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BRIEF COMMUNICATION

Intraocular inflammasome signalling in failed corneal transplants

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Eye (2023) 37:787–789; https://doi.org/10.1038/s41433-022-02236-7

In transplanted corneas, endothelial cells are lost at higher rates and may lead to endothelial dysfunction, which is the overall primary cause of corneal graft failure (CGF) [1]. Furthermore, an elevation of proinflammatory cytokines was found in the aqueous humour (AqH) of patients with endothelial cell loss and CGF following corneal transplantation [2]. However, the mechanism involved is unknown.

Pyroptosis is an inflammasome-dependent proinflammatory programmed cell death mechanism. The inflammasome is a multiprotein complex comprised of the adaptor protein, apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC); a nucleotide oligomerization domain-like receptor (NLRs); and pro-caspase-1 [3]. In response to cellular stress, ASC and the assembled NLRs activate caspase-1, inducing pro-cytokines to be cleaved into bioactive interleukin (IL)-1β and IL-18, which are responsible for signalling propagation [3]. Moreover, caspase-1 activates gasdermin-D (GSDM-D), that forms membrane pores leading to osmotic cell death [3]. Herein, we assessed intraocular inflammasome activation in patients with CGF.

Experiments were conducted in accordance with the tenets of the Declaration of Helsinki for biomedical research involving human tissue and approved by the Institutional Review Board of the University of Miami. Participants signed a written informed consent before specimen collections. AqH was collected from patients with CGF ($n = 7$) prior to repeated keratoplasty. AqH from healthy patients scheduled for cataract surgery were used as controls ($n = 12$). Failed graft tissues of patients with CGF were processed by the Pathology Department of the Bascom Palmer Eye Institute, and human corneoscleral buttons (controls) were procured from donor tissues by the Florida Lions Eye Bank. The expression of inflammasome proteins (ASC, IL-18, and IL-1β) in the AqH was assessed via Simple Plex technology (Protein Simple) as previously described [4]. Intracellular expression of GSDM-D in corneas was assessed by immunofluorescence (Novus Biologicals, NBP2-80427) as previously described [5]. Results were analysed by

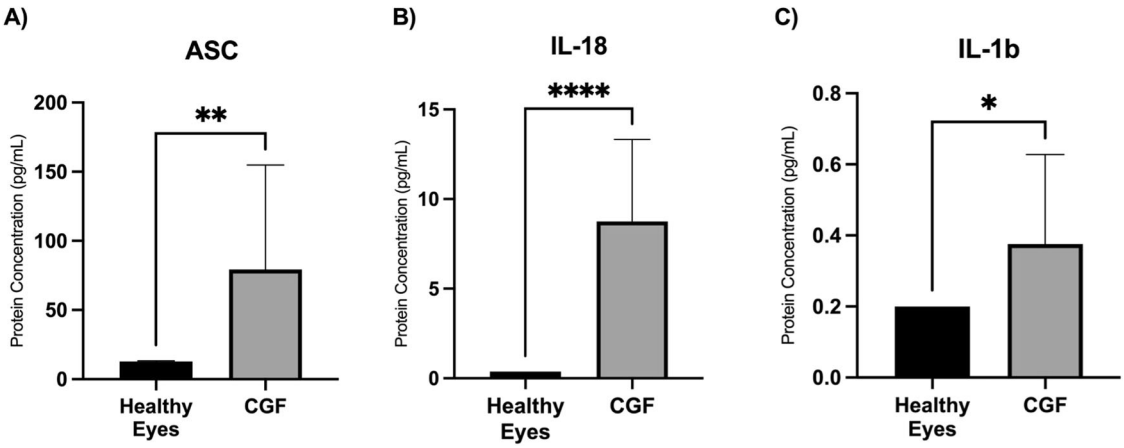

Fig. 1 Inflammasome protein expression in the aqueous humour of patients with corneal graft failure. Expression of ASC (**A**), IL-18 (**B**), and IL-1β (**C**) in the AqH of patients with CGF. N. 12 = Healthy eyes; N. 7 = CGF. Data presented as mean ± SEM. *$p = 0.0072$, **$p < 0.0001$, ****$p = 0.0361$. The lower limit of quantitation of ASC, IL-18, and IL-1β was assumed in samples with undetected levels of these proteins (13.00, 0.39, and 0.20 pg/ml, respectively).

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Received: 12 May 2022 Revised: 29 July 2022 Accepted: 1 September 2022
Published online: 12 September 2022

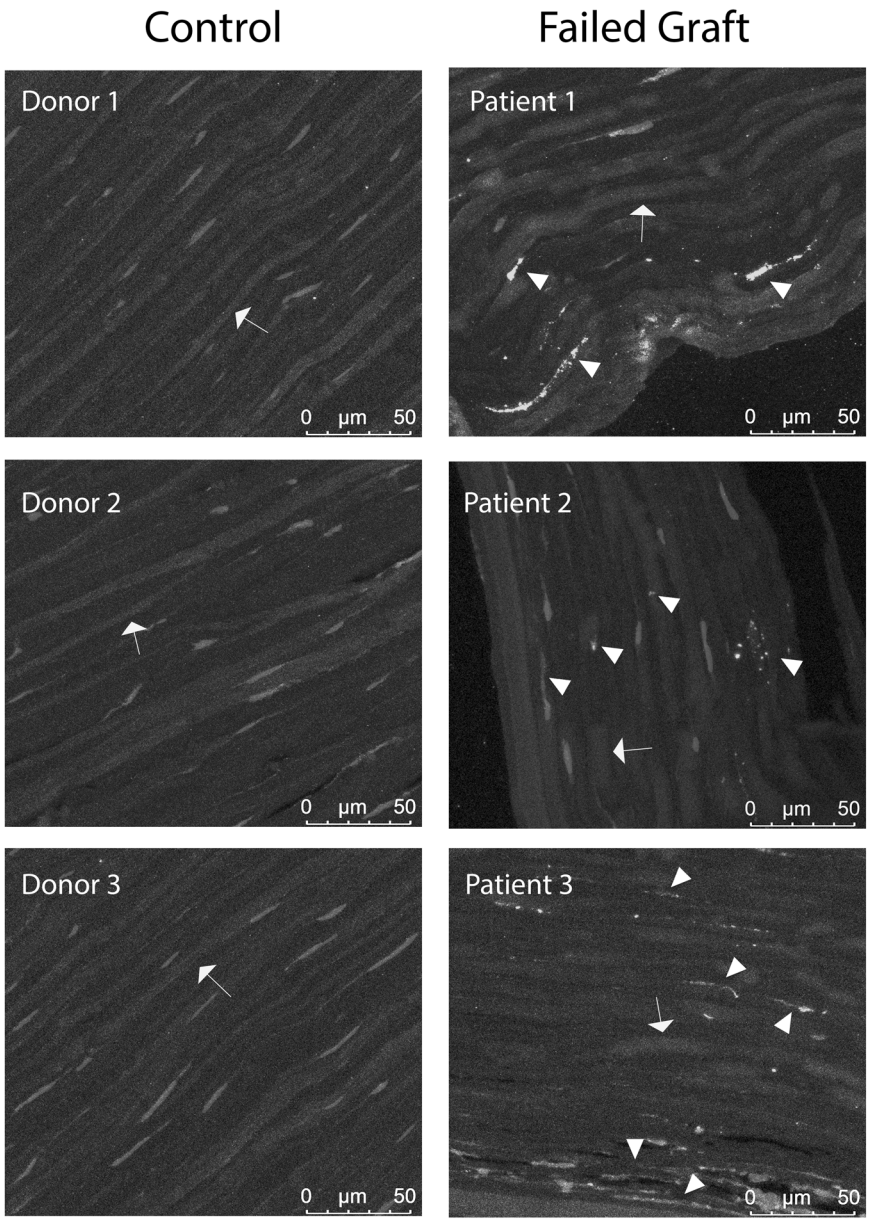

Fig. 2 Expression of GSDM-D in the graft of patients with corneal graft failure. GSDM-D expression (arrow heads) in failed grafts from three different patients compared to healthy donor corneas (controls). A higher expression of GSDM-D was observed in the failed grafts from patients with corneal graft failure. Stromal lamellar thickness was greater in these patients, consistent with the decreased transparency in failed graft corneas. Green: GSDM-D; Blue: nuclear staining (DAPI); Magnification: ×40.

GraphPad Prism® (v.8.04) using the Mann–Whitney test. Significance levels were set as $p \leq 0.05$.

Levels of ASC, IL-18, and IL-1β were significantly elevated in the AqH of patients with CGF compared to healthy eyes (Fig. 1). Moreover, a higher cytoplasmic immunoreactivity of GSDM-D was observed in the failed grafts from patients with CGF (Fig. 2).

A current hypothesis regarding CFG is that a transient increase in proinflammatory cytokines caused by the breakdown of the blood-ocular barrier after an intraocular surgery may cause a proapoptotic milieu and the activation of a nonspecific innate immune response [1]. However, the mechanisms have not yet been identified. Recently, our group showed in an ex vivo model that proinflammatory cytokines induce inflammasome activation in corneal endothelial cells, leading to cell death by pyroptosis [5]. Herein, we demonstrated intraocular inflammasome activation in both AqH and failed grafts of patients with CGF. To the best of our knowledge, this evidence suggests for the first time that pyroptosis may contribute to the pathogenesis of CGF and could establish an association between the intraocular proinflammatory environment found in these patients and the endothelial cell loss leading to CGF. Therefore, further studies may be considered for therapeutic purposes in these patients.

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AUTHOR CONTRIBUTIONS

AS was responsible for sample processing, interpreting the results, statistical data analysis, and writing the manuscript. AGB and AAT contributed to sample processing. MHB was responsible for conducting the immunofluorescence assay and image editing. SRD provided processed healthy tissue samples. ALS, GA, EHK, FC, and RH contributed to the sample collection. ALS and JPdRV were responsible for designing the protocol, statistical analysis, interpreting results, and final version approval.

FUNDING

This work was supported by the Beauty of Sight Foundation (ALS), NIH Center Core Grant P30EY014801 (institutional), and Research to Prevent Blindness Unrestricted Grant GR004596 (institutional).

COMPETING INTERESTS

JPdRV is a co-founder and managing member of InflamaCORE, LLC and has licensed patents on inflammasome proteins as biomarkers of injury and disease and targeting inflammasome proteins for therapeutic purposes. JPdRV is a Scientific Advisory Board Member of ZyVersa Therapeutics.

ADDITIONAL INFORMATION

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