Strategies to Induce Tolerance or Overcome Immune Suppression

Immune Privilege Niche - Subretinal Space

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Immune Privilege

• Immune privilege refers to the fact that foreign tissue grafts placed in the immune privileged site are tolerated and survive for prolonged, often indefinite, intervals, whereas placement of such grafts at conventional body sites leads to acute, irreversible immune rejection.

• Sites include the eye, placenta and testes.

• Immune privilege has evolved from a concept of passive physical barriers to more of an active process.

• Concept of immune privileged site and immune privileged tissue.
Ocular Immune Privilege

Ocular immune privilege was demonstrated by Sir Peter Medawar in 1948 – skin allografts survive when implanted into the anterior chamber of the eye.

In 1993 Streilein showed that the subretinal space was also an immune privileged site for allografts.

Subsequently the RPE has been shown to be a major source of immune privilege in the subretinal space and also an immune privileged tissue.
The subretinal space is a potential space in the living eye but the space may be utilized for a graft by injecting fluid between the photoreceptors and RPE to form a subretinal bleb.
15/19 pancreatic islet cell allografts survive in subretinal space of outbred SD rats – d60

No immune suppression.

Immunoperoxidase stain for insulin.

Survival of Allografted Pancreatic Islets in Subretinal Space in Rats
Physical Factors Promoting Immune Privilege in the Subretinal Space

Lack of lymphatics in retina/RPE (although present in choroid)

Outer blood retina barrier mediated by tight junctions between RPE
RPE express anti-inflammatory factors that promote immune privilege

**Soluble Factors:**
- TGF-beta
- Pigment Epithelium Derived Factor
- AlphaB Crystallin
- Soluble TNF-R

**Cell Surface:**
- TGF-beta
- CD95 (FAS) ligand
- CD59
- CD46

Apical microvilli of RPE projecting into subretinal space
Age-Related Macular Degeneration

Atrophic AMD is characterized by dysfunction and death of RPE in the macula. Confluent areas of RPE loss are termed geographic atrophy.

There is no current effective therapy for atrophic AMD.

HESC represent of source of RPE for cellular therapy of AMD

A clinical trial for hESC-RPE cell suspensions for advanced dry AMD is ongoing (NCT01344993; Advanced Cell Technology)
hESC spontaneously differentiate into pigmented RPE in culture

Pigmented colonies can be picked after 6-8 weeks in culture and can be expanded to pure cultures of RPE.
HLA Class I Expression on HESC-RPE after treatment with Interferon-γ
HLA Class I Expression on HESC-RPE after treatment with Interferon-γ
Our Approach: HESC (H9)-Derived RPE are Grown as a polarized monolayer on Non-Biodegradable Parylene Inserts

Insert with no cells

Insert with H9-derived RPE 4 weeks after seeding
Human Embryonic Stem Cells

Geographic Atrophy
hESC-RPE implanted into the subretinal space of retinal degeneration (RCS) rats survive as an intact monolayer.

The placement of the implant in the subretinal space without damage to Bruch’s membrane and choroid is essential for survival of grafted cells. Grafts with perfect placement may survive without immunosuppression. Current protocols include systemic immunosuppression with cyclosporin and short term therapy with systemic dexamethasone.
hESC-RPE implanted into the subretinal space of RCS rats survive without an inflammatory response, and integrate into the retina where they rescue host photoreceptors.
HLA Expression after Subretinal Implant of HESC-RPE into Rat

**Class I**

![Images for Class I](image1.png)

**Class II**

![Images for Class II](image2.png)
Current Strategies for Immune Suppression in hESC-RPE cell Therapy

1. **ACT Trial: NCT01344993**
   Short term systemic immunosuppression with tacrolimus beginning prior to treatment with mycophenolate mofetil (MMF) added and continued (personal communication with ACT)

2. **London Project to Cure Blindness:**
   Systemic prednisolone (oral) beginning 1 week prior to treatment (taper over one month) with local triamcinolone (intravitreal and scleral) for 3-6 months coverage (personal communication with Pete Coffey).
Current Strategies for Immune Suppression in hESC-RPE cell Therapy

3. Other possibilities:
   Local immunosuppression with Ozurdex (Allergan Inc.) dexamethasone drug delivery system. This biodegradable intravitreal implant provides sustained delivery to the retina and vitreous and is approved by the FDA for treatment of macular edema associated with retinal vein occlusion and noninfectious posterior uveitis.

Major Considerations:
1. Local vs systemic immunosuppression
2. Timing and length of immunosuppression
3. Methods to detect rejection of the transplanted cells
The California Project to Cure Blindness

- USC Doheny Eye Institute (Mark Humayun, PI; David Hinton Co-PI; Vas Sadda, Biju Thomas, Martin Pera)

- UCSB Macular Degeneration and Stem Cell Centers (Dennis Clegg, Co-PI; Lincoln Johnson)

- UCL London Project to Cure Blindness (Pete Coffey, Partner PI funded by MRC)

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