**3629**
**Topic Category:** 4065-ASIP Leukocyte-endothelial cell interactions

**First Author:** Prarthana Dalal
Northwestern University Feinberg School of Medicine
Ward Building Room 3-140 Chicago, IL 60611
United States
**Phone:**
prarthana.dalal@northwestern.edu

**First Author is a:** Medical Student
**First Author is a member of:** American Society for Investigative Pathology
**First Author Degree:** BA, BS, or equivalent

**Presentation Preference:** Oral

**Sponsor:** Prarthana Dalal
**Sponsor Phone:** 9135639600
prarthana.dalal@northwestern.edu
**Sponsor's Society:** Pathology - American Society for Investigative Pathology (ASIP) - Host Society
**Keywords:** 1. Diapedesis 2. Calmodulin
**Awards:** ASIP Trainee Travel Award

## Exploring the Role of Calmodulin and Calcium Signaling in Leukocyte Transmigration

Prarthana J. Dalal, David P. Sullivan, William A. Muller. Northwestern University Feinberg School of Medicine, Chicago, IL

Inflammation results in the dilation of local blood vessels, the activation of endothelium by cytokines and the recruitment of circulating leukocytes into areas of tissue damage. A transient increase in endothelial cytosolic free calcium ($Ca^{2+}$) is required for leukocyte transendothelial migration (TEM) and transient receptor potential canonical 6 (TRPC6) has been found to mediate this required $Ca^{2+}$ influx. Furthermore, we have also found that IQ-domain GTPase-activating protein 1 (IQGAP1) is essential for TEM. Specifically, the actin-binding domain and the calmodulin-binding isoleucine-glutamine (IQ) domain of IQGAP1 are critical for its function during TEM. In endothelial cells re-expression of IQGAP1 truncation mutants lacking the N-terminal actin-binding domain did not localize to endothelial borders and did not support TEM. Re-expression of truncated IQGAP1 constructs containing the actin-binding domain and the IQ domain localized to cell borders and supported TEM while constructs containing the actin-binding domain but lacking the IQ domain localized to the cell borders but did not restore TEM.

Calmodulin (CaM) is a small, highly conserved intracellular calcium-binding protein, which has been shown to interact with IQGAP1 through the IQ domain. It has also been previously found to regulate TRPC channel activation; however, not much is known regarding its role in TEM. Therefore, we hypothesized that it may be involved in the calcium signaling cascade within endothelial cells essential for supporting leukocyte transmigration.

Human endothelial cells treated with calmodulin inhibitor trifluoperazine (TFP) demonstrated a marked reduction in leukocyte TEM. Endothelial monolayers pretreated with 100 mM TFP for 1 hour drastically reduced leukocyte TEM. Only about 13% of leukocytes were able to transmigrate compared to 84% TEM in control, untreated monolayers ($p=0.0025$).

After identifying the potential role of calmodulin, we generated CaM shRNA to deplete endogenous CaM within endothelial cells, wildtype Flag-tagged CaM re-expression constructs and CaM calcium-binding mutants that we are currently in the process of testing. Identifying the specific mechanism by which endothelial $Ca^{2+}$ influx through TRPC6 mediates the events of TEM will provide novel insight into the signaling involved in TEM and also provide potential therapeutic targets for anti-inflammatory therapy.

**Support or Funding Information**
F30HL134202, R01HL046849, R01HL064774