1023 Heterogeneous IL-5 Expression Defines Two Phenotypically Distinct Human Th2 Subpopulations

B. Upadhyaya, Y. Yin, C. Prussin; NIAID/NIH, Bethesda, MD.

RATIONALE: Th2 cells express IL-4, IL-5 and IL-13, each having different functions. We hypothesized that Th2 heterogeneity could yield Th2 subpopulations with different cytokine expression, effector functions and roles in disease pathogenesis.

METHODS: IL-4, IL-5 and IL-13 expression were simultaneously measured in human CD4 T cells using polychromatic cytokine flow cytometry.

RESULTS: In non-allergic healthy controls, IL-4 and IL-13 were respectively expressed by 70% and 81% of Th2 cells, whereas, IL-5 was expressed by only 15% of Th2 cells. In eosinophilic gastrointestinal disease subjects, IL-5 was expressed by 23% of Th2 cells, and IL-4 and IL-13 were similarly coexpressed. In both subject groups, two major Th2 subpopulations were identified: IL-5^+ Th2 (IL-5^+, IL-4^+, IL-13^+) and IL-5^- Th2 (IL-5^-, IL-4^+, IL-13^+). IL-5^+ Th2 cells expressed significantly less CD27 and greater PD-1 than did IL-5^- Th2 cells ($p=0.03$). During five serial rounds of *in vitro* Th2 differentiation, there was a consistent and markedly delayed acquisition of the IL-5^+ relative to the IL-5^- Th2 phenotype. IL-5^+ and IL-5^- Th2 subpopulations were sorted, expanded *in vitro* for 1-2 weeks, and upon analysis maintained the original IL-5 bias of the sorted populations.

CONCLUSIONS: Th2 cells consist of two subpopulations: IL-5^+ and IL-5^- Th2 cells. IL-5^+ Th2 cells display phenotypic markers of highly differentiated memory T cells and require multiple rounds of differentiation. These results suggest that IL-5^+ Th2 cells represent a highly differentiated subpopulation of Th2 cells that have different functional properties and disease association than IL-5^- Th2 cells and may make a unique contribution to allergic inflammation.

1024 Characterization of *Paecilomyces variotii* Allergens

B. J. Green, W. R. Rittenour, J. M. Hettick, E. Janotka, D. H. Beezhold; National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention, Morgantown, WV.

RATIONALE: *Paecilomyces variotii* is a hydrophilic fungal species. Preliminary studies have identified *P. variotii* as an aeroallergen, occupational hazard, and potential biomarker of indoor fungal exposure. In this study, we characterized the allergens derived from *P. variotii*.

METHODS: A diverse collection of participant sera that were positive to a fungal skin prick test or ImmunoCAP (n=24) were selected for analysis. Hyphal extracts derived from *P. variotii* were separated by SDS-PAGE or 2D gel electrophoresis and immunostained with patient serum IgE following western blotting. Immunostained *P. variotii* antigens were excised and sequenced using nanoscale Ultra-Performance Liquid Chromatography Tandem Mass Spectrometry (nanoscale UPLC-MS/MS). De-identified sera from 6 healthcare workers (HCW) and 2 non-atopics were used as controls.

RESULTS: Immunoblotting of *P. variotii* hyphal extracts revealed IgE against 25 antigens within the range 18-132 kDa. IgE reactivity towards 25, 29, 44, and 65 kDa antigens bound IgE from 88%, 96%, 88%, and 58% of study participants, respectively. These immundominant allergens were identified by nanoscale UPLC-MS/MS to be 60S ribosomal protein L10 (25 kDa), electron transfer flavoprotein B subunit (29 kDa), ketol acid reductoisomerase (44 kDa), and heat shock protein 70 (65 kDa). Other antigens that bound IgE did not return significant matches from the protein databank. No IgE antibodies were identified in non-atopic controls.

CONCLUSIONS: Participants with specific IgE to *P. variotii* reacted most commonly with higher molecular weight (MW) antigens. Four immunodominant allergens were identified to bind IgE in greater than 50% of the study participants.

1025 IgE Cross-reactivity to Tropomyosins among Patients with Asthma and/or Rhinitis from Brazil: Analysis by Component-based Allergen Microarray

A. C. S. Santos[1], G. A. Bardini[1], K. S. Santos[2], M. C. R. Barbosa[1], A. B. R. Santos[1], L. K. Arruda[1]; [1]School of Medicine of Ribeirao Preto, Ribeirao Preto, BRAZIL, [2]University of São Paulo School of Medicine, São Paulo, BRAZIL.

RATIONALE: We have previously demonstrated that recombinant tropomyosins from *Ascaris lumbricoides* and cockroach had biological activity. The aim of this study was to evaluate IgE reactivity to tropomyosins by component-resolved allergy microarray.

METHODS: Skin tests to recombinant tropomyosins were conducted in 40 cockroach allergic patients with asthma and/or rhinitis, aged 12 to 75. IgE antibodies to *A. lumbricoides* tropomyosin and rPer a 7 were measured by chimeric ELISA; IgE to purified tropomyosins from cockroach (Bla g 7) and *Anisakis simplex* (Ani s 3, 95% identity to *Ascaris* tropomyosin) were assessed by Immuno Solidphase Allergen Chip (ISAC).

RESULTS: Excellent concordance was found among methods to assess sensitization to cockroach tropomyosin, with the following kappa indexes (95% CI): skin tests to rPer a 7 and IgE to rPer a 7 by ELISA 0.89 (0.76; 1.00); IgE to rPera 7 by ELISA and IgE to Bla g 7 by ISAC, 0.83 (0.66; 1.00); skin tests to rPer a 7 and IgE to Bla g 7 by ISAC, 0.95 (0.84; 1.00). Similarly, high concordance rates were observed for methods to assess sensitization to *Ascaris* tropomyosin, with the following kappa indexes (95% CI): skin tests to recombinant *Ascaris* tropomyosin and IgE to *Ascaris* tropomyosin by ELISA 0.75 (0.55; 0.95); IgE to *Ascaris* tropomyosin and IgE to Ani s 3, 0.66 (0.42; 0.91); skin tests to recombinant *Ascaris* tropomyosin and IgE to Ani s 3, 0.84 (0.70; 1.00).

CONCLUSIONS: Evaluation of IgE responses to tropomyosins from *Ascaris* and cockroach by *in vivo* and *in vitro* methods showed highly concordant results.

1026 Evolution of IgE responses to Bet v 1-like Molecules throughout Childhood: A longitudinal Study in the MAS Birth Cohort

L. Hatzler[1], A. Buongiovanni[1], A. Rohrbach[1], A. Dondi[1], T. Keil[2], S. Lau[1], U. Wahn[1], P. M. Matricardi[1]; [1]Department of Pediatric Pneumology and Immunology, Charité University Medical Center, Berlin, GERMANY, [2]Institute for Social Medicine, Epidemiology, and Health Economics, Charité University Medical Center, Berlin, GERMANY.

RATIONALE: Bet v 1-like molecules, also known as class 10 pathogenesis-related (PR-10) proteins, are characterized by cross-reactivity with the major birch pollen allergen Bet v 1. In this study we aimed to prospectively investigate the evolution of the IgE response to PR-10 proteins throughout childhood.

METHODS: Blood samples from birch sensitized children (IgE-Ab to birch extract >1kU/l) at ≥3 time points, spanning ≥5 years were selected from Multicenter Allergy Study (MAS) birth cohort serum bank. After verifying Bet v 1 sensitization at the last measuring point for each child using ImmunoCAP (Phadia), allergen microarray (ImmunoCAP ISAC, Phadia) was performed.

RESULTS: Our data show that 1) the IgE reaction against Bet v 1 is persistently the strongest one among PR-10 proteins; 2) the number of IgE responses to the other PR-10 proteins is related to the intensity of IgE reaction to Bet v 1 and increases progressively with age; 3) the observed prevalence of IgE sensitization to PR-10 proteins is: Aln g 1 (66%), Mal d 1 (64%), Cor a 1.0401 (60%), Pru p 1 (59%), Cor a 1.0101 (58%), Ara h 8 (47%), Gly m 4 (34%), Api g 1 (29%), Act d 8 (18%), Dau c 1 (5%); 4) this hierarchy is roughly reproduced by the time-sequence of new sensitizations or remissions at the single child level.

CONCLUSIONS: Our results suggest that in the MAS cohort Bet v 1 induced the sensitization to examined PR-10 proteins and the hierarchy of sensitization to these allergens roughly reflects their sequence homology with Bet v 1.