Epithelial TIM-3-Expressing Mast Cells are Associated with Increased Severity of Chronic Rhinosinusitis with Nasal polyposis, Aspirin Exacerbated Respiratory Disease, and Asthma

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Objective

Determine correlation between TIM-3+ mast cells within nasal polyps and clinical severity of chronic rhinosinusitis with nasal polyposis (CRSwNP) with concomitant aspirin-exacerbated respiratory disease (AERD) and asthma.

Background

Mast cells and their activation in CRSwNP is an area that remains poorly understood. It has been reported that mast cells constitutively express cell surface T cell/transmembrane immunoglobulin and mucin domain protein 3 (TIM-3)[1]. It has been suggested that manipulation of TIM-3 activity on mast cells could be a promising target for the development of novel therapeutic modalities for chronic inflammatory diseases[2].

Methodology

- Nasal polyp tissue (n=24) obtained at a tertiary care hospital (2015-2016) was enzymatically digested[3]
- Viable TIM-3+ mast cells identified using flow cytometry markers, while disease severity was assessed using clinical severity scales

Flow Cytometry Markers

- CD45 (hematopoietic cells)
- Live/dead (viable cells)
- c-kit, FcεRI } **mast cells**
- TIM-3

Clinical Severity Scales

- 22-item Sino-Nasal Outcome Test (SNOT-22)
- Lund Mackay staging system
- Lund Kennedy score
- Hematologic counts (eosinophil, basophil)

Demographics

Enrolled patients with CRSwNP, n (%)	24 (100%)
Gender, n (%)	
M	13 (54.2)
F	11 (45.8)
Sinus	
Ethmoid	13 (61.9)
Maxillary	8 (38.1)
Concomitant asthma	13 (54.2)
Concomitant AERD	8 (33.3)
Concomitant allergic rhinitis	13 (54.2)
OCS treatment (384mg/d)	18 (75)

Flow Cytometry

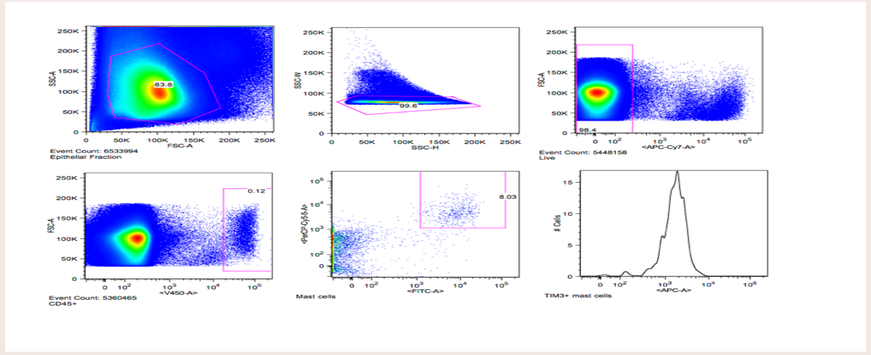

Figure 1. The populations positive for the mast cell flow cytometry markers were gated, with each subsequent population as a sub-population of the previous.

Results

In CRSwNP patients with concomitant AERD and asthma:

- %TIM-3$^+$ mast cells in the nasal polyp **epithelial** layer positively correlated with worsening endoscopic appearance post-operatively (rho=0.949, p=0.014, n=5)
- Higher %TIM-3$^+$ mast cells in the **stromal** layer of nasal polyps correlated with a greater change between pre- and post-operative endoscopic severity scores (rho= –0.894, p=0.041, n=5)

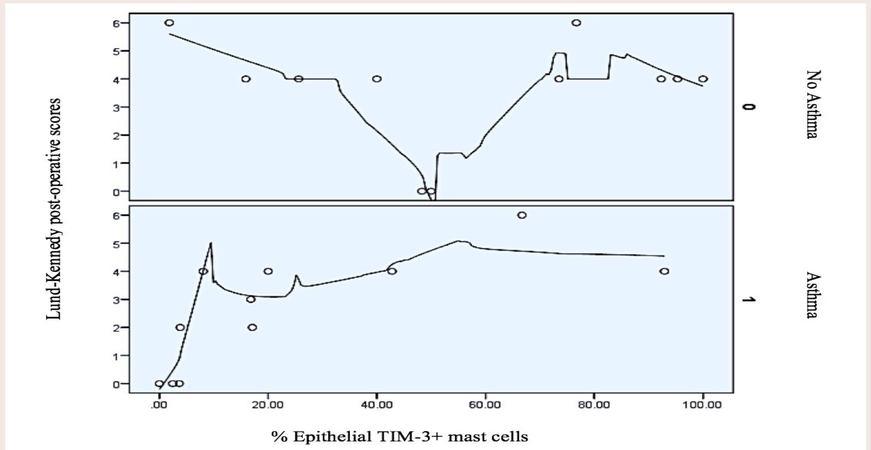

Figure 2. Positive correlation between epithelial %TIM-3$^+$ mast cells and LK post-operative scores in a subgroup of patients with concomitant asthma (rho=0.866, p=0.001, n=11).

Immunohistochemistry

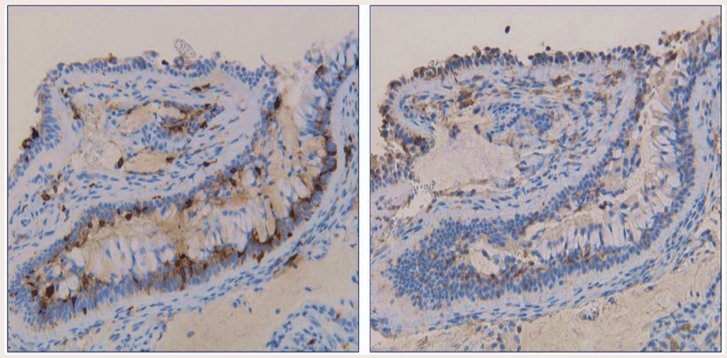

Figure 3. Wilcoxon signed rank test showed greater %TIM-3+ mast cells in the **epithelial** layer of NPs (p=0.001, n=23).

Discussion

Viable mast cells present in nasal polyps with increased TIM-3 expression in the epithelial layer may play a role in perpetuating the inflammatory response after surgical intervention in CRSwNP patients.

Limitations of this pilot study include a small sample size and lack of comparison to healthy sinus control tissue.

Future Direction

Areas for future study include the effects of topical and/or inhaled corticosteroid treatment and environmental factors on the mast cell milieu.

Resources

1 B. L. Phong, L. Avery, T.L. Sumpter, et al., "Tim-3 enhances FcεRI-proximal signaling to modulate mast cell activation," *J. Exp. Med.*, vol. 212, no. 13, pp. 2289–2304, Dec. 2015.
2 A. C. Anderson, D.E. Anderson, L. Bregoli, et al., "Promotion of tissue inflammation by the immune receptor Tim-3 expressed on innate immune cells," *Science*, vol. 318, no. 5853, pp. 1141–1143, Nov. 2007.
3 S. Finotto, J. Dolovich, J. A. Denburg, M. Jordana, and J. S. Marshall, "Functional heterogeneity of mast cells isolated from different microenvironments within nasal polyp tissue," *Clin. Exp. Immunol.*, vol. 95, no. 2, pp. 343–350, Feb. 1994.