The immune response in patients with SARS: Differential gene expression profiling

NUS National University of Singapore

PHYSIOLOGY National University of Singapore
Molecular & Cellular Immunology Laboratory

P.N. Pushparaj M. Jayapal, A. J. Melendez

Department of Physiology, National University of Singapore, Singapore,

Abstract

Severe acute respiratory syndrome (SARS) emerged in 2003, as a new epidemic form of life-threatening infection. As of 26th September 2003, there were 8422 cases of SARS from 29 countries with 908 deaths (WHO). However, the pathogenesis of SARS is poorly understood. To understand the host response to this pathogen, we profiled the gene expression patterns of peripheral blood mononuclear cells (PBMC) from SARS patients compared to healthy controls using one of the latest techniques, high density oligonucleotide expression probe array (HG-Focus array, Gene Chip, Affymetrix, Santa Clara, CA). High-density oligonucleotide microarray is a promising approach for high throughput analysis. It has been extensively used in many areas of biomedical research for different purposes. Thus we could compare the expression levels of thousands of genes which are differentially expressed in SARS patients over healthy controls. Among the most prominent findings, we observed 2 to 200-fold increased expression of transcripts of various genes. This enabled us to classify and cluster genes by functional families as well as to understand known genes in signaling pathways.

Experimental set-up and data analysis

We analyzed the expression pattern of over 8,400 annotated genes from the PBMCs of 10 SARS patients and compared with healthy control samples. Student's T-test (P<0.01) was performed to identify genes that were differentially expressed in SARS patients and normal sample. We selected 186 genes for further analysis that met the following criteria: (i) Changes in expression of at least 2 fold higher or lower comparing the normal (ii) Signal >500 (iii)Detection P <0.01 and (iv) Genes which met the above criteria in at least 30% of samples. Genes were annotated according to biological process using the Gene Ontology Consortium. Results from duplicate normal samples highly correlated with Pearson R >0.95. Unweighted average linkage hierarchical clustering was applied using the 'Genesis' program. Further validation of microaarray results were confirmed with RT-PCR for selected genes.

Results

Fold change scatter plot.

Scatter plot represents fold changes of differentially expressed transcripts.

From the graph panel,
X- axis - SARS sample's signal values
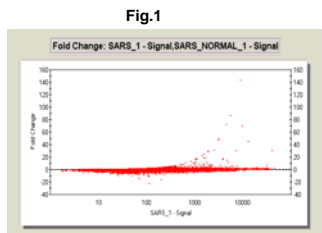Y- axis - fold change of SARS sample over normal control.

Fig.1

Fold Change: SARS_1 - Signal,SARS_NORMAL_1 - Signal

Hierarchical clustering of differentially expressed genes.

Fig.2: Unweighted average linkage hierarchical clustering was applied using Genesis1.2 program. Clustering of gene expression data from 10 SARS patients showing different classes of gene expression profiles. Each row represents a separate gene and each column a separate SARS patient. Student's unpaired T-test (P <0.01) was performed and 248 differentially expressed genes were selected. Expression index for each gene (rows) in each sample (column) is indicated by a color code. The color scale ranges from saturated green for log ratios -5.0 and above to saturated red for log ratios 5.0 and above. Red indicates increased gene expression levels, whereas green indicates decreased levels compared with normal samples.
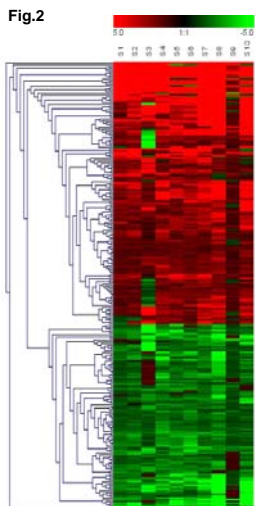
In the figure, S1-S10 denotes SARS samples 1-10.

Fig.2

Functional categorization of differentially expressed genes.
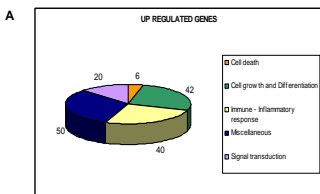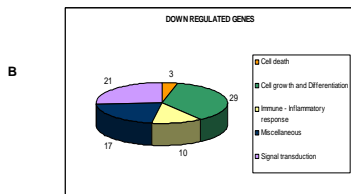
Fig.3

A UP REGULATED GENES

FIG. 3. Functional categorization of differentially expressed genes. Genes were identified as aberrantly regulated as described under Experimental Procedures. Genes were grouped into categories based on information available in the literature, as accessed by PubMed, the Gene Ontology Consortium.

A. Pie-chart showing functional categorization of 125 up-regulated genes.

B. Pie-chart showing functional categorization of 61 down-regulated genes.

B DOWN REGULATED GENES

Real-time PCR: Validation of microarray results

Fig.4

A Lactotransferrin

B Lipocalin

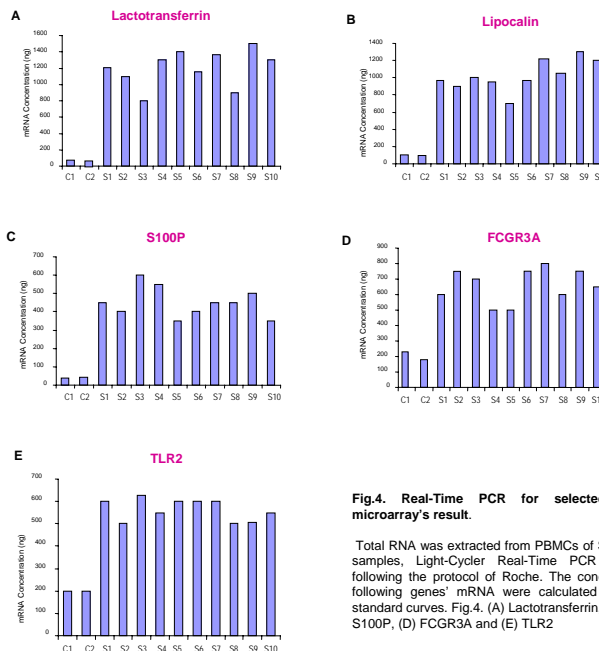**C** S100P

D FCGR3A

E TLR2

Fig.4. Real-Time PCR for selected genes from microarray's result.

Total RNA was extracted from PBMCs of SARS and normal samples, Light-Cycler Real-Time PCR was performed following the protocol of Roche. The concentrations of the following genes' mRNA were calculated using respective standard curves. Fig.4. (A) Lactotransferrin, (B) Lipocalin, (C) S100P, (D) FCGR3A and (E) TLR2

Real-time RT-PCR analysis

Confirmation of expression differences by Real-time RT-PCR. Real-time RT-PCR was used to further verify the relative levels of expression of important genes. A similar expression pattern was obtained for all these genes. The result (table.1) shows significant over expression of genes in SARS samples over controls.

\# mRNA concentration in ng

* Determined by real-time RT-PCR analysis; calculated as follows: (SARS sample)/ (Normal control).

Table.1

Gene name	Control #	SARS sample #	Fold increase*
LTF	75±5	2000±400	26.6
S100P	45±5	475±125	10.5
Lipocalin	95±10	1000±300	10.5
FCGR3A	205±25	650±150	3.17
TLR2	200	563±62	2.8

Expression by microarray analysis compared with Real-time RT-PCR analysis.

Expression of all the five genes by real-time RT-PCR was compared with their expression levels by microarray analysis(Table.2).

The real-time PCR method verified the results of the gene array analysis and showed that those genes that were up-regulated as determined by microarray analysis found to be up-regulated by real-time PCR analysis.

* calculated as (SARS sample)/ (Normal control).

Table.2

Gene Name	Real-time RT-PCR		Microarray	
	mRNA conc.(ng)*	Fold increase	Mean Expression*	Fold increase
LTF	2000/75	26.6	13043/282	46.25
Lipocalin	1000/95	10.5	9015/47	191.81
FCGR3A	650/205	3.17	2543/1145	2.22
S100P	475/45	10.5	3855/205	18.80
TLR2	563/200	2.8	1755/765	2.29

References

1. Rota, P. A., *et al.* Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* **300,** 1394–1399 (2003).

2. Ksiazek, T. G., *et al.* A novel coronavirus associated with severe acute respiratory syndrome. *N. Engl. J. Med.* **348,** 1953–1966 (2003).

3. Guidotti, L. G., & Chisari, F. V. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu. Rev. Immunol.* **19,** 65-91 (2001).

4. M. Ashburner, *et al.* Gene ontology: tool for the unification of biology from the GeneOntology Consortium. *Nat. Genet.* **25,** 25 (2000).

Conclusion

Microarray analysis is a very powerful tool for investigating the genes that are differentially expressed in SARS samples compared with normal control. However, it has become clear that statistical methods are needed, since simple nonstatistical methods have been shown to be inadequate. Reaction of the immune system of SARS affected patients seems to be mainly of innate inflammatory response, rather than a specific immune response against a viral infection. There is no significant level of upregulation of MHC I genes or major cytokines or IFNγ or complement mediated cytolysis suggesting that the immune response against SARS virus might be different from other viral infections or the virus might be using a different strategy to evade the immune system and cause the pathogenesis and mortality.

Acknowledgements

Poster #11