

## POSTER 39

**GENE TRANSFER TECHNOLOGY: A TOOL FOR STUDYING  
GENE FUNCTION AND ROLE IN CORNEAL PATHOGENESIS**

Elizabeth A. Killion (M3)

Ajay Sharma, PhD

Jonathan C.K. Tovey, MD

Ashish Tandon, PhD

Rangan Gupta, PhD

(Rajiv R. Mohan, PhD)

University of Missouri Department of Ophthalmology  
Harry S Truman Veterans' Memorial Hospital

**Purpose:** Transforming growth factor  $\beta$  (TGF $\beta$ ) is associated with many corneal pathologies, diseases and dystrophies. The function of TGF $\beta$  in adult corneas cannot be studied using conventional transgenic approach because TGF $\beta$ 1 and TGF $\beta$ 2 deficient transgenic animals suffer multiple inflammatory diseases, severe developmental defects, and death by 3-4 weeks of age. This study tested the hypothesis that selective tissue-targeted gene transfer approaches will permit examination of TGF $\beta$  gene function in the adult cornea without altering TGF $\beta$  expression in vital organs.

**Methods:** Female black C57 mice were used. Animals were anesthetized with intramuscular injection of ketamine (130mg/kg) and xylazine (8.8mg/kg). Topical solution of 1% proparacaine hydrochloride was instilled to each eye for local anesthesia. Two microliters of AAV5 naked vector or expressing TGF $\beta$ 1 gene (titer  $10^9$  genomic copies/ $\mu$ l) was administered into the cornea. Eyes were collected at various time-points post-AAV application. Visual eye exam, stereomicroscopy, and slit-lamp biomicroscopy were used to monitor corneal health. Immunocytochemistry, western blotting and real-time PCR techniques were used to study corneal tissues.

**Results:** Tissue-selective targeted delivery of TGF $\beta$ 1 gene via AAV5 induced haze and opacity in the mouse cornea in a time-dependent manner as evident from slit-lamp biomicroscopy and preliminary immunocytochemistry experiments. Experiments are underway to study expression of collagens, extracellular matrix proteins and signaling pathways linked to TGF $\beta$ -mediated pathologies.

**Conclusions:** Tissue-specific controlled gene transfer approaches are a powerful tool to study gene function and identify therapeutic targets for mechanism-based innovative therapies to treat and prevent corneal abnormalities.