Corneal Inflammation After Miniature Keratoprosthesis Implantation

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PURPOSE. To compare corneal inflammation after syngeneic and allogeneic penetrating keratoplasty (PK) with miniature Keratoprosthesis (m-KPro) implantation in mice.

METHODS. BALB/C (syngeneic) or C57BL/6 (allogeneic) corneas were transplanted onto BALB/C host beds as part of PK or m-KPro implantation. Corneal inflammation was assessed by determining the frequencies of CD45⁺ leukocytes, CD4⁺ T cells, CD11b⁺ cells, and Gr-1⁺ granulocytes/monocytes by flow cytometry at 2, 4, and 8 weeks post transplantation. In addition, expression levels of the proinflammatory cytokines TNF-α and IL-1β were analyzed using real-time qPCR at 8 weeks post transplantation.

RESULTS. Cell frequencies in the syngeneic (syn) and allogeneic (allo) m-KPro groups were higher compared with the syngeneic and allogeneic PK groups, respectively, at all time points. However, after week 4, frequencies of all analyzed immune cells were higher in the alloPK group as compared with synPK group. At 8 weeks, the expression of TNF-α was higher in synKPro, alloPK, and alloKPro groups compared with the naïve and synPK groups. The expression of IL-1β was significantly higher in both KPro groups as compared with PK groups.

CONCLUSIONS. Although the m-KPro device augments the inflammatory response in the cornea after its implantation, allogenicity (of the carrier tissue) is also a significant contributor to corneal inflammation. These data suggest that using syngeneic or decellularized corneal tissue as a Boston-KPro carrier could reduce the postoperative inflammation response.

Keywords: Boston Keratoprosthesis, penetrating keratoplasty, cornea inflammation, syngeneic, allogeneic

Penetrating keratoplasty (PK) is the mainstay of sight restoration in most severe corneal diseases,1–3 and possesses a good survival rate in low-risk transplants, but has a significantly higher rejection rate in high-risk grafts performed in inflamed host beds.4 When grafts fail, due to rejection or other causes, regrafting yields a poor prognosis for visual rehabilitation, with a survival rate of under 10% after the fourth regraft.5–11 A promising and well-established alternative to regrafting and high-risk transplantation is the Boston Keratoprosthesis (B-KPro), an artificial cornea typically used after multiple PK failures or when PK is determined to be unlikely to succeed. Retention for B-KPro is 80% to 100% at 1 to 2 years12–16 and 2/3 at 7 years, with 50% of patients retaining useful vision.17 Today, glaucoma, optic neuropathy, epiretinal membrane, macular edema, and retinal detachment remain threats to good long-term visual outcomes after B-KPro surgery18–20 and it has been hypothesized that chronic postoperative corneal inflammation may be a contributing factor to these complications.

The immunoinflammatory response after syngeneic and allogeneic PK has been investigated before, showing an early innate immune response that is present in both syngeneic and allogeneic PK and is mostly induced by the surgical procedure, as well as a late immune and inflammatory response seen only in allogeneic PK.21–23 We have recently established a novel murine miniature-KPro (m-KPro) model well suited to investigate corneal inflammation.24 We have used this model in the present study to compare the magnitude and kinetics of the corneal immune response following both syngeneic and allogeneic PK and B-KPro implantation. For the first time, we shed light on potential sources of corneal inflammation after KPro implantation, specifically the relative contribution of an allogeneic graft (or carrier tissue) versus the presence of the KPro device itself.

METHODS

Animals

A total of 306 male mice (8- to 10-weeks old; Jackson Laboratory, Bar Harbor, ME, USA; 162 BALB/C used as recipients and 72 as donors, and 72 C57BL/6 used as donors) were used in this study. They were housed in a climate-controlled animal facility at the Schepens Eye Research Institute (Massachusetts Eye and Ear, Harvard Medical School, Boston, MA, USA) and kept under cyclic light conditions (12 hours on/off). All animal experiments were approved by the Institutional Animal Care and Use Committee, and were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

For this study mice were divided into the following groups: (1) naïve, (2) syngeneic penetrating keratoplasty (SynPK; BALB/
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Cortico stereoid was administered once a day for 2 weeks and then every other day for 2 weeks. The tarsorrhaphy was removed 48 hours after the surgery, and corneal sutures were removed 1 week following surgery. As per standard institutional animal care and use protocols, 0.1 mg/kg Buprenorphine (Reckitt Benckiser Healthcare [UK] Ltd., Hull, England) was applied subcutaneously after the procedure and every 12 hours for 48 hours postoperatively.

The mice were euthanized 2, 4, and 8 weeks after the procedure for further analyses.

Flow Cytometry

Grants and host corneal beds and corneas from naïve mice were collected 2, 4, and 8 weeks post transplantation and digested with DNase I (0.2 mg/mL; Roche, Basel, Switzerland) and collagenase D (0.4 mg/mL; Roche) to create single cell suspensions. Samples from individual animals were prepared and analyzed separately. All cell suspensions were incubated with an Fc-blocking agent (R&D Systems, Minneapolis, MN, USA) before staining. Corneal cells were stained with PE/Cy5-conjugated anti-CD45 (BioLegend, San Diego, CA, USA), Alexa Fluor 488-conjugated anti-CD11b (BD Pharmingen, San Jose, CA, USA), Brilliant Violet 421-conjugated anti-CD4 (BioLegend, San Diego, CA, USA), and PE-conjugated anti-Gr-1 (BioLegend) antibodies. Appropriate isotype matched control antibodies were used in all experiments. Cells were analyzed using a LSRll flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and Summit v4.3 Software (DAKO Colorado, Inc., Fort Collins, CO, USA). The analysis for each time point was performed twice with n = 5 eyes/group.

Reverse Transcription and Real-Time PCR

Corneas were harvested at 8 weeks post surgery. Ribonucleic acid was isolated with the RNeasy Micro Kit (Qiagen, Valencia, CA, USA) and reverse transcribed using Superscript III Kit (Invitrogen, Grand Island, NY, USA). Real-time qPCR was performed using Taqman Universal PCR Mastermix and preformulated primers for murine glyceraldehyde 3-phosphate dehydrogenase (GAPDH), TNF-α, and IL-1β (Applied Biosystems, Foster City, CA, USA). The results were normalized to the expression level in naïve mice and analyzed by the comparative threshold cycle method, using GAPDH as an internal control. Real-time PCR was repeated four times for each cytokine. Corneas of three mice were pooled and duplicates for each group were analyzed.

Statistics

The two-tailed ANOVA test was employed to analyze flow cytometry data for all time points and one-tailed ANOVA test was used to analyze real-time qPCR data at week 8. P less than or equal to 0.05 was considered statistically significant. Results are presented as the mean ± SEM.

Results

Cellular Corneal Inflammation After Syngeneic or Allogeneic PK and m-KPro Implantation

To analyze the magnitude and kinetics of the corneal immune response after PK and m-KPro surgery, we analyzed the frequencies of CD45+ leukocytes, CD4+ T cells, CD11b+ cells,
and Gr-1⁺ granulocytes/monocytes in the cornea of mice after SynPK, AlloPK, SynKPro, AlloKPro, and naïve mice at 2, 4, and 8 weeks post transplantation.

At week 2, we found higher frequencies of all analyzed immune cells in the syngeneic and allogeneic m-KPro groups as compared with the syngeneic and allogeneic PK groups, respectively (Figs. 1A–D). At week 4, cornea infiltration of CD45⁺ and CD4⁺ cells significantly increased in allogeneic PK and m-KPro groups as compared with syngeneic PK and m-KPro groups, respectively, and as well as when compared with the allogeneic PK and m-KPro group at 2 weeks, respectively (Figs. 1A, 1B). Additionally, CD11b⁺ and Gr1⁺ cells showed a tendency to increase in allogeneic PK and m-KPro groups compared with syngeneic PK and m-KPro groups, respectively. Both cell types also increased in allogeneic PK and m-KPro groups at week 4 compared with week 2, respectively, although the differences were not significant (Figs. 1C, 1D). At week 8, frequencies of most analyzed immune cells decreased or remained similar to week 4, except CD45⁺ and CD4⁺ cells increased in the SynKPro group and AlloKPro group, respectively (Figs. 1A–D). Based on flow cytometry data, chronic inflammation 8 weeks after surgery was enhanced in the AlloPK group compared with the SynKPro group.

**Inflammatory Cytokine Expression After Syngeneic or Allogeneic PK and m-KPro Implantation**

To evaluate inflammation at the cytokine level following PK and m-KPro, we quantified mRNA expression of the proinflammatory cytokines TNF-α and IL-1β in the cornea at week 8 post surgery. We detected increased TNF-α expression in SynKPro, AlloPK, and AlloKPro groups compared with naïve and SynPK group (Fig. 2A). The expression of IL-1β was
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DISCUSSION

In a recently published retrospective study about glaucoma in B-KPro patients, we reported that 7% of the eyes with normal or low IOP had progressive cupping of the optic nerve. The etiology of the optic nerve damage in those eyes is unclear. Likewise, the mechanisms inducing other chronic complications after B-KPro surgery such as optic neuropathy, epiretinal membrane, macular edema, and retinal detachment remain unknown. However, it is known that corneal inflammation can induce posterior segment damage, for example, massive apoptosis of the retinal ganglion cells can occur within 24 hours after alkali burns of the cornea. This is most likely caused by posterior diffusion of inflammatory cytokines from the injured anterior segment, indicating that corneal inflammation can have wide-reaching effects. With this in mind, we sought to analyze the extent and kinetics of the corneal inflammatory response following PK and m-KPro surgery, and perhaps shed light on a mechanism by which postoperative complications arise after B-KPro implantation. Comparing corneal inflammation after syngeneic and allogeneic PK and m-KPro implantation, we found that the use of an allogeneic carrier graft contributes to chronic inflammation to a greater degree than the presence of the KPro device itself.

Using flow cytometry, we assessed the frequencies of corneal immune cells at 2, 4, and 8 weeks after surgery and observed similar kinetics for CD45 leukocytes, CD4 T cells, CD11b cells, and Gr-1 granulocytes/monocytes. The frequencies of all investigated immune cells in the cornea were higher after syngeneic and allogeneic m-KPro implantation compared with syngeneic and allogeneic PK, respectively, at 2 weeks post transplantation. At week 4, the frequencies of all observed immune cells increased after allogeneic PK and m-KPro, and were higher in comparison to syngeneic PK and m-KPro implantation, respectively. Finally, at week 8, the highest frequencies of all analyzed inflammatory cells were found after AlloKPro implantation, followed by AlloPK, SynKPro, and SynPK groups.

Given our data on cellular corneal inflammation, we conclude that the KPro device contributes more to acute inflammation than chronic inflammation. Moreover, at weeks 4 and 8, we found immune cell infiltration to be higher after AlloPK than after SynPK, suggesting that in the long-term, allogeneic immunity contributes to corneal inflammation to a greater extent than the KPro device. This suggests using patient’s own cornea as a carrier for the B-KPro if possible. When an auto graft carrier is not an option, methods to reduce corneal inflammation, and blockade of IL-1 and TNF-α expression was also significantly higher in the SynKPro as compared with AlloPK group (Fig. 2B).

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REFERENCES


