208 The Mechanisms Involved in IL-2 Production By Regulatory Mast Cells in Chronic Allergic Dermatitis

Alon Hershko, MD, PhD[1], Itay Moshkovits[2], Ariel Munitz, PhD[3], Yoseph A. Mekori, MD, FAAAAI[4], Pazit Salamon, PhD[1]; [1]Meir Medical Center, Kfar Saba, Israel, [2]Department of Clinical Microbiology and Immunology, The Sackler School of Medicine, Tel-Aviv University, Tel Aviv, Israel, [3]Tel Aviv University, Israel, [4]Meir Hospital, Kfar-Saba, Israel.

RATIONALE: We have previously reported that MCs dampen inflammation in oxazolone-induced chronic allergic dermatitis in mice. This suppression occurs by a previously unrecognized regulatory mechanism in which MC's secrete IL-2 which, in turn, supports regulatory T cell activity at the site of inflammation. However, since IL-2 is not a typical product of MC's, we sought to define conditions that support its production in the setting of allergic dermatitis.

METHODS: Isolated bone marrow-derived MCs (BMMCs) were incubated with various stimulators and IL-2 release was assessed by ELISA. Phosphorylation of signaling molecules was studied by Western blot analysis. Allergic dermatitis was induced in mice by 10 repeated exposures of the ear skin to 0.5% oxazolone in acetone over 4 weeks. Immunohistochemistry was done on paraffin embedded skin specimens.

RESULTS: IL-33 is an exclusive inducer of BMMC IL-2 production. Presensitization of cells with IgE further enhanced secretion of IL-2. IL-33 induced phosphorylation of the MAPKs family members ERK, p38 and JNK, and the inhibitors for these MAPKs dampened IL-2 release. The levels of IL-33 in ear homogenates from oxazolone-treated mice was greater than those of naïve ears. Staining of ears disclosed *in vivo* co-localization of IL-33 and MC in ears with dermatitis.

CONCLUSIONS: MC-derived IL-2 was previously shown to stimulate Tregs in allergic dermatitis, and here we show that its production is stimulated by IL-33. In dermatitis, the levels of IL-33 increase and it co-localizes with MC. We suggest that IL-33 is a pivotal player in the induction of regulatory MC's.

209 A New Disease Cluster: Mast Cell Activation Syndrome, Postural Orthostatic Tachycardia Syndrome, and Ehlers-Danlos Syndrome

Ingrid Cheung, Peter Vadas, MD, PhD; St. Michael's Hospital, Toronto, ON, Canada.

RATIONALE: Patients with postural orthostatic tachycardia syndrome (POTS) and hypermobility often describe symptoms suggestive of mast cell activation. Herein, we describe a new, unique phenotype, characterized by the co-segregation of three disorders: POTS, Ehlers Danlos syndrome (EDS) and mast cell activation syndrome (MCAS).

METHODS: Participants with diagnoses of POTS and EDS were recruited from throughout North America through a patient support group and evaluated by questionnaire and supporting documentation. A formal diagnosis of POTS by a cardiologist included confirmation via tilt-table test. A formal diagnosis of EDS required assessment by a dermatologist, a Beighton score of $\geq$ 5/9 and a diagnostic skin biopsy. A questionnaire for MCAS was based on diagnostic criteria and validated symptoms as reported by Akin, Valent and Metcalfe (2010).

RESULTS: 15 participants completed questionnaires with required documentation. All eligible participants were female. 12 of these people had formal diagnoses of POTS (80%), 9 were diagnosed with both POTS and EDS. 6 of 9 patients with both POTS and EDS had validated symptoms of a mast cell disorder (66%), suggestive of MCAS.

CONCLUSIONS: From these pilot data, it appears that a mast cell disorder may frequently co-segregate with POTS and a collagen disorder such as EDS.

210 Microbiome Effects on Hematopoietic Eosinophil/Basophil Progenitor Phenotype: Implications for the Pathogenesis of Allergic Inflammation

Elli Rosenberg, MD, PhD[1], Pia Reece, PhD[1], Michael G. Surette, PhD[2], Paul Poayyedi, MD[2], Judah A. Denburg, MD, FRCPC, FAAAAI[1]; [1]Division of Clinical Immunology and Allergy, Department of Medicine, McMaster University, Hamilton, ON, Canada, [2]Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada.

RATIONALE: $CD34^+$hematopoietic eosinophil/basophil (Eo/B) progenitors express TLRs and cytokine receptors (CyR) differentially in both newborns and adults, depending on allergic risk or disease, thus providing evidence for allergy as a systemic disease. We hypothesized that Eo/B progenitor phenotype would be affected by the host's gut microbiome, examining this in ulcerative colitis (UC), a prototypical systemic inflammatory disease.

METHODS: Flow cytometry assessment of TLR and CyR surface expression on peripheral blood $CD34^+$ progenitors as well as *ex vivo*examination of effects on these receptors of exposure to supernatants of bacterial cultures representative of the gut microbiome in adults with UC and age-matched healthy controls.

RESULTS: UC subjects showed a distinct pattern of TLR and CyR expression in comparison to healthy controls, with down-regulation of TLR-4 (MFI of 3.03 ± 0.45 vs 1.87 ± 0.33, P value 0.02) and GM-CSFR (1.71 ± 0.56 vs 0.28 ± 0.26, P value 0.01) and up-regulation of the Eo/B progenitor IL-5R (0.04 ± 0.02 vs 0.77 ± 0.24, P value 0.01). This pattern could be reproduced *ex vivo* upon exposure of $CD34^+$ progenitors to both normal- and UC-derived gut microbiome supernatants.

CONCLUSIONS: Products of the gut microbiome affect $CD34^+$ progenitor expression of TLR and CyR, with potential effects on Eo/B differentiation. These findings suggest that the gut microbiome may modulate allergic inflammation and disease through systemic effects on hemopoietic progenitors in early life and into adulthood.