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

Anne Fitzpatrick, Emory University
X Li
E Ampleford
T Howard
D Torgerson
H Li
W Moore
W Busse
M Castro
S Erzurum

Only first 10 authors above; see publication for full author list.

Journal Title: World Allergy Organization Journal
Volume: Volume 5, Number
Publisher: (publisher) | 2012-02, Pages S38-S39
Type of Work: Article
Publisher DOI: 10.1097/01.WOX.0000411861.60664.a3
Permanent URL: https://pid.emory.edu/ark:/25593/s5nmk

Final published version:
http://dx.doi.org/10.1097/01.WOX.0000411861.60664.a3

Accessed December 31, 2017 9:40 PM EST
range of physiologic and pathological processes including clearance of hemorrhagic infections, and allergy. They are closely associated with recruiting and amplifying T helper 2 (Th2) lymphocyte response in contrast to Th1-associated CAMs. Wide donor-to-donor variability of human primary monocytes and their limited life span in vitro is a current impediment to investigating human AAM biology and their contribution to enhancing Th2-mediated pathologic inflammation found in asthmatic lungs.

Methods: 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-a, 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, IFR4 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

Jia-Wang Wang, PhD,1 Kunyu Li, BS,1 Richard F. Lockey, MD,2 Subhra Mohapatra, PhD,1 and Shyam Mohapatra, PhD.1 1Internal Medicine, University of South Florida, Tampa, FL; 2Division of Allergy & Immunology, University of South Florida and James A. Haley Veterans’ Hospital, Tampa, FL; 3Nanomedicine Research Center and Division of Translational Medicine, Department of Molecular Medicine and Internal Medicine, University of South Florida and James A’ Haley Veterans’ Hospital, Tampa, FL; 4USF Nanomedicine Research Center, University of South Florida College of Medicine & VA Hospital, Tampa, FL.

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 microRNAs 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/NLPR3 Inflammasome

Ruan Cox, Jr,1 Salman Aljubran, MD,2 Richard F. Lockey, MD,3 and Narassiah Kolliputi, PhD.1,4 1Internal Medicine, and; 2Allergy and Immunology, University of South Florida, Tampa, FL; 3Division of Allergy & Immunology, University of South Florida and James A. Haley Veterans’ Hospital, Tampa, FL; 4Division of Allergy and Immunology.

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 (NLPR3) is a nucleotide binding domain-leucine rich repeat (NLR) family protein responsible for sensitzation and oligomerization of the NLPR3 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 NLPR3 inflammasome is unknown. We therefore investigated whether caspase-4 plays a role in the activation of the NLPR3 inflammasome.

Methods: Inflammasomes in THP-1 macrophages were activated using Nigericin (10 μg/mL), a bacterial pore causing toxin and NLPR3 inflammasome activator, 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 hallmark of inflammasome activation.

Conclusions: These findings suggest that caspase-4 is important to the activation of the NLPR3 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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Xingnan Li, PhD, MS,1 Elizabeth Ampleford, PhD,1 Timothy Howard, PhD,2 Tara Jorgenson, PhD,2 Huashi Li, MS,3 Wendy Moore, MD,3 William Busse, MD,1 Mario Castro, MD, MPH,1 Seripil Erurum, MD,3 Anne Fitzpatrick, PhD,6 Benjamin Gaston, MD,7 Elliot Israel, MD,8 Nizar Jarjour, MD,7 W. Gerald Teague, MD,7 Sally Wenzel, MD,10 Gregory Hawkins, PhD,1 Carole Ober, PhD,2 Dan Niclaue, PhD,2 Eugene R. Bleecker, MD,1 and Deborah Meyers, PhD.1 1Center for Genomics and Personalized Medicine Research, Wake Forest University School of Medicine, Winston Salem, NC; 2Department of Human Genetics, University of Chicago, Chicago, IL; 3Department of Medicine, University of Wisconsin, Madison, WI; 4Division of Pulmonary and Critical Care Medicine,

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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, ILIRIL/IL1R1, RAD50-DI.13, 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 TNAIP3 interacting protein 1 (TNIP1) on chromosome 5q32-3q3.1 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 TNAIP3 and inhibit TNFα-induced NFκB inflammation pathway. Joint analyses were performed on 6 SNPs in GSDMB (rs2872507), IL33 (rs3939286), ILIRIL (rs1431828), IL13 (rs20541), TSLP (rs1837253), and HLA-DR (rs2395185) in STAMPEED and TENOR populations, but only limited variance can be explained (percentage of deviance = 1.9–1.9%); 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 asthma but the risk allele for rheumatoid arthritis, Crohn’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 EGCG Downregulates Mucin Gene Expression Through the MAPK Signaling Pathway in Asthma

Yong-Dae Kim, Sang Baik Ye, Chang Hoon Bae, and Si-Youn Song.

Yeungnam University College of Medicine, Daegu, South Korea.

Background: Mucus plays an important role in protecting human airway regions. Mucus plays an important role in protecting human airway regions. TSLP (mucus cell transport protein 1) of the airway is expressed in mucus cells and is a major component of mucus, responsible for its viscoelastic properties. Excessive mucus is an important factor in the development of respiratory diseases. Epigallocatechin-3-gallate (EGCG) is a major component of green tea extract and is known to provide numerous functions, such as anti-oxidant effect, anti-inflammatory 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 effect of EGCG on the expression of MUC5B, we performed activation study of mitogen-activated protein kinase (MAPK) pathways, which is well-known to the signaling pathway of the PMA-induced mucus 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, EGCG 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 stain was performed using MUC5B specific antibody. MUC5B mRNA and protein level was measured using extracted lung tissues.

Results: PMA-induced MUC5B mRNA and protein level was significantly decreased after treatment of EGCG at all doses in NCI-H292 cells. PMA-induced phosphorylation of p38 MAPK was significantly decreased after treatment of EGCG at all doses in NCI-H292 cells. Results from in vivo studies showed that decreased bronchiolar mucin distribution in the group of pretreated with EGCG in asthmatic mice. MUC5B mRNA and protein levels were significantly decreased in the group of pretreated with EGCG in asthmatic mice.

Conclusions: PMA-induced MUC5B mRNA and protein over-expression in both NCI-H292 cells and extracted tissues from asthmatic mice was significantly decreased with the treatment of EGCG. We demonstrated that