Diffrential Gene Expression of Human Mast cell Activation Reveals Gene profiles of Innate and Adaptive Immunity

Department of Physiology, National University of Singapore, Singapore,

Abstract

High-density oligonucleotide microarray is a promising approach for high throughput analysis. It has been extensively used in many areas of biomedical research. Immunoglobulin E (IgE) mediated allergic response (type-1 hypersensitivity) is one of the most powerful reactions of the immune system. Tissue Mast Cells (MCs) and circulating basophils are the major effector cells in these reactions. By dissecting the regulatory circuitry of mast cells by analyzing the genome wide effects of antigen stimulation triggered by FcεRI, offers a potential for finding novel genes as 'targets' for therapeutic intervention. In this work, we tried to study the gene expression pattern in IgE sensitized and FcεRI cross linked cord blood derived MCs using one of the latest techniques, high-density oligonucleotide expression probe array (HG-Focus array, Gene Chip® Affymetrix, Santa Clara, CA). Microarray hybridization of RNA from cord blood derived MCs revealed coordinated changes in gene expression in response to IgE stimulation and receptor cross linking at different time points. Among the most prominent findings, we observed 2 to 32-fold increased expression of different transcripts. Real-time PCR confirmed reliability of microarray data. This enabled us to classify and cluster genes by functional families as well as to understand known genes in signaling pathways. These results defined a list of primary candidates for finding novel genes as 'targets' for therapeutic intervention.

Experimental set-up and Data Analysis

Total RNA isolated from cord blood derived human mast cells was processed and hybridized to each array of the HG-Focus GeneChip® according to the protocols described in the GeneChip® Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA). GeneChip image files were processed using the Microarray Analysis Suite 5.0.1 and further analyses was performed with Micro DB 3.0 & Data Mining Tool 3.0 (Affymetrix). Data of 8400 genes from each treatment were scaled to an average intensity of 500. Two chips were used for each time point and the results from duplicate chips highly correlated with R value > 0.91. In this work, genes termed "significantly changed" in response to IgE were those that meeting the following criteria: All genes induced at a given time points to a fold change >2 and p value < 0.05 by the MicroarraySuite (Affymetrix, Santa Clara, CA). Microarray hybridization of RNA from cord blood derived MCs revealed coordinated changes in gene expression in response to IgE stimulation and receptor cross linking at different time points. Among the most prominent findings, we observed 2 to 32-fold increased expression of different transcripts. Real-time PCR confirmed reliability of microarray data. This enabled us to classify and cluster genes by functional families as well as to understand known genes in signaling pathways. These results defined a list of primary candidates for finding novel genes as 'targets' for therapeutic intervention.

Hierarchical clustering of differentially expressed genes

Changes in gene expression in human mast cells stimulated via FcεRI. Results: A) Clustering of 395 genes that exhibited a 2–32 fold change in expression over control in duplicates of human cord blood derived mast cells that were activated by IgE sensitization and FcεRI cross linking for different time points (2hr, 6hr and 12hr). Hierarchical clustering was applied using Genesis. Genes were selected for this analysis if their expression level deviated from that in the unstimulated mast cells by 2 fold changes in at least 1 time point. The values from different time points and its duplicates were analyzed. Changes in gene expression were depicted according to the color scale shown at the bottom (E). The results are displayed in a table format, in which each row represents a series of measurements of mRNA levels for a single gene, and each column represents the measured mRNA levels for all of the genes in a single sample of cells. Each cell is colored to reflect expression of the corresponding gene in a specific cell sample, relative to its expression level prior to sensitization. Green color represents decreased expression; red color represents increased expression. As indicated, the scale extends from ratios of -3 to 3 in fold change units. Genes for (B) Cytokines, (C) Chemokines and (D) Adhesion molecules whose expression changed significantly.

Electrophoresis of PCR products

Validation of real-time PCR amplicons were checked by agarose gel electrophoresis. Figure 2 shows 210bp amplicon of MIP3α gene. Results: A) Negative control, Lane-1: RNA from Control, Sensitized, Cross-linked 2hr, Cross-linked 6hr and Cross-linked 12hr respectively. Lane M: Molecular size marker 100bp DNA ladder.

Results

Hierarchical clustering of differentially expressed genes

Real-time PCR: Validation of microarray results

In our study, we compared the levels of expression of thousands of genes; even from the sensitization stage of mast cells. Sensitization with IgE triggers the upregulation of several chemokines and cytokines, involved in chemotaxis, adhesion and TH1 activation. Even though there are some reports on mast cells gene expression profile after activation, they have not focused on the overall picture on the different stages of mast cell sensitization and activation. Although mast cells have been viewed as mediators of allergy, anaphylaxis and immune dysfunction, the findings we report here show mast cells capable of triggering an array of genes essential in triggering adaptive immune responses. The data we present here suggest that mast cells, possibly play a role in the initiation of innate and adaptive immunity, and is pointing to a different view of mast cells, which has traditionally been linked to their role in immune dysfunctions widely demonstrated for allergies and autoimmunity. Thus, it is one of the differential gene expression pattern of human cells, we suggest that mast cells may not only involved in innate immune responses, but also may play a key role in initiating adaptive immune responses. Future studies should be focused on models that can validate the potential roles of mast cells in overall immunity, and in identifying novel molecules as potential targets for therapeutic intervention in allergic and inflammatory diseases.

Conclusion


References

Acknowledgements

We are greatful to BFIC, National University of Singapore. This work was supported by BMRC Young Investigator Award (R-185-000-044-305).