STIM/Orai-mediated store operated Ca^{2+} entry may contribute to overproduction of TSLP in epidermal cells isolated from atopic dermatitis mice

Shuhei Kobayashi¹, Rieko Matsui¹, Yumeka Yamada¹, Hiroaki Kito^{1,2}, Susumu Ohya^{1,2}, Masanori Fujii¹

¹Department of Pharmacology, Kyoto Pharmaceutical University, Japan, ²Department of Pharmacology, Graduate School of Medical Sciences, Nagoya City University, Japan

[Background] Atopic dermatitis (AD) is a highly pruritic, chronic inflammatory skin disease. Thymic stromal lymphopoietin (TSLP) is highly expressed in the epidermis of AD patients. Also, TSLP is known to induce Th2 immune responses and itch; however, the mechanism of how TSLP is produced in AD conditions is not fully understood. We have reported a unique, diet-induced AD mouse model (Fujii et al., *Exp. Dermatol.*, 14, 460-468, 2005). In the present study, we examined the TSLP production capability of epidermal cells isolated from the AD model mice and determined its mechanism of action.

[Methods] HR-1 hairless mice were fed a normal or the special (called HR-AD) diet. Epidermal sheet was obtained by treatment with dispase overnight at 4 ° C, and then was treated with trypsin at 37 ° C to isolate epidermal cells. The isolated epidermal cells were incubated in the normal medium for 24 h at 37 ° C. At the indicated time point in cultivation, mRNA and intracellular and extracellular protein of TSLP were determined by real time PCR and ELISA, respectively.

[Results] TSLP mRNA and intracellular and extracellular TSLP protein were significantly increased with a peak at 3 h, 6 h, and 24 h, respectively, after the cultivation in epidermal cells isolated from special diet-fed AD mice, whereas such TSLP increase was not observed in normal mice. The TSLP overproduction was almost completely inhibited by the Ca^{2+} chelator ethylene glycol tetraacetic acid, suggesting a requirement of extracellular Ca^{2+} . Treatment with transient receptor potential channel inhibitors, such as Gd^{3+} , ruthenium red and La^{3+} , did not affect the TSLP overproduction. On the other hand, the TSLP overproduction was partly but significantly suppressed by 2-aminoethoxydiphenylborate, which blocks IP_3 receptors. Furthermore, the TSLP overproduction was almost completely inhibited by treatment with either ML-9, which inhibits plasma membrane interaction of stromal interaction molecule 1 (STIM1), preventing store-operated Ca^{2+} entry (SOCE), or BTP-2, which blocks the Ca^{2+} release-activated Ca^{2+} channel Orai-1.

[Conclusions] Epidermal cells isolated from AD mice can produce high amount of TSLP, perhaps by STIM/Orai-mediated SOCE.