ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 2, Issue 9, September 2017

Toll-like receptors and calcium-activated chloride/potassium channels as immunomodulators of allergic airway inflammation and asthma: a public health research experience in State of Nebraska, USA

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***Abstract*— Allergic airway inflammation and asthma have emerged as major public health problem in the Midwest, United States of America. Targeting Toll-like receptor (TLR) signaling and Calcium-activated Chloride (ClCa)/Potassium (KCa) Channels in unraveling the cellular/molecular mechanisms underlying genetic susceptibility to inflammatory diseases in specific human patient population subset(s) is emerging as an attractive immunotherapeutic pharmacological strategy in management/prevention of asthma. Objectives: My pilot study aimed to investigate the role of TLRs and ClCa/KCa ion channels in human bronchial epithelial cells (NHBEC, BEAS-2B from ATCC), and eosinophils-derived from asthma patients of North American ethnicity. Methods: Whole cell patch clamp electrophysiological recordings were conducted for ClCa and KCa currents in cultured cells (passages P3-P7) grown on cover-slips. RNA and Protein were extracted from NHBECs, BEAS-2B and eosinophils using Trizol and RIPA methods. Pipette and bath solutions for electrophysiology were subjected to stringent pH and osmolality checks prior to current recordings. Borosilicate patch pipettes were fabricated using Sutter instrument; micromanipulators were adjusted for gigaseal recordings. The study was approved by Institutional Review Board.**

Results: Cell viability assays demonstrated >80% viability of NHBECs, BEAS-2B cells (ATCC), and eosinophils. Mean age of American patients (N=7; White N=2, African American N=4, Caucasian N=1) was 47.0 years (S.D±5.0 years). Receptor/ion channel studies demonstrated the expression of TLR2 and intermediate conductance IKCa3.1 mRNA transcripts; beta-actin was used as internal control. Patch clamp electrophysiology recordings detected Chloride and Potassium channel current spikes in cultured cells in presence of intracellular Calcium, and DIDS, Chloride channel inhibitor(s). My preliminary data implicates the public health impact of TLR-ClCa/KCa mediated immunomodulation in asthma management. Future gene-epidemiology studies with larger sample size are warranted for development of cost-effective predictive biomarkers for asthma susceptible populations of diverse ethnicities. Index Terms- Asthma, Biomarkers, Calcium activated Chloride/Potassium Channels, Toll-like receptors

Acknowledgement: Dr. Saumya Pandey (Ph.D.) acknowledges the fellowship support from Department of Biomedical Sciences, Creighton University, Omaha, NE, USA during her tenure

I. INTRODUCTION

Allergic airway inflammation and asthma have emerged as major public health problem in the Midwest, United States of America. Allergic asthma is one of the most common chronic conditions in Western world, including the State of Nebraska; the clinical sequelae in allergic airway inflammation/asthma include goblet cell metaplasia, mucus hypersecretion, bronchial hyper-responsiveness, smooth muscle hypertrophy, reactive oxygen species generation, eosinophil infiltration, airway obstruction, NF-KB activation and production of inflammatory cytokines [1]. Toll-like receptors, a family of evolutionarily conserved pathogen recognition receptors, initiate inflammatory responses to foreign pathogens; thirteen TLRs are known till

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date (TLR1-13) and their complex signaling mechanisms involve various intermediaries in the signal transduction pathway for an inflammatory/immune response in the target cell [2]. Calcium-activated chloride channels (CaCC) are primarily expressed in excitable/non-excitable cells, and their functional roles are defined by anion selectivity, activated by intracellular calcium and modulated by Calmodulin kinase (CaMK) II and calcineurin; chloride channels are involved in diverse physiological processes viz. cell migration, proliferation and apoptosis and interestingly, the immunobiological significance of CACC in asthma pathophysiology has been demonstrated recently [3]. Ion channel electrophysiology is an intriguing research area with immense immunotherapeutically relevant clinical/public health impact in deciphering the intricacies of aberrant physiological conditions and/or human diseases. Calcium-activated Potassium channels (KCa) are broadly of three classes: the large conductance (BKCa), intermediate conductance (IKCa) and small conductance (SKCa), and KCa inhibitors have been implicated in human diseases, including asthma, neuronal degeneration and cardiovascular diseases [3]. Racial and ethnic disparities in asthma management and health care utilization are emerging as thrust areas of public health policy-related research in contemporary times; cost-effective predictive research models with biomarkers are warranted to decrease the increasing burden of allergic airway inflammation, atopy and asthma amongst disease-susceptible at-risk individuals of ethnically disparate populations. Surveillance of asthma risk and outcomes-based timeline based strategies may be developed for prevalence and disease outcome assessments involving risk factors, asthma management, asthma control and prevention. Targeting TLR signaling and Calcium-activated Chloride (ClCa)/Potassium (KCa) Channels in unraveling the cellular/molecular mechanisms underlying genetic susceptibility to inflammatory diseases in specific human patient population subset(s) is emerging as an attractive immunotherapeutic pharmacological strategy in management/prevention of asthma. My pilot study aimed to investigate the role of TLRs and ClCa/KCa ion channels in human bronchial epithelial cells (NHBEC, BEAS-2B from ATCC), and eosinophils-derived from asthma patients of North American ethnicity.

II. METHODS

Cell culture: Human bronchial epithelial cells (NHBEC, BEAS-2B from ATCC), and eosinophils-derived from asthma patients of North American ethnicity were subjected to routine cell culture in a humidified 5% CO2 incubator at 37 degree Celcius; basal media supplemented with 10% Fetal bovine serum (FBS) was utilized for growing cells to 70-80% confluence. MTT assay was performed for cell viability; cell passages P3-P7 were used for pilot experiments.

Electrophysiology: Whole cell patch clamp electrophysiological recordings were carried out for ClCa and KCa currents in cultured cells of passages P3-P7 grown on cover-slips; cells were checked for confluence, contamination and viability by microscopically observing their membranes, shape and density, prior to ion channel electrophysiology. Pipette and bath solutions with adjusted Calcium ion concentrations for electrophysiology were freshly prepared and stored at 4 degree Celcius after initial rounds of stringent pH and osmolality checks prior to performing current recordings. Borosilicate patch pipettes were fabricated using the sophisticated Sutter instrument; background noise interfering with current recordings was eliminated, cover-slips with confluent cells were reexamined, and micromanipulators were thereafter adjusted for gigaseal recordings for detection of outwardly and inwardly rectifying chloride and potassium currents.

Molecular biology experiments: RNA and Protein were extracted from NHBECs, BEAS-2B and eosinophils using Trizol and RIPA methods. Nuclease-free water was used for RNA and protein isolation from cell lysates; spectrophotometric determinations of relevant concentrations were performed prior to RT-PCR and Agarose gel electrophoresis for RNA, and Western blot for protein, respectively. The study was duly approved by Institutional Review Board.

III. RESULTS AND CONCLUSIONS

The preliminary findings were convincing and significantly appreciable, considering the study timeline of six months. Cell viability assays demonstrated >80% viability of NHBECs, BEAS-2B cells (ATCC), and eosinophils. The cell-specific membrane dynamics and structural integrity were observed prior to electrophysiologic assessments; cell shape, size and membrane structure were stringently observed, and rigorous pH checks were conducted for measuring accurate, error-free reliable current recordings. Patch clamp electrophysiology recordings detected Chloride and Potassium channel current spikes in adherent cultured cells in presence of intracellular Calcium, and DIDS, anionic Chloride channel inhibitor(s). Inwardly and outwardly rectifying current recordings and subsequent data-curves/plots were reviewed for subsequent software-based analysis. Interestingly, the mean age of clinically diagnosed asthma patients of North American ethnicity (N=7; White N=2, African American N=4, Caucasian N=1) was 47.0 years (s.d. ±5.0 years); the core tenets of good practice research and bioethics, including written informed consent, were followed. Receptor/ion channel molecular biology-based RNA/Protein-related data demonstrated the expression of TLR2 and intermediate conductance IKCa3.1

mRNA transcripts; beta-actin was used as internal control/referent. Experiments were performed in triplicates so as to rule out the possibility of ambiguous/erroneous findings.

TLR2 and TLR4 have been implicated in the pathogenesis of asthma and the inflammatory responses underlying asthmatic exacerbations; TLR4 detects Gram-negative bacteria through their lipopolysaccharides (LPS); while TLR2 plays a pivotal role in recognizing Gram-positive bacteria [2]. Further, TLR2 promotes Th2-biased immune responses, which may be correlated to the Th1/Th2 imbalance in asthma. Genetic polymorphisms affect susceptibility, severity, and responsiveness of asthmatic patients to specific allergens; oxidative stress contributes to the sensitization of allergens by generating an enhanced allergic immune response [4], thus exacerbating the development of allergic asthma.

TLR-based immunotherapeutics and Calcium activated Chloride and Potassium channels alongwith TLR agonists and/or antagonists as well as ion channel inhibitors are emerging as burning areas of asthma research in contemporary times; interrelated immune/inflammation-related biochemical signaling cascades may be targeted for eventual design of immunomodulatory and anti-inflammatory drugs.

The immunobiological role of TLRs has been well-documented, and polymorphisms of the TLR genes can result in significant alterations in the severity and susceptibility of respiratory inflammatory diseases amongst disease-susceptible populations of varying genetic landscapes. Recent studies strongly implicate the intriguing role of TLRs in allergic airway inflammation and asthma; TLR2 activation by its synthetic ligand Pam3Cys, in contrast to the activation of TLR-9 by immunostimulatory DNA, induces a prominent Th2-biased immune response and aggravates experimental asthma [2, 5]. The TLR-4 (Asp299Gly) polymorphism is associated with an increased prevalence of asthma in Swedish children [6]. The promising preliminary findings of my public biomedical research study with a public health perspective implicates the clinical impact of TLR-ClCa/KCa mediated immunomodulation in asthma management. Future gene-epidemiology studies with larger sample size are warranted for development of cost-effective predictive biomarkers for asthma susceptible populations of diverse ethnicities. TLR-ClCa-KCa may eventually prove to be a boon in rationalized asthma gene therapy in the American cohort of State of Nebraska, USA.

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