

**967 Thymic Stromal Lymphopoietin Directly Activates Eosinophils**

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**RATIONALE:** Cumulative evidence shows that epithelial-cell derived thymic stromal lymphopoietin (TSLP) may initiate Th2-type immune responses through dendritic cell activation. TSLP has also been shown to directly activate Th2 cells and mast cells. Direct action of TSLP on eosinophils, major effector cells in allergic inflammation, however, is not well known.

We studied possible direct effects of TSLP on effector functions of eosinophils.

**METHODS:** Gene expressions of TSLP receptor (TSLPR) and IL-7 receptor  $\alpha$  in eosinophils were examined by means of real-time RT-PCR. Expression of TSLPR was also immunohistochemically determined by using confocal laser microscope. Eosinophils were incubated with recombinant human TSLP at various concentrations. A panel of cytokines and chemokines in the culture supernatants were measured with a multiplex beads array system. In some experiments, combinations of TSLP with other cytokines were examined for induction of TSLPR and cytokine/chemokine production from eosinophils. Effects of TSLP on eosinophil survival, degranulation and superoxide generation were also examined.

**RESULTS:** Eosinophils expressed TSLPR and IL-7R $\alpha$  and the expression of the former was significantly enhanced by IL-3 and/or TNF- $\alpha$ . TSLP induced production of MIP-1 $\alpha$ , MIP-1 $\beta$ , MCP-1, TNF $\alpha$ , IL-6, and IL-8, not IL-4 and IL-5, from eosinophils in a dose-dependent manner. IL-3 and TNF- $\alpha$  synergistically enhanced TSLP-induced production of the cytokines/chemokines. In some cases eosinophil survival was enhanced by TSLP. Superoxide production and degranulation were not induced by TSLP. **CONCLUSIONS:** TSLP directly activates eosinophil functions. Induced inflammatory cytokines and chemokines from eosinophils by TSLP may be involved in host defense and chronic inflammation, which may be distinct from typical Th2 inflammation.

**968 Rhinovirus Enhances Eosinophil Activation through the Production of IL-5 in Acute Exacerbation of Childhood Asthma**M. Kato<sup>1,2</sup>, H. Tsukagoshi<sup>1</sup>, M. Yoshizumi<sup>1</sup>, M. Saitoh<sup>1</sup>, K. Kozawa<sup>1</sup>, Y. Yamada<sup>2</sup>, K. Maruyama<sup>2</sup>, Y. Hayashi<sup>2</sup>, H. Kimura<sup>3</sup>; <sup>1</sup>Gunma Prefectural Institute of Public Health and Environmental Sciences, Maebashi, Gunma, Japan, <sup>2</sup>Gunma Children's Medical Center, Shibukawa, Gunma, Japan, <sup>3</sup>National Institute of Infectious Diseases, Musashimurayama, Tokyo, Japan.

**RATIONALE:** Since little information is available on eosinophil activation and cytokine response in virus-induced asthma, we previously examined respiratory viruses and 17 serum cytokines/chemokines, and eosinophil cationic protein (ECP) in acute as well as stable asthma. Here, we further investigated ECP, and 27 cytokines/chemokines in both nasal secretions and serum from children with asthma.

**METHODS:** We detected viruses in nasal secretions from 174 patients with acute asthma using antigen detection kits or PCR, followed by direct DNA sequencing analysis. We measured peripheral eosinophil counts, and serum concentrations of ECP and 27 cytokines/chemokines (IL-1 $\beta$ , IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, IFN- $\gamma$ , IP-10, TNF- $\alpha$ , GM-CSF, G-CSF, MCP-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , eotaxin, RANTES, PDGF-bb, FGF basic, VEGF) using a multiplex beads-based assay in patients with acute (85) and stable (79) asthma. We also examined nasal ECP and 27 cytokines/chemokines in acute asthma.

**RESULTS:** Of the 174 acute asthma samples, rhinovirus was detected in 59; RS virus in 44; enterovirus in 17; other viruses in 19; and no viruses in 35. The concentrations of serum ECP, IL-5, IL-6, IL-1ra, and IP-10 were significantly elevated in rhinovirus-induced acute asthma compared with stable asthma. Similarly, serum ECP, IL-5, and IP-10 were significantly higher in rhinovirus-induced acute asthma than in controls. Furthermore, only IL-5 was significantly elevated in the rhinovirus-group compared with the RS virus-groups in both serum and nasal secretions.

**CONCLUSIONS:** Virus-induced asthma, especially via rhinovirus, might enhance eosinophil activation through IL-5 production.

**969 Hypereosinophilia in Two Patients with Ventriculoperitoneal Shunts**

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**RATIONALE:** Silicone is often used in ventriculoperitoneal shunts (VPS) and contains soluble oils which bind anti-silicone antibodies and may cause hypersensitivity.

**METHODS:** We present 2 patients with eosinophilia after VPS placement likely resulting from silicone hypersensitivity.

**RESULTS:** A 22-month-old male with schizencephaly and porencephalic cyst developed rash and edema at the VPS site with central and peripheral eosinophilia. CSF cultures were negative. The peripheral eosinophil (E) count was 0% and CSF 3% on the day of shunt placement. Three days later the peripheral E were 6% (normal 0-5%), with 700 K/uL (normal 0-0.3 K/uL), and CSF E 39%. CSF E were 36% and peripheral E 51% at 2 months, and 6.3% (0.58 k/uL) at 7 months. Total IgE was 161 (0-120 IU/mL).

The second patient was a 15 month old with post-natal meningitis resulting in neurologic dysfunction and hydrocephalus. Following silicone VPS placement he developed peripheral eosinophilia to peak of 18% (2 K/uL). Total IgE high was 2766 IU/mL. Peripheral eosinophilia decreased over 7 months to 11.2%, and is now 5% while on steroids for refractory seizures. In both patients parasitic and hematologic causes of eosinophilia were excluded. Despite these findings the patients were clinically well with functioning shunts.

**CONCLUSIONS:** CSF and peripheral eosinophilia coinciding with VPS placement may suggest silicone hypersensitivity. However, anti-silicone antibody testing is not readily available, necessitating diagnosis of exclusion. The diagnosis raises a clinical dilemma regarding the need for shunt replacement in hypersensitive patients who are clinically well.

**970 Characterization Of Cell Surface Proteins Involved In Lipoxin A4 Inhibition Of Eosinophils Activation.**

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**RATIONALE:** IL-3, IL5 and GM-CSF are main cytokines that activate eosinophils in asthma and allergy. Since activation plays an important role in eosinophil-related pathologies, we examined the molecular mechanism of the anti-inflammatory and pro-resolution eicosanoid lipoxin A4's involvement in the inhibition of eosinophil activation.

**METHODS:** Eosinophils isolated from the blood of healthy donors were incubated with GM-CSF (30 ng/ml) for varying times with or without pretreatment with lipoxin A4 (100 nM). Cell surface proteins were isolated using a biotinylation pull down assay and resolved by 2-D SDS PAGE. CD69 expression on the cell surface was measured by flow cytometry. Cytokine secretion was analysed by multiplex cytokine assay.

**RESULTS:** GM-CSF induced a three-fold expression of CD69 on eosinophils ( $p < 0.01$ ). This effect was inhibited by pretreatment with 100 nM lipoxin A4. The organization of cell surface proteins was changed considerably after treatment with pro-inflammatory cytokines. Lipoxin A4 partly reversed GM-CSF action, but also had its own unique influence on cell surface protein composition. Lipoxin A4 also significantly inhibited the secretion of pro-inflammatory cytokines stimulated by GM-CSF.

**CONCLUSIONS:** Lipoxin A4 inhibits eosinophil activation elicited by strong proinflammatory cytokines. Cell surface proteins were altered by activation and by lipoxin treatment.