SUNDAY

572 The Production and Characterization of Monoclonal Antibodies against Toluene Diisocyanate (TDI)-conjugated Proteins

D. Schmechel, T. B. Ruwona, J. M. Hettick, E. Janotka, F. M. Blachere, D. H. Beezhold, P. D. Siegel; CDC/Natl. Inst. Occup. Safety & Health, Morgantown, WV.

RATIONALE: Toluene diisocyanate (TDI) is a highly reactive chemical and one of the most commonly reported causes of occupational asthma. The production of TDI-specific monoclonal antibodies (mAb) will allow the development of standardized immunoassays for exposure assessment.

METHODS: Mice were immunized with TDI-keyhole limpet hemocyanin (KLH) conjugates and hybridomas were screened by ELISA using TDI conjugated to human serum albumin. MAbs were characterized by western blot against various mono- and diisocyanates conjugated to different carrier proteins using carbamate or thiocarbamate linkages.

RESULTS: A total of 35 mAbs were produced (25 IgG_1, 8 IgG_{2a}, 2 IgG_{2b}). Several mAbs were found to be specific for 2,4-TDI-conjugated proteins and did not react with 2,6-TDI-, monoisocyanate- or isothiocyanate -conjugated proteins. Other mAbs cross-reacted with the above conjugates, methylene diphenyldiisocyanate- and hexamethylene diisocyanate protein conjugates. All mAb reactivities were carrier protein independent.

CONCLUSIONS: The mAbs can differentiate between specific isocyanates in a carrier independent fashion and may be useful as hapten-specific reagents for immunoassay development.

573 Genetic Variability Of Crth2 Polymorphism Is Associated With The Required Dose Of Antihistamines In Patients With Chronic Urticaria

N. Shrestha Palikhe, S. H. Kim, Y. M. Ye, G. Y. Hur, H. S. Park; Ajou University, School of Medicine, Department of Allergy and Rheumatology, Suwon, Republic of Korea.

RATIONALE: Chronic urticaria (CU) is a common disorder associated with mast cell activation, degranulation and histamine release. Skin mast cell and basophils are the key cells involved in CU. We investigated the association of PGD2 receptor, *CRTH2* gene promoter polymorphism with the CU phenotype and antihistamines drug requirement in patients with CU.

METHODS: Two promoter polymorphisms of the *CRTH2* gene, -466 T/C and 129C/A were genotyped using primer extension methods from 384 CU patients and 231 as normal controls (NCs). The requirement of antihistamines was calculated from total antihistamine doses taken for control of CU and presented as the equivalent dose of loratadine. *In-vitro* functional study was carried out by dual luciferase system.

RESULTS: No significant differences were observed in the genotype, allele frequencies and clinical parameters (atopy status, serum total IgE, prevalence of autoantibodies, and duration of CU) of the two *CRTH2* polymorphisms between the CU and NC groups, However, CU patients with the TT genotypes had significantly higher dose requirements of antihistamines to control the CU symptoms (164.56 $\pm$ 115.62 versus 137.38 $\pm$ 90.15 loratadine equivalents, mg/week) than those with the CT and CC ($P = 0.025$). The promoter activity was significantly enhanced in the *CRTH2*-466C compared to the -466 T allele in HMC-1 cells ($P < 0.001$). Co-transfection with GATA-3 revealed that the *CRTH2* -466 T allele produced a greater increase in induction of luciferase activity than the -466C allele ($P < 0.001$).

CONCLUSIONS: The *CRTH2* -466 T/C gene polymorphism may contribute to the required dose of antihistamines in patients with CU.

574 Variants in the Tight Junction Gene, *Claudin-1* are Associated with Atopic Dermatitis in Two American Populations and May Contribute to Skin Barrier Dysfunction

A. De Benedetto[1], N. M. Rafaels[2], D. Y. M. Leung[3], A. I. Ivanov[1], T. Hand[2], L. Gao[2], M. Yang[2], M. Boguniewicz[3], T. R. Hata[4], L. Schneider[5], J. M. Hanifin[6], R. L. Gallo[4], K. L. Barnes[2], L. A. Beck[1]; [1]University of Rochester Medical Center, Rochester, NY, [2]Johns Hopkins University School of Medicine, Baltimore, MD, [3]National Jewish Medical and Research Center, Denver, CO, [4]University of California San Diego, San Diego, CA, [5]Children's Hospital Boston, Boston, MA, [6]Oregon Health & Science University, Portland, OR.

RATIONALE: Claudins are components of epithelial tight junctions (TJ), which control paracellular movement of water, solutes and immune cells. We have previously shown that claudin-1 expression is markedly reduced in Atopic Dermatitis (AD) skin which suggests that the barrier defect observed in this disease may also be at the level of TJs.

METHODS: We screened 27 haplotype-tagging single nucleotide polymorphisms (SNPs) in *CLDN1* to identify variants that confer susceptibility for AD in independent groups of patients and healthy-controls, a European American (EA; N = 321) and African American (AA; N = 307) group. Single SNP analyses were performed using the Cochran-Armitage trend test. *In vitro*, we investigated the modulation of claudin-1 protein in differentiated primary keratinocytes (dPHFK). The barrier function of claudin-1 in dPHFK monolayers was evaluated using a knockdown approach (siRNA).

RESULTS: Significant associations were observed with a noncoding SNP (rs17501010; P = 0.003) and a 5'SNP (rs9290927; P = 0.04) and AD among AA. While a SNP in the promoter region (rs16865373; P = 0.03) was significantly associated with AD among EA. Claudin-1 protein expression was significantly upregulated by IL-4 (2.$\pm$ 0.7 fold;n = 3), IL-13 (1.6 $\pm$ 0.5 fold;n = 3) and TLR-2 ligands (1.4 $\pm$ 0.1 fold;n = 3) but not by TNFα, IFNγ or IL-17. Knockdown of claudin-1 reduced transepithelial electrical resistance by 34.3 $\pm$ 9.8% (p <0.02;n = 3).

CONCLUSIONS: Results from this study suggest that genetic or acquired defects in claudin-1 may be observed in AD subjects. Knockdown experiments confirm the critical importance of claudin-1 in epithelial barrier function. This is the first study to implicate *CLDN1* as a strong candidate gene for AD.

575 Molecular Profiling Distinguishes Patients With Active Idiopathic Anaphylaxis From Normal Volunteers And Reveals Novel Aspects Of Disease Biology

D. L. Howell, C. Jacobs, G. Metz, C. Rhodd, J. Zhang, S. Dave, P. Lugar; Duke University, Durham, NC.

RATIONALE: Idiopathic anaphylaxis (IA) is a disorder characterized by episodes of anaphylaxis and spontaneous remission. There is heterogeneity within the disease with respect to severity, symptoms and response to treatment. We sought to investigate whether gene expression profiles of mononuclear cells (MC) obtained from IA patients demonstrate distinct differences compared to those obtained from controls. We reasoned that the molecular profile of patients with IA would reveal key elements of the biology of the disease and allow for the identification of novel markers and treatment.

METHODS: 9 patients with IA and 5 non-atopic controls were enrolled. MC were isolated from peripheral blood using Ficoll density centrifugation. MC were profiled for gene expression at the whole-genome level using Affymetrix U133plus DNA microarrays. The scanned data were normalized and the gene expression of IA patients was compared to that of controls. We further analyzed the data for expression of genes associated with CD203c.

RESULTS: We found 53 genes predicted IA from controls. The genes in the predictor are differentially expressed (P < 0.001) with a minimum 2-fold difference between IA patients and controls. The predictor was 100% successful in distinguishing patients with IA from controls, and tested using leave one out cross-validation.

Among these genes are regulators of cell activation and mast cell/basophil degranulation. We also found 106 genes that strongly correlated with the level of CD203c (r = >.75, p < 0.01).

CONCLUSIONS: Gene expression profiling can reliably distinguish patients with IA from controls. The gene analysis suggests potential for understanding mechanism of disease, biomarker development and targeted treatment.