Oral Session 15

Novel developments in immunogenetics and immunogeneomics

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Quantitative proteomics: a strategy to identify respiratory disease biomarkers in bronchial biopsies

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Background: Recent proteomic studies on respiratory conditions investigate proteome changes to identify protein biomarker candidates for diagnosis, monitoring and treatment. A major challenge in clinical proteomics is the limited patient material. This is usually addressed by pooling patients into disease groups. With sensitive quantitative proteomics methods, like iTRAQ (isobaric tag for relative and absolute quantitation) technology, to identify and quantify proteomic profiles with mass spectrometry, it may be possible to determine individual patient proteome differences without the need for pooling. The feasibility of this strategy is examined with the identification of proteins in bronchial biopsies of asthma and COPD patients, in an effort to identify disease and treatment specific protein biomarker candidates.
Methods: Bronchial biopsies per patient, per timepoint, were taken from asthmatic ($n = 14$) and COPD ($n = 16$) subjects, treated with budesonide or placebo, before and after treatment. Healthy subjects ($n = 4$) and healthy smokers ($n = 4$) were used as controls. Biopsies were randomised over 20 sets of 4-plex experiments with the operator being blinded to the disease status. Each 4-plex experiment contained a common reference pool for normalisation purposes. Proteins extracted from the biopsies were digested, labelled with iTRAQ reagents and fractionated using cation exchange chromatography, before each of the fractions was subjected to reversed phase nanoLC-MS/MS. The resulting spectra were analysed by Proteome Discoverer and the Swissprot database was searched for protein identifications.
Results: Bronchial biopsies yielded a small, but sufficient amount of protein to conduct proteomic experiments using iTRAQ technology. Sixty-five micrograms of protein was digested, fractionated and analysed by nanoLC-MS/MS. Early results showed that more than 1000 proteins could be identified with high statistical confidence.
Conclusion: Small amounts of patient bronchial biopsy material may be used to identify and quantify proteins using the iTRAQ technology with a highly sensitive MS system, without the need for pooling of samples from several patients. This method may be used to identify airway disease specific mucosal biomarkers.

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Genetic markers in chronic urticaria

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Background: The cellular role in chronic urticaria (CU) has been gradually recognised, however the genetic determinism is not well identified. The aim of this work was to evaluate the influence of HLA haplotype and the association of genetic polymorphism of some cytokines in CU.
Methods: After signed informed consent, in a 15 patients population (10 female, mean age 45.7 ± 8.8 years and disease evolution time of 6.5 ± 5.9 years), with clinical criteria of CU and previous clinical and laboratory evaluation, blood samples were collected to assess class I (locus A and B) and class II (locus DR) HLA genetic typing and evaluate cytokine single nucleotide polymorphisms (SNP's) in the promoter region of IL-1 (α,β,R,RA), IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, TGF-β and TNF-α. The results were compared with a group of 174 healthy individuals (group C).
Results: It was observed a higher genotype frequency in locus A for A*26 (NS) and A*68 ($P = 0.048$), while for A*01 (NS) it was identified a lower frequency, comparing with control group. In respect to locus B, the allele B*51 was the most frequent (NS); there was a higher frequency of B*38 in CU comparing to group C ($P = 0.047$). For class II, the alleles DRB1*04, DRB1*07 and DRB1*15 were the most frequent, comparing to group C, but without statistical significance. The most frequent haplotype was the A*01 B*08 DRB1*03, as in group C; however the second most frequent was A*02 B*51 DRB1*11, corresponding to the fifth most frequent of the controls (4.7% *versus* 1.4%, P 0.106). It was found a higher phenotype frequency for the following cytokine SNP's: IL10-592CC ($P = 0.021$), IL10-819CC ($P = 0.021$), TNFα-308GG ($P = 0.018$), IL1a-889CC ($P = 0.043$), TGFβ + 869CT ($P < 0.0001$), TGFβ + 915CG ($P = 0.001$) and IL2 + 166TT and GT ($P = 0.014$ and 0.020). On the other hand, there were lower frequencies for IFNγ + 874TT ($P = 0.001$), TGFβ + 915GG ($P = 0.023$), IL2-166GG ($P < 0.0001$). In group CU there were not find heterozygous for TNFα-238AG ($P < 0.0001$) and homozygous for TNFα-308AA ($P < 0.0001$), TGFβ + 869TT and +915CC ($P < 0.0001$ and 0.030), IL2-330TT ($P = 0.002$) and IL4-1098TT and -33TT ($P = < 0.0001$ and 0.014).
Conclusion: The haplotype and cytokine SNP's differences found suggest an immunogenetic contribution in CU pathogenesis. There were identified significant changes in cytokines of Th1 and Th2 response, explaining the immunologic and clinical complexity of this syndrome.

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Differential gene expression in the sensitisation and tolerance to olive pollen: microarray analysis

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Background: Microarrays studies could be very useful in the identification of new routes or pathways that can discriminate sensitization and natural or induced tolerance against allergens.
Methods: Subjects living in an area with extremely high load of olive pollen were collected in two exposition moments, high pollen levels (pollen season) and low (outside pollen season) distributed in 5 Groups: