116 Genome-Wide Association Studies of Asthma Indicate Opposite Immunopathogenesis Direction From Autoimmune Diseases

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Using the human promonocytic cell line, THP1, we have successfully established a THP1-derived and committed CAM and AAM populations demonstrating typical macrophage-oriented morphological characteristics.

Results: Quantitative PCR and ELISA demonstrated that THP1-AAM cell model express classic pathogen neutralizing dectin receptors such as scavenger type mannose receptor (MRC1) and Th2-associated signature chemokines including CCL13, 17, 18 and 22, and are tolerant to TLR4 challenge by LPS treatment in contrast to THP1-CAM which expressed an LPS enhanced expression of pro-inflammatory mediators such as TNF-α, CXCL10 and -11. Furthermore, THP1-AAM cell model expressed 50- to 100-fold lower expression IFN-alpha 4, IFN-beta, and IFN-lambda1 compared to THP1-CAM. Quantitative PCR array revealed that a select group of interferon regulatory factors (IRFs), antiviral genes such as Mx1, and interferon stimulated genes such as ISG15 are down-regulated only in THP-1 AAM cell model upon differentiation or LPS treatment emphasizing its classic infection tolerant phenotype. In addition, IRF4 was found to be up-regulated only in the THP1-AAM model which may point towards its critical role in orchestrating the macrophage lineage commitment towards an alternatively activated phenotype as well as governing its unique cytokine and chemokines expression profile.

Conclusions: Compared to the donor variability of primary human monocytes, establishing THP1-AAM and CAM cell models will enable a more rapid and efficient investigation of a spectrum of molecular mechanisms governing innate, classic, and alternative phenotypes in macrophage populations and their role in pathologic processes, in particular allergic inflammation of the upper airways.

114 A Highly Sensitive and Specific Universal Mirna Profiling Method

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Background: miRNAs can be used as robust biomarkers for diagnosis, staging, prognosis and the response to therapy in various diseases. Although a wide spectrum of miRNA detection techniques have been developed, none can accurately and sensitively perform genome-wide high-throughput miRNA profiling (Chen C, Ridzon DA, Broomer AJ, Zhou Z, Lee DH, Nguyen JT, Barbisin M, Xu NL, et al 2005. Real-time quantification of miRNAs by stem-loop RT-PCR. Nucleic Acids Res. 33:e179). This problem stems from that miRNAs are only ~22 bases, and multiple species of nucleic acids that contain the mature miRNA sequences are present in the total RNA samples that are usually used for miRNA detection.

Methods: A novel RT-qPCR miRNA assay (UQmiR, universally quantitating miRNA) was developed to overcome the difficulty. This assay requires only one RT reaction and one universal set of multiple hydrolysis probes to detect all miRNAs, using one universal RT primer, a common reverse primer, and individual miRNA-specific forward primers. A computer program (MSPPD, miRNA-specific primer and probe designer) was developed for the assay.

Results: The UQmiR has the advantages, but not the disadvantages, of the 2 mostly used miRNA assays. It has the specificity of hydrolysis probe assay and the universal detection of SYBR Green assay. This assay is more sensitive and specific than the commercially available hydrolysis probe assay and SYBR Green assay. Using this method, we have successfully detected 91 out of 96 miRNAs in 0.8 ml of plasma for each miRNA.

Conclusions: This approach affords a highly specific, sensitive, economical and convenient system to profile the expression of all known miRNAs.

115 Caspase-4 Plays a Role in the Activation of the Cryopyrin/ NLRP3 Inflammasome

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Background: The inflammasome is a multi-protein complex which regulates the activation of caspase-1. This activation results in the cleavage and secretion of the IL-1β super family cytokines, IL-1β, IL-18, and IL-33. NLR family-pyrin domain containing- 3 (NLRP3) is a nucleotide binding domain-leucine rich repeat (NLR) family protein responsible for sensitization and oligomerization of the NLRP3 inflammasome complex. Although various damage and pathogen associated patterns have been implicated as stimuli, the exact mechanism of activation has yet to be elucidated. Caspase-5, an inflammatory caspase with similar homology to caspase-1, is a key molecule activation of the NLRP1 inflammasome. Caspase-4, an evolutionary duplicate in humans to murine caspase 12 along with caspase 5, is important in IL-1β processing; its involvement with the NLRP3 inflammasome is unknown. We therefore investigated whether caspase-4 plays a role in the activation of the NLRP3 inflammasome.

Methods: Inflammases in THP-1 macrophages were activated using Nigericin (10 μg/mL), a bacterial pore causing toxin and NLRP3 inflammasome-activating factor, in the presence or absence of various concentrations (0.1 μM, 1 μM, and 10 μM) of caspase-4 inhibitor, Z-YVAD-FMK. We analyzed the inflammasome activation, caspase-1 cleavage, and IL-1β release by western blot and ELISA analysis.

Results: Our results indicate that inhibition of caspase-4 leads to a dose dependent decrease in IL-1β secretion. In addition, our results show that caspase-4 contributes to IL-1β and caspase-1 cleavage, both of which are hall marks of inflammasome activation.

Conclusions: These findings suggest that caspase-4 is important to the activation of the NLRP3 inflammasome. In modulating the inflammasome, caspase-4 appears to be a druggable target for treatment of chronic inflammatory pulmonary conditions such as allergy and asthma.

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Background: Genome-wide association studies (GWAS) of asthma and asthma-related traits, including our previous TENOR study, have consistently identified ORMDL3-GSDMB, IL33, IL1RL1-IL18R1, RAD50-IL13, TSLP-WDR36, and HLA-DR/DQ regions. Methods: In this study, GWAS of asthma was performed in non-Hispanic white population from STAMPEED study (813 cases and 1564 controls). Our GWAS results were compared with the published GWAS of asthma and autoimmune diseases (AD).

Results: Multiple SNPs in TNFAIP3 interacting protein 1 (TNIP1) on chromosome 9q32-q33.1 were associated with asthma in STAMPEED: rs1422673 (P = 3.44 × 10^-7) and rs10036748 (P = 1.41 × 10^-6). rs1422673 was weakly associated with asthma in the published Gabriel study (P = 0.018 for meta-analysis) but not in the TENOR study (P = 0.18 but same trend). TNIP1 may interact with TNFAIP3 and inhibit TNFα-induced NFκB inflammation pathway. Joint analyses were performed on 6 SNPs in GSDMB (rs2872507), IL33 (rs3939286), IL1RL1 (rs1341828), IL13 (rs20541), TSLP (rs1837253), and HLA-DRA (rs2395185) in STAMPEED and TENOR populations, but only limited variance can be explained (percentage of deviance = 1.5–1.9%); the area under the receiver operating characteristic curve (AUC) = 0.58–0.59. Minor allele T of rs20541 in IL13 is the risk allele for asthma but the protective allele for psoriasis. Minor allele A of rs2872507 in GSDMB is the protective allele for asthma but the risk allele for rheumatoid arthritis. Cronb’s disease and ulcerative colitis. T allele of rs10036748 in TNIP1 is the minor protective allele for asthma, but the minor or major risk allele for systemic lupus erythematosus in non-Hispanic white or Chinese population, respectively.

Conclusions: Our study provides genetic evidence that asthma and AD have opposite immunopathogenesis directions.

REFERENCES


118 EGCGB Downregulates Mucin Gene Expression Through the Mapk Signaling Pathway in Asthma

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Background: Mucus plays an important role in protecting human airway from external environments. Highly glycosylated mucin proteins are the major components of mucus, responsible for its viscoelastic properties. Excessive mucus is major manifestation of inflammatory respiratory diseases. Epigallocatechin-3-gallate (EGCG) is major component of green tea extract and known to provide numerous functions, such as anti-oxidant effect, anti-tumor effect, anti-diabetic effect and anti-inflammatory effect. But precise mechanisms are still unclear.

Methods: Using NCI-H292 human airway epithelial cells, we measured phorbol 12-myristate 13-acetate (PMA)-induced MUC5B mRNA expression with the treatment of indicated doses of EGCG. We also measured PMA-induced MUC5B protein secretion with the treatment of indicated doses of EGCG using ELISA technique in NCI-H292 cells. To test the brief signaling pathways, we performed activation study of mitogen-activated protein kinase (MAPK) pathways, which is well-known to signaling the PMA-induced mucin gene over-expression, using Western blot technique in NCI-H292 cells. And then we performed in vivo study using ovalbumin-induced asthmatic mice model and control mice group. In ovalbumin-sensitized asthmatic mice model, EGCGB was treated with indicated dose. And then ovalbumin was challenged and we sacrificed the mice. Tissue samples from the mice were stained with PAS (periodic acid-Schiff) for mucin distribution in bronchioles of each group. Immunocytochemical analyses supported a very rapid traffic through the epithelium in allergic patients, but not in healthy subjects. A striking specificity is observed when birch pollen allergic subjects were also challenged with timothy grass pollen and no entry of this pollen allergen Phl p 1 into epithelial cells was detected. While the specific transport mechanism for birch pollen remains unsolved the first hints of the role of caveolae in this have been obtained. In the double immunofluorescence analyses caveolin 2, but not caveolin 1 or 3, was present on the conjunctival epithelial surface in the same clusters as Bet v 1. Transcriptomics indicated that the health epithelium displayed a strong immune response against pollen allergens while this response was absent in the epithelium of allergic patients.

Conclusions: Active transport of allergens through the epithelium might be incorporated to the pathogenesis of allergy. It is possible that the healthy epithelium displays a strong immune response against pollen allergens and thus escapes from becoming allergic. If allergy turns out to be, at least in part, a result of epithelial hyporesponsivity, it could have major consequences in the strategies of prevention and treatment of these diseases. Towards this end, a national allergy program has been launched in Finland, which changes the basic idea of trying to avoid allergens to the concept of natural exposure and tolerance.

MECHANISMS OF ASTHMA