

405 Evaluation of Indoleamine 2,3- Dioxygenase Gene Expression and Activation in Chronic Spontaneous Urticaria

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RATIONALE: Chronic spontaneous urticaria (CSU) is a common skin disorder characterized by the emergence of hives for at least six weeks without any known etiologic agent. Indoleamine 2,3- dioxygenase (IDO) which catalyzes tryptophan (Trp) to kynorenic acid (KYN) is an immunomodulatory enzyme and complicated in immunological diseases. This study, Trp, KYN and IDO gene expression in CSU patients were analyzed.

METHODS: We studied 20 CSU patients (mean age: 28±6 years, mean duration: 27±4 month) and 20 healthy individuals (mean age: 28±9). Peripheral blood mononuclear cells (PBMCs) were isolated from both patients and healthy control and stimulated by phytohemagglutinin (PHA). Real-time PCR was applied to quantify IDO gene expression and its activity was estimated by KYN/Trp ratio in supernatant of PBMCs.

RESULTS: Our study results showed that the gene expression of IDO was significantly higher in CSU patients (4.56±0.91) compare to healthy individuals (1.36±0.52) (p=0.006). Interestingly, the activity of IDO (KYN/Trp) was decreased in CSU patients (195.63±39.59) contrast of healthy ones (663.60±123.10) (p=0.001).

CONCLUSIONS: Previous studies documented the impaired of IDO gene expression in CSU patients, however in the present study we observed a decrease activity of IDO in CSU patients which might suggest the function of this factor is impaired in CSU patients.

406 Functional Expression of CRTH2 on Blood Eosinophils from Chronic Idiopathic Urticaria Subjects

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RATIONALE: Lesions of chronic idiopathic urticaria (CIU) demonstrate mast cell (MC) activation and recruitment of lymphocytes, eosinophils, and basophils. CRTH2/DP2 are receptors for Prostaglandin D2 (PGD2) expressed on infiltrating cells and induce chemotaxis and activation. Since activated skin MC release PGD2, we explored the effects of *in vitro* PGD2 exposure alone or with a CRTH2 receptor antagonist (AZD1981) on the shape change of eosinophils from active CIU patients.

METHODS: Blood was obtained from CIU patients (n=15) using a JH IRB approved protocol. Whole blood samples were incubated with buffer or AZD1981 (0.1 or 1 μM), and then stimulated with PGD2 (10⁻⁸ to 10⁻⁵ M). Eosinophil shape change was examined using flow cytometry scatter movement.

RESULTS: A net scatter movement at each PGD2 dose was plotted on a concentration curve, and analyzed using an area under the curve (AUC) method. Cells incubated in buffer alone had an AUC value of 119.9. Cells exposed to 1 μM AZD1981 showed a concentration curve of 52.9 AUC (p<0.001 from buffer alone). The cell curve at 0.1 μM AZD1981 was 76.5 AUC (p<0.001). The 50% of maximal response concentration of PGD2 required for eosinophils was 10⁻⁷ M (buffer), 10⁻⁶ M (1 μM AZD1981), and 10^{-6.5} M (0.1 μM AZD1981).

CONCLUSIONS: In CIU patients, PGD2 induces eosinophil shape change at concentrations similar to healthy subjects. Preincubation with AZD1981 markedly reduced the PGD2 mediated shape change response indicating functional expression of CRTH2 on circulating CIU eosinophils and suggesting CRTH2 as a therapeutic target in CIU.

407 The Expression of CRTH2 on Blood Basophils and Eosinophils in Chronic Idiopathic Urticaria

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RATIONALE: Evidence of mast cell (MC) degranulation and infiltration by leukocytes such as basophils, eosinophils, and T lymphocytes is observed in lesions in chronic idiopathic urticaria (CIU). Since activated MCs release Prostaglandin D2 (PGD2), a possible recruitment pathway for these leukocytes is CRTH2, a receptor for PGD2. We compared CRTH2 expression on leukocytes in the blood of CIU and healthy subjects.

METHODS: We recruited adult CIU subjects (n=20) and nonatopic subjects (n=8) and examined basal expression of CRTH2 via flow cytometry. Basophils and eosinophils were gated using scatter and specific markers. Values are reported as Median Net MFI (± SEM). Data were analyzed using Mann-Whitney Test.

RESULTS: CRTH2 expression was significantly decreased on basophils from CIU subjects as compared with healthy controls (307.1 ± 21.11 vs 398.4 ± 29.41, p=0.0395). CRTH2 surface expression on eosinophils from CIU subjects also trended lower as compared to controls (61.18 ± 4.197 vs 70.88 ± 6.77, p=0.0524). CRTH2 expression levels were more variable on basophils and eosinophils in CIU subjects as compared to controls. There was no difference between the percentage of basophils or eosinophils expressing CRTH2 in CIU compared with controls.

CONCLUSIONS: CRTH2 expression on leukocytes from CIU subjects is more variable than healthy controls. Levels on blood basophils are significantly reduced, compared to nonatopic controls. Eosinophil CRTH2 levels were overall lower than basophils and trended lower in CIU subjects. These findings suggest that the CRTH2 pathway may be engaged in the recruitment of eosinophils and basophils to CIU lesions.