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

Manikandan J, Renji R, Pushparaj. P.N, Alirio J. Melendez.
Department of Physiology, National University of Singapore, Singapore,

Nature Precedings : doi:10.1038/npre.2007.1148.1 : Posted 27 Sep 2007

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 An

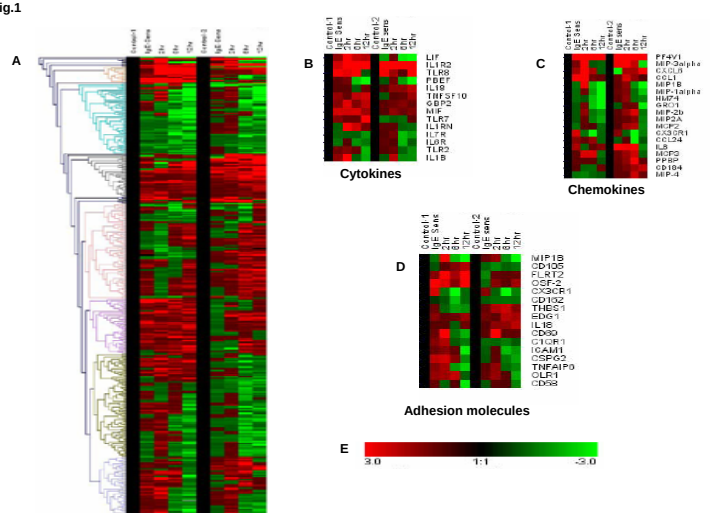
Total RNA isolated from cord blood derived human mast cells was processed and hy
Chip® according to the protocols described in the Gene Chip® Expression Analysis
CA). Gene Chip image files were processed using the Microarray Analysis suite 5.
Micro DB 3.0 & Data Mining Tool 3.0 (Affymetrix). Data of 8400 genes from each trea
500. Two chips were used for each time point and the results from duplicate chips
work, genes termed "significantly changed" in response to IgE were those that meetin
given time point had to be called 'present'(P <0.05) by the Microarray Suite and gene
(increase) or "D" (decrease), but not "NC" (no change). A further constraint was that
in at least one experimental condition were included in subsequent analyses. Expressi
criteria were clustered by average linkage hierarchical clustering using the Genesis
clustering prior to hierarchical clustering yielded similar results (data not shown). Gen
their biological process as described in the NetAffx analysis centre database (Affym
selected genes to confirm the reliability of microarray results.

ach array of the HG-Focus Gene
Manual (Affymetrix, Santa Clara,
ier analysis was performed with
scaled to an average intensity of
ted with R value = 0.91. In this
g criteria: All genes induced at a
change call (P<0.05) of either "I"
wing a change of 2-fold or more
95 unique probes meeting these
am. Self-organizing map (SOM)
tated and classified according to
– time PCR was performed for

Results

Hierarchical clustering of differentially expressed genes

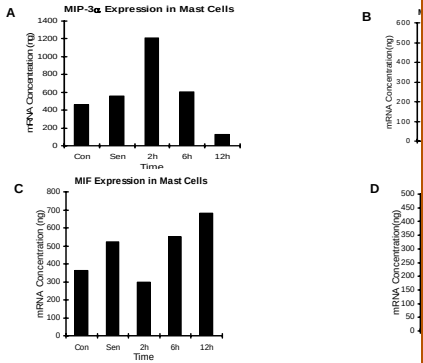
Fig.1



Changes in gene expression in human mast cells stimulated via FcεRI. Fig.1 (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 change 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.

Real-time PCR: Validation of microarray results

Fig.3



Real-Time PCR for some genes (selected from microarray's result) expre
sensitized by human IgE, and then cross-linked with anti-human IgE for 2
extracted. Light-Cycler Real-Time PCR was performed following the protocol
mRNA were calculated using respective standard curves. Fig.3. (A) MIP-3α
expression and (D) COX-2 expression.

n in Mast Cells

nan mast cells. Mast cells were
h, respectively. Total RNA was
e concentrations of these genes'
(B) MCP-3 expression, (C) MIF

Conclusion

In our study, we compared the levels of expression of thousands of genes; ev
Sensitization with IgE triggers the upregulation of several chemokines and cyt
TH1 activation. Even though there are some reports on mast cells gene exp
focused on the overall picture on the different stages of mast cell sensitization
viewed as mediators of allergy, anaphylaxis and immune dysfunction, the findi
triggering an array of genes essential in triggering adaptive immune responses
cells, potentially play a role in the initiation of innate and adaptive immunity, a
which has traditionally be linked to their role in immune dysfunctions widely
Thus, in view of the differential gene expression pattern of human mast ce
involved in innate immune responses, but may also play a key role in initiatin
should be focused on models that can validate the potential roles of mast cell
molecules as potential targets for therapeutic intervention in allergic and inflamm

sensitization stage of mast cells.
ved in chemotaxis, adhesion and
e after activation, they have not
. Although mast cells have been
here show mast cells capable of
e present here suggest that mast
to a different view of mast cells,
l for allergies and autoimmunity.
st that mast cells may not only
mune responses. Future studies
mmunity, and on identifying novel
es.

Electrophoresis of PCR products

Validity of real-time PCR amplicons were checked by agarose gel electrophoresis. Figure.2 shows 210bp amplicon of MIP3α gene.

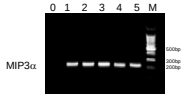


Fig.2

Fig.2. Lane 0 :Negative control,
Lane1-5: RNA from Control, Sensitized, Cross-linked 2hr, Cross-linked 6hr and Cross-linked 12hr respectively.
Lane M :Molecular size marker 100bp DNA ladder.

References

1. Melendez,A.J., and Khaw, A.K., (2002) Dichotomy of Ca2+ Signals Triggered in Antigen Stimulation of Human Mast Cells. *J Biol Chem,* 277(19): 17255-17

2. Sturn A, Quackenbush J, Trajanoski Z., (2002) Genesis: cluster analysis of mi 18(1):207-8.

3. Metcalfe, D.D., Baram, D. & Mekori, Y.A. (1997), Mast cells. *Physiol. Rev.* 77,

4. Echtenacher, B., Mannel, D.N. & Hulter, L. (1996) Critical protective role of ma peritonitis. *Nature* 381, 75-77.

phospholipid Pathways

Bioinformatics,

model of acute septic

Acknowledgements

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

Poster #20