiography. Subretinal hyperpigmentation suggesting cell engraftment at the site of transplantation was observed in all patients.	BCVA showed borderline improvement in four patients which were unsustained or were same as the untreated eye.	AEs related to immunosuppressive medications occurred during the period of their administration but had no impact subsequently.
Sung, 2021[32]	Phase 1 (South Korea); NCT01625559	Allogenic MA09-hESC-RPE: 50k cells per eye Cell suspension (SRS)	STGD N=3 Age: 40–45 years	3 years	Low-dose tacrolimus (initial dose, 0.1 mg/kg daily, adjusted to achieve a target through level of 3–7 ng/mL) and mycophenolate mofetil (1.0 g per day orally) were administered and continued for 6 weeks up to 1 year after transplantation.	No observed signs of inflammation or immune rejection , except to mild vascular leakage on fluorescein angiography from week 7 until 9 months after transplantation in one patient. No obvious AEs were seen using fundus photography, OCT or autofluorescence imaging.	One patient showed BCVA improvement, while the other patients had stable BCVA during the 3-year follow-up period.	No SAEs were observed related to transplanted cells.
da Cruz, 2018[20]	Phase 1 (UK); NCT01691261	Allogenic hESC-RPE (GMP): 100–120k cells per eye Sheet/patch (SRS)	Exudative AMD (wet AMD) N=2 Age: 60–84 years	1 year	Local immunosuppression: fluocinolone (0.19 or 0.59 mg) implant post-surgery and oral prednisolone (1 mg/kg) up to 60 mg maximum was used 2–4 days pre-surgery and continued for at least 2 weeks followed by a tailing off over at least 1 further week.	Biomicroscopy and OCT confirmed the successful delivery and survival of graft. Graft survived at least 1 year with only local immunosuppression.	BCVA improved with increased VA and reading speed which was sustained or improved over 1-year post-transplant.	Three SAEs were reported related to immunosuppression. The first was exposure of the suture of the fluocinolone implant in patient 1, which required conjunctival revision surgery. Two SAEs (worsening of diabetes following oral prednisolone and a retinal detachment) were both observed in patient 2.
Liu, 2018[34] and Li, 2021[33]	Phase 1 (China); NCT02749734 (ChiCTR-OCB-15006423)	Allogenic O-CTS-hESC-2-RPE: 100k cells per eye Cell suspension (SRS)	Exudative AMD (wet AMD) N=3 Age: 60–71 years	1- and 5-year follow-up	Tacrolimus (target 10 ng/mL) was given one week before surgery, mycophenolate mofetil (200 mg, twice/day), and prednisone (30 mg, once/day) were administered 1 week before surgery and continued for 1-month post-surgery. All drug dosages were decreased and eventually discontinued 12 weeks post-transplant.	There were no signs of graft rejection (cystoid macular edema, or retinal neovascularization). Small high reflective foci in the RPE layer were displayed after the transplantation and maintained until the last visit. Three categories of patients, classified based on autofluorescence, exhibited distinctive patterns of morphological and functional change.	All seven patients had transiently increased or stable visual function 1–4 months after transplantation. At last follow-up visit, 2/7 patients showed visual function loss and 1/7 stable VA relative to baseline.	No patients experienced any notable immune responses or inflammation due to systemic immunosuppressive drugs. At 5-year follow-up, there were no SAEs recorded such as abnormal cell proliferation, tumor genesis, immune rejection or retinal detachment.

AMD, age-related macular degeneration; BCVA, best corrected visual acuity; CFP, colour fundus photograph; CPCB, California Project to Cure Blindness; ERM, epiretinal membrane formation; FAF, fundus autofluorescence; GA, geographic atrophy; GMP, Good Manufacturing Practice; hESC, human embryonic stem cell; OCT, optical coherence tomography; RPE, retinal pigment epithelial; SAE, serious adverse events; SRS, subretinal space; STGD, Stargardt disease; VA, visual acuity