Characterization of Immune Cell Populations in a Murine Model of Pediatric Penetrating Keratoplasty

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Introduction

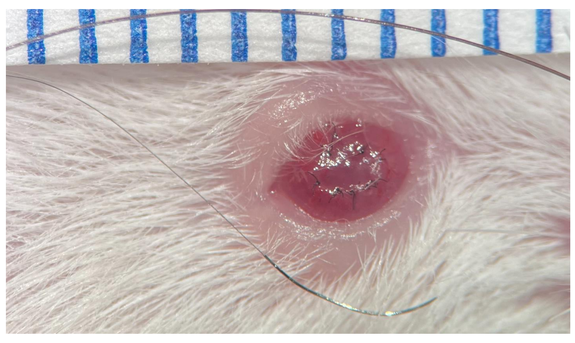
- Clinical and laboratory data suggest lower corneal transplant survival in pediatric penetrating keratoplasty (PK), but the underlying immunologic mechanisms are not known.[1]
- The murine corneal transplantation model allows investigation of mechanisms of graft rejection.[2]
- Corneal immune cells are key responders to inflammation, shaping innate and adaptive immunity of the cornea.[3]
- Flow cytometry enables precise characterization of resident and infiltrating immune cells in corneal transplantation.[4]

Purpose

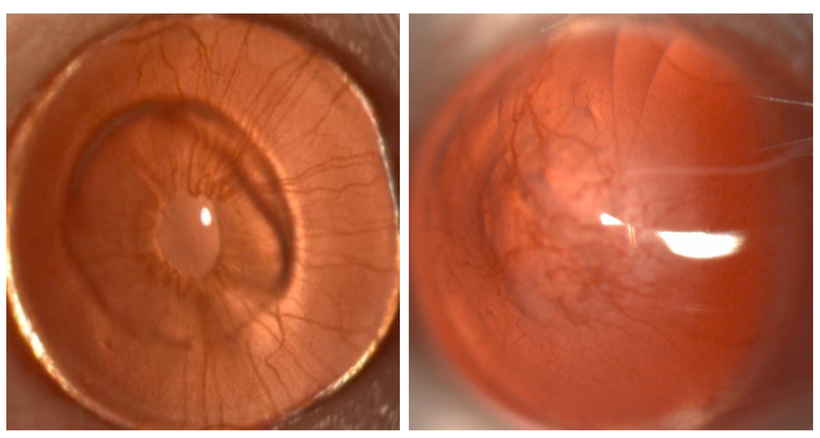
- The purpose of this study was to quantify antigen-presenting cell and graft-infiltrating immune cell frequencies in a murine model of pediatric PK.

Methods

- Corneal buttons were harvested from 10-week-old C57BL/6 mice and transplanted into the eyes of 3.5-week-old (young) or 10-week-old (adult) BALB/c male mice.



- Grafts were monitored for eight weeks using slit lamp evaluation to assess corneal opacity.



- At 14 days post transplantation, transplant recipients were euthanized and cell populations analyzed by flow cytometry.
- The frequencies of CD11c+ antigen-presenting cells (APCs) were assessed in the ipsilateral cervical and submandibular lymph nodes.[5]
- Corneal grafts were also collected and the frequency of CD45+ leukocytes was quantified, along with the frequency of CD45+IFNγ+ (Th1), CD45+IL-4+ (Th2), CD45+IL-17 (Th17) and CD45+CD49b+ (NK) cells.

Results

Figure 1: Allogeneic Graft Survival

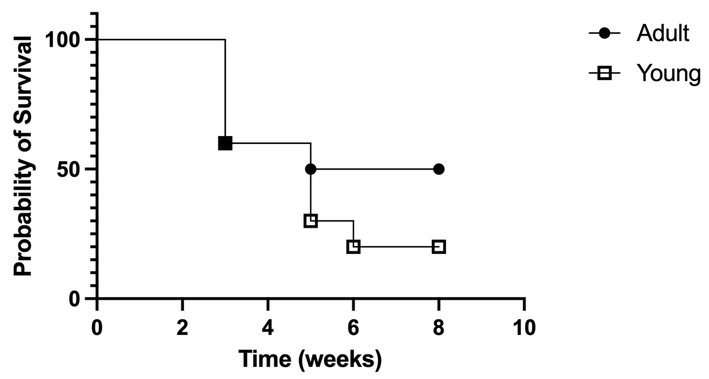


Figure 1:Trend towards lower eight-week graft survival in young mice as compared to adult mice (20% vs. 50%, p=0.276).

Figure 2: Flowcytometric analysis of Corneal population

A

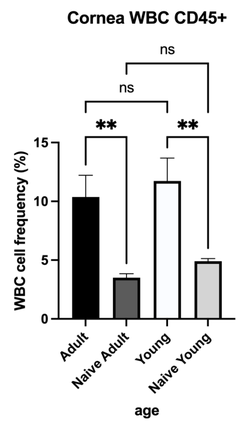


Figure 2A: At 14 days post-transplantation, there was a statistically significant increase in CD45+ leukocytes in both Adult and Young mice compared to age matched controls.

B

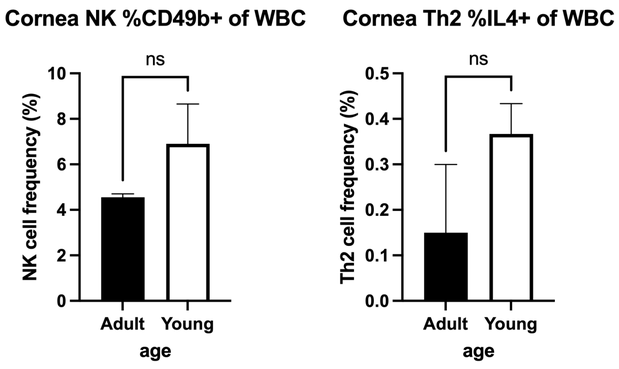


Figure 2B: Young mice exhibited trends toward higher percentages of graft infiltrating NK and Th2 cells compared to adult mice: NK cells: 6.9±3.03% vs. 4.55±0.21% (p=0.38, % of CD45+ cells); Th2 cells: 0.37±0.11% vs. 0.15±0.21% (p=0.22)

C

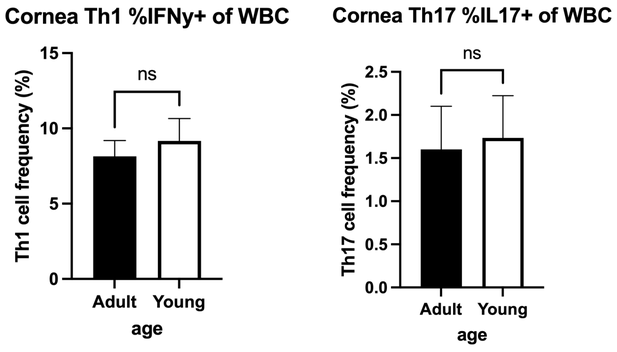


Figure 2C: Similar frequencies of Th1 (9.17±2.59% vs 8.15±1.49%, p=0.66) and Th17 (1.73±0.85% vs. 1.6±0.7%, p=0.86) cells were observed between groups.

D

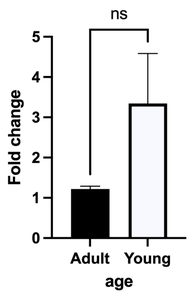


Figure 2D: There was a 3.3- fold increase in NK cells in young transplant recipient mice and a 1.22-fold increase in NK cells in adult transplant recipient mice compared to age-matched controls (p=0.28).

Figure 3: Flowcytometric analysis of Draining lymph node population

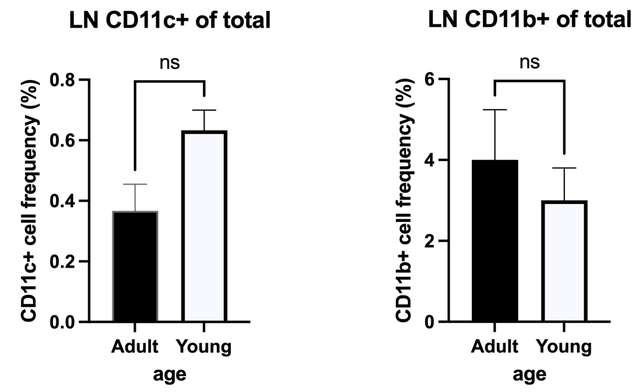


Figure 3: Trend toward a higher frequency of CD11c+ APCs (0.63 ± 0.11% in young mice vs. 0.37 ± 0.15% in adults, p = 0.078) and a lower frequency of CD11b+ APCs (3.0 ± 1.38% in young mice vs. 4.0 ± 2.15% in adults, p = 0.268) in the draining lymph nodes, as % of total cells.

Conclusion

- In a murine model of PK, we observed trends toward lower corneal transplant survival in young mice as compared to adult mice
- There were trends towards higher frequencies of APCs and immune cells, in particular NK cells, in young mice.
- These findings recapitulate the poor outcomes seen clinically in pediatric PK, and suggest further investigation of both the afferent and efferent arms of the alloimmune response in young mice to better understand the underlying immune mechanisms leading to graft failure in pediatric PK
- Further studies will focus on functionality and specifically the potential role of NK cells in corneal transplant rejection

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