UNIVERSITI TEKNOLOGI MARA

GENE EXPRESSION PROFILING OF HUMAN BRONCHIAL SMOOTH MUSCLE AND LUNG FIBROBLASTS CELLS STIMULATED WITH IL-1β, IL-4, IL-5 AND IL-13 CYTOKINES THROUGH THE APPLICATION OF cDNA MICROARRAY.

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as reference work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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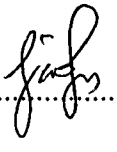
Signature of Student	:	
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ABSTRACT

Both Th1 and Th2 cytokines are known to be potent stimulants acting on resident airway cells by inducing inflammatory response. Th1 cytokines were known as proinflammatory mediators while Th2 cytokines, in particular play critical role in eliciting the activation and recruitment of the IgE antibody producing B cells, mast cells and eosinophils which are the key features of allergic asthma. However, the molecular mechanisms involved are still poorly understood. The aim of this study is to investigate the molecular mechanism underlying the mechanism of the cytokines in inducing asthma. In this study, cultured normal human bronchial smooth muscle cells (BSMC) and normal human lung fibroblasts (NHLF) were stimulated with cytokines namely IL-1ß, IL-4, IL-5, IL-13 and combination of all four cytokines at two timepoint (1 and 24 hours) which represent the early and late onset of inflammatory response . The application of cDNA microarray in this study has generated a gene expression database for both BSMC and NHLF. Some of the key-genes involved in the stimulatory response were selected for further validation by real-time PCR and protein assays. Microarray data was generated by using third party software. Two algorithms were chosen to summarize the data; RMA and PLIER. Multi-way ANOVA test was conducted at p value= 0.01 with >2 fold change. Results clearly showed that in NHLF stimulation, IL-1ß one hour showed high number of expressed genes for 89 and 67 genes for both PLIER and RMA analysis. In IL-1ß 24 hours stimulation, the expressed genes are 313 and 246 for both algorithm respectively. In contrast, Th2 cytokines of IL-4, IL-13 and IL-5 merely generating non-significant genes compared to IL-1ß and combination of all four cytokines. The combination stimulation generated more to IL-1ß like expression. In BSMC stimulation, IL-5 surprisingly showed a similar pattern of expression to that of IL-1ß in both 1 and 24 hours stimulation. There are about 199 genes expressed common to both IL-5 and IL-1ß stimulation. RMA analysis proved to be more stringent in data generation compared to PLIER. Despite activating in a common signaling pathway, IL-5 when compared to other Th2 cytokine type like IL-4 and IL-13 induced dramatically different patterns of gene expression in primary cultures of airway smooth muscle cells, and lung fibroblasts, with little overlap among cell types. The most prominent effects of IL-1ß and IL-5 were observed on the airway smooth muscle stimulation. A better understanding on the relationship of IL-5 and IL-1ß was further investigated and found to be that IL-5 is acting through the same pathway as in the IL-1ß cytokines which is responsible for the inflammatory response in modulation of the smooth muscle expression. However, the stimulation of IL-4 and IL-13 on the other hand did not yield to a significance result. This might be due to the low concentration of cytokine used in the stimulation. IL-1ß on the other hand is taken as positive control due to its ability as potent stimulant in both NHLF and BSMC. On the overall, effects of cytokine on the airway resident cells in this study are able to provide molecular insight especially during inflammatory response.

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