Genome activity profiling of monomeric-IgE and FcεRI-aggregation on human mast cells reveals a complex network of genes involved in inflammatory responses

M. Jayapal, A. J. Melendez, P.N. Pushparaj
Department of Physiology, National University of Singapore, Singapore.

Abstract

Mast cell activation, mediates type-1 allergic responses, one of the most powerful reactions of the immune system. However, mast cells activation is becoming increasingly linked to inflammatory, autoimmunity, and to adaptive immunity by regulating T-cell activation. Here we analyzed the gene expression pattern in IgE-sensitized and FcεRI aggregation on human mast cells. Our data revealed coordinated changes in gene expression. We observed increased expression of gene-transcripts involved in allergic, innate and adaptive immune responses. Among the most prominent findings is the increased expression of transcripts encoding for MIP3a, SPARCL1, AREG, IL18, CCL1, TNFRSF9, IL1b, CX3CR1, PTGER3, MIF, MMP12, ADORA3, IL8RB, and other genes involved in innate and cell-mediated immunity. These results represent a substantial advance in our understanding of the genome-wide effects triggered by "passive sensitization" or active stimulation of human mast cells, and how this relate to mast cells involvement not only in allergic responses but also in innate and adaptive immunity.

Experimental set-up and Data Analysis

Human umbilical cord blood samples were collected form normal full-term deliveries of informed individuals with formal consents progenitor cells were separated and differentiated in to MCs. Cells, except the control, were sensitized with IgE for overnight and crosslinked the receptors using anti IgE for 2hr, 6hr and 12hr. Total RNA isolated from these cells was processed and hybridized to HG-Focus GeneChip® according to the protocols described in the GeneChip® Expression Analysis Technical Manual. Chip image files were processed using the Microarray Analysis suite; version 5.0.1 (Affymetrix) and further analysis were performed with Micro DB 3.0 & Data Mining Tool 3.0. Global scaling was performed to compare genes from chip to chip; thus, each chip was normalized to an arbitrary value 500. Results from duplicate chips (*n=2*) highly correlated with Pearson R value = 0.91. Expression levels of 395 significantly changed unique probes meeting our filter criteria were clustered by average linkage hierarchical clustering for genes using the Genesis 1.2.2 program. Self-organizing map (SOM) clustering prior to hierarchical clustering yielded similar results. Genes were annotated and classified according to their biological process as described in the NetAffx analysis centre database. Real time RT-PCR was performed for selected genes to confirm the reliability of microarray results.

Results

Hierarchical clustering of differentially expressed genes

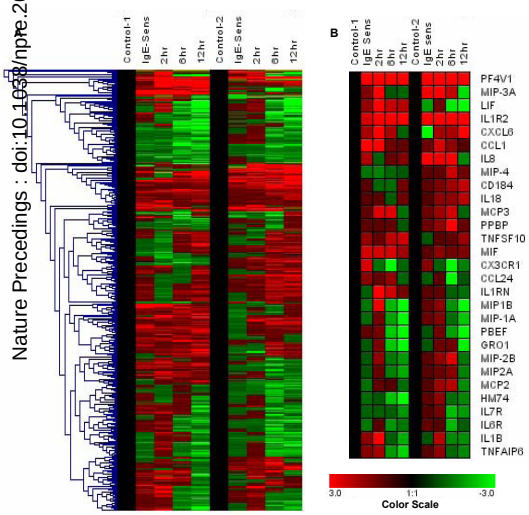

Fig. 1. Hierarchical clustering of Changes in gene expression in human mast cells stimulated via FcεRI (A) Clustering of 395 genes that exhibited a 2–32 fold change in expression over control in duplicates of human cord blood derived mast cells that were activated by IgE sensitization and FcεRI cross linking for different time points (2hr, 6hr and 12hr). Average linkage hierarchical clustering for genes was applied using Genesis program. Changes in gene expression were depicted according to the color scale shown at the bottom. 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 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 results are displayed in a table format, in which columns indicate different treatments and rows indicate individual genes. (B) Genes for cytokines, chemokines and their receptors whose expression changed significantly.

Table.1

GenBank ID	Fold Change	Gene name	Gene description
NM_002345.1	12.91	*LUM*	Lumican
M31933.1	6.96	*FCGR2B*	Low affinity IgG Fc region receptor II-b
D13665.1	4.38	*OSF-2*	Osteoblast specific factor 2
NM_000045.2	4.29	*ARG1*	Arginase
NM_005001.1	3.92	*NDUFA7*	NADH dehydrogenase 1 a subcomplex, 7
NM_005165.1	3.81	*ALDOC*	Aldolase C
NM_000935.1	3.68	*PLOD2*	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase
X86401.1	3.68	*GATM*	Glycine amidinotransferase
AF135268.1	3.41	*P8*	p8 protein
NM_013315.1	2.89	*TPTE*	Transmembrane phosphatase with tensin homology
NM_014584.1	2.68	*ERO1L*	ERO1-like
U20350.1	2.58	*CX3CR1*	Chemokine (C-X3-C motif) receptor 1
X83858.1	2.51	*PTGER3*	Prostaglandin E receptor 3
NM_002415.1	2.51	*MIF*	Macrophage migration inhibitory factor
NM_002426.1	2.45	*MMP12*	Matrix metalloproteinase 12
NM_021778.1	2.3	*ADAM28*	A disintegrin and metalloproteinase domain 28
NM_004385.1	2.27	*CSPG2*	Chondroitin sulfate proteoglycan 2
NM_000677.2	2.23	*ADORA3*	Adenosine A3 receptor
X51757	2.19	*HSPA6*	Heat-shock protein HSP70B
NM_000917.1	2.17	*P4HA1*	Proline 4-hydroxylase alpha polypeptide I
NM_000265.1	2.07	*NCF1*	Neutrophil cytosolic factor 1
NM_001557.1	2.03	*IL8RB*	Interleukin 8 receptor, beta
AL021977	-2.04	*MAFF*	V-maf musculoaponeurotic fibrosarcoma oncogene homolog F
AF073890.1	-2.4	*CTSZ*	Cathepsin Z
NM_006732.1	-2.08	*FOSB*	FBJ murine osteosarcoma viral oncogene homolog B

Table.1.Genes which were differentially expressed by MCs upon sensitization with IgE (during 'passive pre-sensitization' stage).

Table.2

GeneBank ID	Gene name	IgE sensitized	FcεRI cross linked		
			2hr	6hr	12hr
M13436.1	*INHBA*	*	32.00	*	*
NM_002309.2	*LIF*	*	11.88	*	*
NM_002620.1	*PF4V1*	*	*	*	11.00
BF433902	*TNFRSF11B*	*	*	*	10.27
NM_004591.1	*CCL20*	*	7.21	*	*
M31933.1	*FCGR2B*	6.96	6.28	3.78	1.69
J03189.1	*GZMB*	*	*	5.58	*
NM_001557.1	*IL8RB*	2.03	*	5.54	*
NM_016610.1	*TLR8*	*	*	5.46	5.46
L07555.1	*CD69*	3.37	3.73	-1.33	-2.10
NM_002993.1	*CXCL6*	1.97	3.39	2.04	3.66
U65590	*IL1RN*	*	3.20	2.46	1.03
NM_000963.1	*PTGS2*	*	2.95	*	*
NM_002984.1	*CCL4*	-1.08	2.63	-2.19	-4.96
M15330	*IL1B*	1.65	2.79	-1.61	-1.69
NM_006273.2	*CCL7*	1.30	2.62	2.55	-1.20
NM_002981.1	*CCL1*	*	2.57	*	*
NM_002415.1	*MIF*	2.52	2.51	2.57	1.69
U08015.1	*NFATC1*	*	2.38	*	-1.76
NM_012072.2	*C1QR1*	1.56	2.10	*	-1.44
NM_000265.1	*NCF1*	2.07	2.01	*	-1.49
NM_000361.1	*THBD*	-1.63	2.01	-3.68	-3.02
NM_003853.1	*IL18RAP*	*	2.00	*	*
U20350.1	*CX3CR1*	2.58	*	-3.78	*
X83858.1	*PTGER3*	2.51	*	*	*
NM_002426.1	*MMP12*	2.45	1.55	1	1.65
NM_002000.1	*FCAR*	2.00	1.60	*	-3.51
NM_001243.1	*TNFRSF8*	*	*	*	3.78
NM_016562.1	*TLR7*	*	*	*	3.36
NM_021057.1	*IFNA7*	*	*	*	2.99
NM_003810.1	*TNFSF10*	1.74	*	2.25	2.48
NM_001562.1	*IL18*	*	*	*	2.20
AA780381	*MAP2K3*	-1.31	1.74	-1.5	-203
M57731.1	*CXCL2*	*	*	1.02	-2.06
NM_002748.1	*MAPK6*	*	*	-1.39	-2.13
AI078167	*NFKB1A*	-1.23	-1.55	-2.06	-2.27
NM_002341.1	*LTB*	*	*	-2.23	-2.27
NM_004556.1	*NFKB1E*	*	*	-2.38	-2.31
NM_007115.1	*TNFAIP6*	1.57	1.82	-1.50	-2.51
NM_000201.1	*ICAM1*	*	1.56	-2.25	-2.85
NM_001511.1	*CXCL1*	*	1.35	-2.36	-3.10
NM_002983.1	*CCL3*	-1.28	1.44	-1.60	-3.32
AF043337.1	*IL8*	1.43	1.97	*	-3.53
BF575514	*PBEF*	*	1.38	-2.41	-4.69
NM_000565.1	*IL6R*	-1.39	*	-2.29	-1.43
NM_002090.1	*CXCL3*	*	1.74	-2.66	-1.60
AI984980	*CCL8*	*	1.87	-2.16	-1.29
NM_002991.1	*CCL24*	*	*	-3.78	*
Y13710	*CCL18*	1.63	*	-2.4	*

Table 2. Selected genes which play a role in adaptive and/or innate immune response were found to be differentially expressed by activated MCs followed by IgE sensitization and FceR1 crosslinking at different time points.
* represents 'No Change'

Real-time RT- PCR: Validation of microarray results

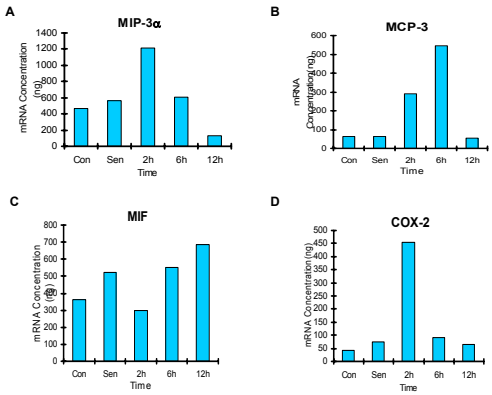

Fig.2. Real-Time PCR for some genes (selected from microarray's result) expression in human mast cells. Mast cells were sensitized by human IgE, and then cross-linked with anti-human IgE for 2h, 6h and 12h, respectively. Total RNA was extracted. Light-Cycler Real-Time PCR was performed following the protocol of Roche. The concentrations of these genes' mRNA were calculated using respective standard curves. Fig.3. (A) MIP-3α expression, (B) MCP-3 expression, (C) MIF expression and (D) COX-2 expression.

Conclusion

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, potentially play a role in the initiation of innate and adaptive immunity, and is pointing to a different view of mast cells, which has traditionally be linked to their role in immune dysfunctions widely demonstrated for allergies and autoimmunity. Thus, in view of the differential gene expression pattern of human mast cells, we suggest that mast cells may not only be involved in innate immune responses, but may also play a key role in initiating adaptive immune responses. Future studies will be focused on models that can validate the potential roles of mast cells in overall immunity, and on identifying novel molecules as potential targets for therapeutic intervention in allergic and inflammatory diseases.

References

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