SUNDAY

447 Analysis of Eosinophil, Mast Cell, and Basophil Siglec-8 Expression on Human Cell Lines and Hematologic Malignancies

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RATIONALE: Engagement of Siglec-8 receptors induces apoptosis in human eosinophils and inhibits IgE-receptor mediated release of histamine and prostaglandin D2 from human mast cells. Siglec-8 therefore represents a unique therapeutic target for mast cell and eosinophil-related diseases. We evaluated the surface expression of Siglec-8 on human eosinophil, mast cell, and basophil cell lines. Also evaluated were eosinophils and basophils from patients with hypereosinophilic syndrome (HES), chronic myelogenous leukemia (CML), and chronic eosinophilic leukemia (CEL), as well as bone marrow mast cells in indolent mastocytosis.

METHODS: Siglec-8 cell surface expression was evaluated on human mast cell lines (HMC-1.1, HMC-1.2, LUVA), bone marrow mast cells, an eosinophilic leukemia cell line (EOL-1), and a basophilic precursor line (KU812) by indirect immunofluorescence and flow cytometry. In addition, Siglec-8 was evaluated on blood eosinophils and basophils from patients with HES, CML, and CEL.

RESULTS: Siglec-8 was consistently detected on human eosinophils and basophils from subjects with HES, CEL, and CML (>95% positive, n=2-4). It was weakly detected on HMC-1.1 (10-20% positive), but not on EOL-1 or KU812 cells (<5% positive) (n=1-5). Siglec-8 was brightly and consistently expressed on HMC-1.2 (>90% positive, n=5) and LUVA cells (>70% positive, n=3) as well as bone marrow mast cells in indolent mastocytosis (>95% positive, n=3).

CONCLUSIONS: Siglec-8 is expressed on normal and neoplastic human eosinophils, basophils and mast cells, including certain mast cell lines. Siglec-8-based therapies should target mature and malignant eosinophils, mast cells, and basophils.

448 Cord Blood (CB) Eosinophil/Basophil (Eo/B) Progenitors Predict Respiratory Outcomes Until The Age Of Two

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RATIONALE: It has been shown that in infants at high risk of atopy cord blood hematopoietic progenitor cells predict the development of acute respiratory illnesses during the first 12 months. In the present study we investigated the predictive value of CB Eo/B progenitors regarding respiratory outcomes independently from the atopy risk. Furthermore, we analysed whether the Th1/Th2 balance at birth is of relevance for CB Eo/B progenitor cell recruitment.

METHODS: In a sub cohort of 40 children of the LINA study (Lifestyle and environmental factors and their Influence on Newborns Allergy risk) frozen cord blood PBMCs were used for methylcellulose assays to assess Eo/B differentiation by colony formation (CFU) in the presence of IL-3, IL-5 or GM-CSF. Standardized questionnaires were recorded during 34th week of pregnancy and annually thereafter till the age of two. Ex vivo stimulated CB cytokines were measured using the cytometric bead array (CBA).

RESULTS: For the CB Th2 cytokines IL-4 and IL-13 a positive correlation was seen with the number of IL-5 responsive Eo/B CFUs ($p<0.05$). Enhanced CB IL-5- (but not IL-3- or GM-CSF-) responsive Eo/B CFU numbers predicted the occurrence of bronchitis and treated wheezing within the first 12 or 24 months ($p<0.05$).

CONCLUSIONS: Our data confirm the hypotheses that a modified Th2 milieu at birth may contribute to the recruitment and differentiation of Eo/B progenitors. We could further show that the predictive value of CB Eo/B progenitors in terms of respiratory illnesses is not restricted to high-risk children.

449 Mechanisms Of Tlr-mediated Cord Blood Cd34+ Progenitor Cell Eosinophil Differentiation: Signaling And Autocrine Pathways

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RATIONALE: Eosinophils are multi-functional leukocytes that play a role in allergic inflammation, and their lineage commitment has been shown to be dependent on hematopoietic cytokine signaling and the activation of STAT5 and MAPK signaling pathways. We have previously reported that LPS can induce eosinophil-basophil (Eo/B) differentiation of cord blood (CB) progenitors; however, the mechanism for this was unclear.

METHODS: CB CD34+ cells were stimulated with LPS and assessed for Eo/B colony forming units (CFU) by methylcellulose cultures, cytokine production by luminex and phosphorylation of STAT5 and MAPK proteins by phospho-flow cytometry. The importance of LPS induced hematopoietic cytokines and signaling molecules in Eo/B CFU formation was determined by neutralizing Abs and pharmacological inhibitors respectively.

RESULTS: Stimulation with LPS increased the formation of IL-3-, and GM-CSF-responsive Eo/B CFU compared to hematopoietic cytokine stimulation alone ($p=0.017$). Overnight stimulation of CB CD34+ cells resulted in the secretion of IL-3 and GM-CSF which stimulated Eo/B CFU production ex vivo, since Ab blockade of these cytokines significantly reduced Eo/B CFU formation ($p=0.016$). Likewise, LPS induced the phosphorylation of p38 MAPK, STAT5, and ERK 1/2 in a time- dependent manner ($P=0.043$); blocking these proteins resulted in suppression of LPS mediated Eo/ B CFU formation ($p=0.025$).

CONCLUSIONS: We show for the first time that LPS stimulation of human CB CD34+ cells can influence Eo/B differentiation directly through STAT5 and MAPK signaling pathways and hematopoietic cytokine secretion. Since LPS-mediated immuno-modulation of CB CD34+ progenitors can shape neonatal immunity, understanding microbial influences on eosinophilopoiesis may aid the development of therapies for eosinophil-related allergic disorders.

450 Differential Effect of TGF-β1 and Eotaxin on Novel CLC3 Ion-Channel Variants in Human Peripheral Blood Eosinophils

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RATIONALE: Chloride channels are implicated in differentiation, proliferation, apoptosis and migration of cells. Previously, we reported the involvement of CLC3 in TGF-β-induced migration, presence of CLC3b and CLC3e transcript variants and membrane expression of the CLC3 in human peripheral blood eosinophils. In this study, we examined the effect of eotaxin in CLC3 expression in eosinophils.

METHODS: Eosinophils were isolated and purified (>99% pure >98% viable) from venous blood of healthy donors with negative selection. Cells were incubated with either TGF-β1 (10ng/ml) or eotaxin (10ng/ml) for 24 hours. Total RNA was isolated using mirVana kit and 500ng RNA was reverse transcribed using Improm RT II. The qPCR data was analyzed using GAPDH as the reference gene with $2^{-\Delta\Delta Cq}$ as fold increase.

RESULTS: There was a 2-fold increase in CLC3 mRNA transcripts (exon 10-exon 11) with TGF-β1 ($n=4$, $p<0.05$) relative to GAPDH. Eotaxin has no statistically significant effect on CLC3 mRNA transcripts (exon 10-exon 11). CLC3b mRNA (exon 12-exon 14) was increased 3-4-fold with TGF-β1 ($n=4$, $p<0.05$) compared to 2-fold increase with eotaxin. Eotaxin increased the mRNA transcripts CLC3e (exon 13-exon 14) 3-4-fold ($n=4$, $p<0.05$) compared to 2-2.5-fold increase with TGF-β1. However, CLC3e mRNA transcripts decreased in combination of TGF-β1 and eotaxin.

CONCLUSIONS: mRNA transcript levels of CLC3 with different primer sets suggest the presence of more transcript variants in human blood eosinophils than the known variants, CLC3b and CLC3e. The significant increase in the transcript level of CLC3e with eotaxin suggests the role of CLC3e ion-channel in eotaxin-induced migration of eosinophils in allergic asthma.