Mast Cells and Lipid Cross-Talk in Skin Inflammation

By

Piper A. Wedman

Atopic dermatitis (AD) is an inflammatory skin disease whose pathogenic mechanisms remain unclear. We describe a novel method of computer-assisted image analysis for *in situ* quantification of mast cells (MC) activation. We also define quantitative parameters to measure skin remodeling in early-stage AD (eczema) using a validated human-like mouse model. Remarkably, skin thickening was substantiated after a single application of ovalbumin (OVA), antigen used in this model, with cellular infiltration of the hypodermis. Cell recruitment correlated with chemokine production, MC activation, and increased levels of sphingolipid sphingosine-1-phosphate (S1P), produced by sphingosine kinase-1 (SphK1), we showed could be produced by and also activate MC-derived chemokine production. MC or SphK1 deficiency significantly hindered these inflammatory responses.

Skin barrier dysfunction observed in chronic AD correlates with decreased ceramide (CER) content. Skin lipidomics revealed significant increase of local CER species C16 and C24 in OVA-treated samples, compared to controls. We analyzed skin CER synthase (CerS, synthesis) and ceramidase (CER catabolism) mRNA expression. CerS4, 5 and 6 mRNA levels were statistically augmented after OVA exposure. We found that OVA treatment triggered apoptosis through proapoptotic CER, correlating with elevation of key molecules of endoplasmic reticulum stress. Surprisingly, primary mouse bone marrow-derived MC activated *in vitro* by S1P also exhibited elevation of C16 CER. To substantiate the importance of MC in these processes, CER profiling was similarly conducted in treated skin samples collected from MC-deficient mice. MC deficiency prevented OVA-induced CER increase and local apoptosis.

Epigenetic regulation of AD is unknown. MicroRNA profiling was conducted at different time-points following skin treatments. We identified a microRNA triad whose downregulation promoted the AD-related pathogenic pathways we have unraveled. Predicted target genes were validated further confirming the relevance of this triad in AD regulation.

We conclude that MC could initiate AD by driving both early skin remodeling and cell recruitment through local chemokine production and S1P interaction, and CER-elicited apoptosis. Moreover, epigenetic studies highlight the down-regulation of a microRNA triad that de-repressed newly identified key players of AD pathogenesis. Targeting these pre-symptomatic effector mechanisms may offer new prophylactic strategies for AD whose treatment remains a clinical challenge.