499 Mycoplasma Pneumoniae Cards Toxin Regulates NLRP3 Inflammasome Activation

Jesus A. Segovia, Jr, PhD[1], Santanu Bose, PhD[2], Sudha R. Somarajan[3], Te-Hung Chang[1], Thirumalai Kannan, PhD[1], Joel Barry Baseman, PhD[1]; [1]UT Health Science Center at San Antonio, San Antonio, TX, [2]Washington State University, Pullman, WA, [3]UT Health Science Center at Houston, Houston, TX.

RATIONALE: *Mycoplasma pneumoniae* is a common bacterial airway pathogen that possesses the Community-Acquired Respiratory Distress Syndrome (CARDS) toxin. CARDS toxin is capable of reproducing the robust inflammation and cytopathology associated with *M. pneumoniae* infection. We hypothesized that CARDS toxin interacts with components of the host innate immune response to trigger inflammation mediated by the cytokine interleukin-1β (IL-1β).

METHODS: WT or NLRP3 knockout bone-marrow derived macrophages were treated with WT CARDS toxin or different CARDS mutants for different time points. Supernatants and lysates were assayed for IL-1β maturation and caspase-1 activation. NLRP3-overexpressing 293 cells were treated with CARDS toxin and immunoprecipitation was performed on the cell lysates to study the interaction of CARDS toxin with NLRP3. NLRP3-overexpressing 293 cell lysate was also used for an ADP-ribosylation assay.

RESULTS: Treatment of macrophages with CARDS toxin triggers inflammasome complex formation, resulting in caspase-1 activation and IL-1β secretion. CARDS mutants deficient for cellular entry or ADP-ribosylation failed to activate the inflammasome complex. CARDS toxin was found to interact directly with NLRP3. Furthermore, CARDS toxin modifies NLRP3 by ADP-ribosylation.

CONCLUSIONS: We have uncovered a novel mechanism by which *M. pneumoniae* CARDS toxin ADP-ribosylates NLRP3, triggering inflammasome activation and enhanced inflammation. This is the first report of ADP-ribosylation as a post-translational modification that mediates inflammasome activation.

500 Fcγ-Fragment and IgG Monoclonal Antibody Polarization of Human Macrophages; A Novel Immunomodulatory Mechanism

Ryan A. Steele, DO, MS[1], Michael J. Littlefield, BA[2], Iryna Voloshyna, PhD[2], Mark A. Davis-Lorton, MD, FAAAAI[3], Marcella R. Aquino, MD, FAAAAI[1], Luz S. Fonacier, MD, FAAAAI[1], Allison B. Reiss, MD[2]; [1]Winthrop University Hospital, Allergy & Immunology, Mineola, NY, [2]Winthrop Research Institute, Department of Medicine, Winthrop University Hospital, Mineola, NY, [3]Winthrop University Hospital, Mineola, NY.

RATIONALE: Omalizumab (mAb), a humanized IgG1k monoclonal antibody, is an indicated treatment for allergic asthma and chronic urticaria. Efficacy in non-atopic asthma patients suggests alternative non-IgE mechanisms. Our laboratory has shown mAb helps to drive polarization of THP-1 human monocytes. We investigate if this polarization is a Fab or Fcγ mediated phenomenon.

METHODS: THP-1 monocytes were differentiated with PMA, then stimulated with IFNγ/LPS and IL-4 to polarize them into the M1 and M2 state, respectively. Cells were stimulated with mAb or human Fcγ ± IFNγ/LPS or IL-4 (during and after polarization). Then, mRNA was isolated and subjected to QRT-PCR to examine profile markers for M1 (CCR7 and IL12), and M2 (CD163 and CCL17). Data was normalized to GAPDH.

RESULTS: Expression of CCR7 was increased over IFNγ/LPS controls in cells costimulated with IFNγ/LPS+mAb (26-fold, $p<0.0001$) and IFNγ/LPS+Fcγ (31-fold, $p<0.0001$). CD163 expression was reduced in cells costimulated with IL-4+mAb (by 59%, $P<0.0001$) and IL-4+Fcγ (by 56%, $p>0.0001$) versus IL-4 alone. Expression was also reduced in cells polarized first with IL-4 then stimulated with mAb (by 36%, $p<0.0005$) and Fcγ (by 70%, $p<0.0001$).

CONCLUSIONS: The upregulation of M1 marker CCR7, and downregulation of M2 marker CD163 is consistent with our previous study with mAb alone. Fcγ closely paralleled the use of mAb and suggests that the polarizing shift observed is Fcγ mediated. To our knowledge this study is the first to demonstrate Fcγ mediated polarization of human macrophages. This may represent a novel immunomodulatory mechanism for monoclonal antibody and IVIG therapy.

501 Effects of Maternal Geohelminth Infections on the Risk of Allergy during the First 3 Years of Life: Findings from a Birth Cohort in Rural Ecuador

Philip J. Cooper[1], Martha Chico[2], Leila Amorim[3], Carlos Sandoval[4], Laura Rodrigues, PhD[5], Mauricio L. Barreto, PhD[6], David P. Strachan[7]; [1]Pontificia Universidad Catolica del Ecuador, [2]Laboratorio de Investigacion FEPIS, Quito, Ecuador, [3]Universidade Federal da Bahia, Brazil, [4]Laboratorio de FEPIS, Ecuador, [5]London School of Hygiene and Tropical Medicine, London, United Kingdom, [6]Instituto de Saúde Coletiva, UFBa, Salvador, BA, Brazil, [7]St George's University of London, London, United Kingdom.

RATIONALE: Maternal geohelminths during pregnancy may protect against allergy development in childhood. We investigated the effect of maternal geohelminths during pregnancy on the development of eczema, wheeze, and atopy during the first 3 years of life in children.

METHODS: A cohort of 2,404 neonates followed to 3 years of age in rural Ecuador. Data on wheeze and eczema were collected by questionnaire and physical examination at 13, 24, and 36 months, and allergen skin prick test reactivity (SPT) to 10 allergens was done at 36 months. Maternal stool samples were examined for geohelminths by microscopy. Data on potential confounders was collected at birth by questionnaire.

RESULTS: Geohelminths were observed in 46.1% of mothers. Eczema and wheeze during the first 3 years was reported for 17.7% and 25.9%, respectively, of 2,069 (86.1%) children with complete follow-up. At 3 years, SPT to any allergen was present in 17.2% and to house dust mites in 8.7%. Maternal geohelminths were not significantly associated with eczema (adjusted OR 1.23, 95% CI 0.97-1.56, $P=0.082$), wheeze (adj. OR 1.04, 95% CI 0.84-1.29, $P=0.727$), and SPT to any allergen (adj. OR 0.84, 95% CI 0.67-1.06, $P=0.142$). However, in a sub-group analysis, maternal ascariasis was associated with an increased risk of eczemna but reduced SPT.

CONCLUSIONS: Our data do not support a protective effect of maternal geohelminths on development of eczema and wheeze in early childhood, although *in utero* exposures to ascariasis may reduce allergic sensitization.