SARS-CoV Regulates Immune Function-Related Gene Expression in Human Monocytic Cells

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Abstract

Severe acute respiratory syndrome (SARS) is characterized by acute respiratory distress syndrome (ARDS) and pulmonary fibrosis, and monocytes/macrophages are the key players in the pathogenesis of SARS. In this study, we compared the transcriptional profiles of SARS coronavirus (SARS-CoV)-infected monocytic cells against that infected by coronavirus 229E (CoV-229E). Total RNA was extracted from infected DC-SIGN-transfected monocytes (THP-1-DC-SIGN) at 6 and 24 h after infection, and the gene expression was profiled in oligonucleotide-based microarrays. Analysis of immune-related gene expression profiles showed that at 24 h after SARS-CoV infection: (1) IFN- α/β -inducible and cathepsin/proteasome genes were downregulated; (2) hypoxia/hyperoxia-related genes were upregulated; and (3) TLR/TLR-signaling, cytokine/cytokine receptor-related, chemokine/chemokine receptor-related, lysosome-related, MHC/chaperon-related, and fibrosis-related genes were differentially regulated. These results elucidate that SARS-CoV infection regulates immune-related genes in monocytes/macrophages, which may be important to the pathogenesis of SARS.

Introduction

BETWEEN NOVEMBER 2002 AND JULY 2003, the outbreak of severe acute respiratory syndrome (SARS) greatly impacted public health around the world. A total of 8096 cases and 774 deaths were reported (50). About 20% of SARS patients developed acute respiratory distress syndrome (ARDS) (40). Chest x-rays revealed bilateral diffuse consolidation in these patients (15). Massive macrophage infiltration is a prominent feature in the lung sections of patients who died of SARS (36). We reported that SARS coronavirus (SARS-CoV) infects human monocytes (9), and that monocytic cells infected by SARS-CoV produce chemokines that attract the migration of neutrophils, macrophages, and activated T lymphocytes (64). Patients who recovered from SARS often suffered from pulmonary fibrosis (3), and macrophages play a role in fibroblast accumulation (30). Thus it is apparent that the immune response plays an important role in the pathogenesis of SARS, and that monocytes/macrophages are key players in the immunopathogenesis of SARS.

The microarray is a tool that is useful in revealing the host response to an infectious agent at the genomic level. Microarray methodology has been used to examine immune cell gene expression profiles after infection by *Mycobacterium leprae*, cytomegalovirus (CMV), human immunodeficiency virus (HIV), *Escherichia coli*, *Chlamydia pneumoniae*, *Plasmodium*

falciparum, Japanese encephalitis virus, and influenza virus H5N1 (5,8,12,16,37,43,56,65). The gene expression profile in peripheral blood mononuclear cells (PBMCs) of convalescent SARS patients has been reported in a microarray study (31). It has also been shown by microarray analysis that SARS-CoV infection of primary human monocyte-derived macrophages is abortive and does not induce IFN- β (8). The molecular signature and disease severity index thus identified are useful for the diagnosis and prognosis of SARS-CoV infection if another SARS outbreak should occur (31). However, the expression of immune function-related genes in SARS-CoV-infected monocytes/macrophages has never been revealed.

In the present study, oligo-microarrays were used to profile the expression of immune function-related genes. We used DC-SIGN stably-transfected monocytic THP-1 cells as targets to model the alveolar environment, as it has been shown that interstitial alveolar macrophages in histologically normal adult lung tissue constitutively express DC-SIGN (51). The gene expression profile induced by SARS-CoV was compared to that expressed by human coronavirus 229E, a group I coronavirus that causes the mild common cold (13), on the basis that the infectivity of CoV-229E of DC-SIGN-transfected THP-1 cells is comparable to that of SARS-CoV (64). Since SARS-CoV-induced chemokine gene expression in monocytic cells peaks as early as 24 h (64), we chose to study

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the gene expression profiles at both 6 and 24 h after infection. The results of the present study showed that after SARS-CoV infection of monocytes, the expression of (1) IFN- α / β -inducible and cathepsin/proteasome genes were downregulated; (2) hypoxia/hyperoxia-related genes were upregulated; and (3) TLR/TLR-signaling, cytokine/cytokine receptor-related, chemokine/chemokine receptor-related, lysosome-related, MHC/chaperon-related, and fibrosis-related genes were differentially regulated.

Materials and Methods

Monocytic cell culture

The THP-1 cell line human monocytic cell line THP-1 stably transfected with DC-SIGN (THP-1-DC-SIGN) was kindly provided by Dr. Vineet N. Kewal Ramani (Model Development Section, HIV Drug Resistance Program, National Cancer Institute, U.S. National Institutes of Health). The cell line was maintained in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) in 5% CO₂ at 37°C.

Virus infection

THP-1-DC-SIGN cells were seeded in 15-mL TPP culture tubes at a density of 1×10^6 cells/mL. The cells were left in medium or infected with SARS-CoV TW1 (22) or CoV-229E, at 100-fold 50% tissue culture infective doses (TCID₅₀, titrated in Vero E6 or MRC-5 cells, respectively), and incubated for 6 or 24 h. The infectivities of SARS-CoV TW1 and CoV-229E were compared and they were comparable (63). Morphologically, most of the cells remained semi-adherent to the wall of the culture tubes up to 24 h after infection, with estimated viability of about 90%. At different time points post-infection, cells were harvested and culture supernatants were collected and stored at -70°C. For chip hybridization assays, cell pellets were resuspended in TRIzol. Experiments that required handling SARS-CoV were performed in the P3 facility in the National Taiwan University College of Medicine. All procedures were performed according to the Centers for Disease Control and Prevention P3 Biosafety Guidelines.

Monitoring of virus titer

To monitor SARS-CoV and CoV-229E titers, Vero E6 and MARC-5 cells were seeded at 1×10^4 cells/well in 96-well plates. Two days after seeding, 1:10 serial dilutions (from 10^{-1} to 10^{-8}) of supernatants harvested from SARS-CoV- or CoV-229E-infected cell cultures were added to quadruplicate wells. Cytopathic effects were assessed 3 d later. The TCID₅₀ was defined as the dosage at which two out of four quadruplicate wells exhibited cytopathic effects.

RNA extraction

Total RNA was extracted from uninfected, CoV-229E-infected, and SARS-CoV-infected THP-1-DC-SIGN cells. After chloroform was added to each tube, the samples were centrifuged, and RNA contents were isolated from the upper aqueous phase. Glycogen and isopropanol were added to precipitate the total RNA. After washing with 75% ethanol, total RNA was spun down and stored in DEPC-water. The A260/A280 OD ratios of all RNA samples were >1.800, as

determined by nanodrop-spectrophotometry (NanoDrop Technologies, Wilmington, DE). Capillary RNA gel electrophoresis (Agilent, Palo Alto, CA) was performed to ensure that the total RNA samples had clear 18S and 28S ribosomal RNA bands.

Chip hybridization

Oligonucleotide-based microarrays, validated by the U.S. National Cancer Institute (54), were provided by Compugen (Jamesburg, NJ), and printed by an OmniGrid arrayer (San Carlos, CA). A total of 19,137 oligos were used in the microarrays. QIAGEN (Valencia, CA) RNA clean-up columns containing DNase were used to further purify RNA samples. Then 20 μ g of RNAs from SARS-CoV- and CoV-229E-infected DC-SIGN-THP-1 cells, and 40 μ g of RNA from uninfected controls, were labeled with cyanine 3-dUTP (Cyt3), and cyanine 5-dUTP (Cyt5), respectively. The Cyt3- and Cyt5-labeled samples were then mixed with 1 μ L of human Cot1-DNA (10 μ g/ μ L), polyA (8–10 μ g/ μ L), and yeast tRNA (4 μ g/ μ L) each, to block non-specific binding. The sample mixture was then denatured at 100°C for 1 min. Then 20 μ L of 2 $\times$ hybridization buffer (50% formamide, 10 $\times$ SSC, and 0.2% SDS) was added to each pre-hybridized slide. Hybridization was performed in a humid chamber in a water bath at 42°C overnight. Two arrays for both SARS-CoV and CoV-229E for each time point were obtained.

Image and data analysis

After hybridization, the slides were scanned on a GenePix 4000A scanner (Axon Instruments, Foster City, CA). The TIFF images were then analyzed by GenePix Pro software and GPR files were generated. Signal dots were aligned with the grid first, and signal dots that were too small were deleted. If bubbles or severe scratches appeared in the slide images, the signal dots were considered invalid. The photomultiplier tube gain of red light (635 nm) intensity and green light (532 nm) intensity were adjusted according to the following principles. First, a general normalization method was applied to ensure that the ratio of total correct light intensity to green light intensity was 1:1. Second, all the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) dots were adjusted so they appeared to be yellow, as GAPDH should not have been up- or down-regulated. All the data were uploaded to the US National Cancer Institute Microarray Website for data processing, transformation, and annotation. The transformed numerical data, with gene annotations, were then downloaded from the website. Genes were highlighted when the average expression ratios (fold change) were >1.5 (>1.5-fold up-regulation) or <0.67 (>1.5-fold downregulation). When the intensity ratio was positive from one replicate and negative from the other replicate, the images from the GPR files were rechecked. If the data were still inconsistent, they were excluded from further analysis. A heat map of the microarray results is shown in Fig. 1.

Pathway network analysis

The gene network and pathway analyses were generated with IPA (Ingenuity Systems; www.ingenuity.com). Ingenuity pathway analysis software was used to identify

specific upregulated or downregulated gene-to-gene networks at 24 h after SARS-CoV infection. Data including gene name and fold change levels were input into the software. Genes that are immune response related were selected in the pathway analysis. In this analysis, two major immunological pathway networks were identified, including an IL-8-centered network and a proteasome-related gene network (Fig. 2).

Results

The expression of IFN- α/β -inducible genes is downregulated

IFN- α/β is the first line of host defense against virus infection. The expression of IFN- α 14, and RNA-activated protein kinase, an enzyme that phosphorylates EIF2 to inhibit cellular protein translation (47), were slightly upregulated after CoV-229E infection at 6 h, and remained upregulated at 24 h. IFN-inducible guanylate binding proteins (GIP2 and GIP3) and IFN-inducible myxovirus resistance-1 (MX1) were moderately downregulated at 24 h after infection (Table 1). Interestingly, almost all interferon-related genes were downregulated by >1.5-fold in SARS-CoV-infected monocytic cells at 6 h. These genes included IFN-induced protein 35 (IFI 35), GIP2 and GIP3, 2',5'-oligoadenylate synthetase 2 (2,5OAS2), MX1, and IFN- α -inducible protein 44. At 24 h after SARS-CoV infection, more IFN-related genes were downregulated, and these included IFN- α 14, IFN-stimulated 3 gamma (ISGF3g), IFN-induced exonuclease, and RNA-activated protein kinase (eukaryotic translation initiation factor 2 α kinase, EIF2AK), in addition to IFN-induced protein 35, GIP2 and GIP3, 2,5OAS2, MX1, and IFN- α -inducible protein 44. It is worth noting that GIP3, 2,5OAS2, MX1, and IFN- α -inducible protein 44 were downregulated by >2-fold at both 6 and 24 h after SARS-CoV infection. These results indicate that while CoV-229E induces IFN- α responses, SARS-CoV infection of macrophages not only does not induce IFN- α/β responses, but it also downregulates IFN-related genes. Thus it appears that the type 1 interferon response is intact in CoV-229E-infected monocytes, but is significantly downregulated in SARS-CoV-infected cells.

The expression of toll-like receptors and toll-like receptor signaling molecules are differentially regulated

Triggering the toll-like receptor (TLR)-signaling pathway is important to the induction of innate immune responses (23). CoV-229E infection induced upregulation of toll-like receptor adaptor molecule-1 (TRIF-1), but most TLR-related genes remained unchanged at 6 h and 24 h post-infection (Table 2). While only slight changes in most TLR-related

FIG. 1. Heat map image of microarray analysis result of SARS-CoV- and CoV-229E infected THP-1-DC-SIGN cells (s6M, SARS 6 h mean; s6A, SARS 6 h sample A; s6B, SARS 6 h sample B; e6M, CoV-229E 6 h mean; e6A, CoV-229E 6 h sample A; e6B, CoV-229E 6 h sample B; s24M, SARS 24 h mean; s24A, SARS 24 h sample A; s24B, SARS 24 h sample B; e24M, CoV-229E 24 h mean; e24A, CoV-229E 24 h sample A; e24B, CoV-229E 24 h sample B). Color images available online at www.liebertpub.com/vim

FIG. 2. Pathway analysis of the gene-to-gene networks of the IL-8 and proteasome families. Red and green colors denote upregulation and downregulation, respectively. Darker color indicates greater up- or downregulation, and lighter color mild to moderate up- or downregulation. Solid and dotted lines indicate direct and indirect upregulation from upstream gene A to downstream gene B, respectively. Straight lines with blunt ends indicate downregulation from upstream gene A to downstream gene B. Color images available online at www.liebertpub.com/vim

genes were observed at 6 h after SARS-CoV infection, both the negative regulator of TLR signaling IL-1 receptor-associated kinase M (IRAK-M) and TLR7 were >2-fold upregulated at 24 h. In the meantime, the downstream molecules of the TLR pathway MyD88 and TRIF, and the toll pathway evolutionary conservative (SITPEC), were all downregulated. The expression of other TLRs, including TLR5, TLR4, and TLR2, were also moderately upregulated at 24 h after SARS-CoV infection. Strong expression of IRAK-M and downregulation of the downstream signal molecules of TLRs suggest that although TLR7 was upregulated, the TLR7 downstream signals may not be transduced in SARS-CoV-infected monocytes.

The expression of cytokines, receptors, and signaling molecules are differentially regulated

As cytokines are important immune mediators, we studied the cytokine, receptor, and signal molecule gene expression in monocytes (Table 3). At 24 h after SARS-CoV infection, the genes that were upregulated were tumor necrosis factor receptor superfamily member 21 (TNFRSF21) and TNF- α inducible protein 1 (TNFAIP1). Also, the gene expression of TNF receptor 21 was upregulated >2-fold. In the TGF family, transforming growth factor- β 2 (TGF- β 2) and IL-2 receptor-enhancing thioredoxin were >1.5-fold downregulated by SARS-CoV at 24 h after infection; endoglin was downregulated >2-fold. Interestingly, Th-1-related IFN- γ inducible adhesion regulating molecule 1 (ADRM1) and Th-2 key cytokine IL-13 were downregulated.

The expression of lysosome-related genes are differentially regulated

Lysosomal enzymes responsible for degrading foreign proteins are important to the biological functions of macrophages (2). While not much change was observed in the expression of lysosome-related genes after CoV-229E infection except ATPase 21KDV0C, the expression of GlcNAc

TABLE 1. INTERFERON GENE EXPRESSION

Gene	GeneBank accession number	Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
IFN- α 14	NM002172	0.95	0.96	0.93	1.34	1.78	0.90	0.66	0.41	0.91	1.11	1.10	1.12
ISGF3	NM006084	1.04	0.97	1.10	1.00	1.00	1.00	0.62	0.54	0.69	1.01	1.05	1.11
IFN exonuclease	NM002201	0.93	0.96	0.90	1.03	1.51	0.56	0.62	0.43	0.81	0.99	1.01	0.98
EIF2AK	NM002759	0.98	0.88	1.08	2.05	2.55	1.55	0.58	0.54	0.62	1.25	1.18	0.97
IFI 35	NM005533	0.58	0.49	0.67	1.09	1.00	1.18	0.55	0.61	0.49	0.80	0.83	1.32
GIP3	NM002038	0.28	0.25	0.31	0.96	0.84	1.08	0.39	0.32	0.46	0.57	0.57	0.77
2,5OAS2	NM002535	0.28	0.30	0.26	1.47	1.00	1.95	0.34	0.42	0.26	0.76	0.73	0.58
MX1	NM002462	0.40	0.35	0.44	1.07	0.95	1.19	0.28	0.31	0.24	0.64	0.65	0.80
IFN- α 44	NM006417	0.32	0.27	0.37	1.28	0.96	1.59	0.26	0.34	0.18	0.93	0.90	0.62
GIP2	NM005101	0.61	0.55	0.66	0.64	0.70	0.57	0.20	0.19	0.21	0.58	0.57	0.95

A and B represent duplicate samples.
Figures in bold face type indicates >1.5-fold upregulation; those in bold face underlined type indicates <0.67 (>1.5-fold) downregulation.
GIP2, IFN-inducible guanylate binding protein 2; GIP3, IFN-inducible guanylate binding protein 3; MX1, IFN-inducible myxovirus resistance-1; IFI 35, IFN-induced protein 35; 2,5OAS2, 2',5'-oligoadenylate synthetase 2; IFN- α 44, IFN- α -inducible protein 44; IFN- α 14, IFN- α -inducible protein 14; ISGF3g, IFN-stimulated 3 gamma; EIF2AK, eukaryotic translation initiation factor 2 α kinase.

TABLE 2. TOLL-LIKE GENE EXPRESSION

Group/Gene	GeneBank accession number	Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
Toll-like signaling:													
IRAK-M	NM007199	1.00	1.00	1.00	1.00	1.00	1.00	2.35	2.51	2.19	1.36	1.40	1.31
MyD88	NM002468	0.90	0.78	1.01	0.77	1.00	0.53	0.63	0.68	0.59	0.98	1.01	0.94
TRIF-1	NM014261	0.73	0.66	0.81	1.57	1.73	1.40	0.60	0.40	0.80	1.12	1.07	1.16
SITPEC	NM016581	0.73	0.79	0.67	1.00	1.00	0.99	0.51	0.43	0.59	0.93	0.91	0.95
TLR molecules													
TLR7	NM016562	1.17	1.35	1.00	1.00	1.00	1.00	2.10	1.84	2.36	1.03	1.01	1.04
TLR5	NM003268	0.78	0.72	0.83	0.97	1.10	0.83	1.32	1.16	1.48	1.01	1.05	0.97
TLR4	NM003266	1.19	1.39	1.00	1.00	1.00	1.00	1.27	1.54	1.00	0.98	0.96	1.00
TLR2	NM003264	1.06	1.13	1.00	1.00	1.00	1.00	1.12	1.22	1.01	1.03	1.02	1.04
TLR9	NM017442	0.88	0.95	0.82	1.11	1.00	1.22	0.71	0.81	0.61	1.00	1.03	0.98

A and B represent duplicate samples.

Figures in bold face type indicates >1.5-fold upregulation; those in bold face underlined type indicates <0.67 (>1.5-fold) downregulation. TRIF-1, toll-like receptor adaptor molecule-1; TLR, toll-like receptor; SITPEC, toll pathway evolutionary conservative.

phosphotransferase (GlcNAcp) was downregulated at 6 h after SARS-CoV infection (Table 4). At 24 h after SARS-CoV infection, all but ATPaseV01 and glucosamine-6-sulfatase (G-6-S) lysosome-related genes were downregulated. These genes included GlcNAcp, palmitoyl-protein thioesterase 2 (PPTE2), mannosidase, lysosome ATPase subunits (14KDV1F and 21KDV0C), lysosomal-associated protein (LAMP5), mu-1 subunit (AP2M1), and prosaposin. It is

worth noting that lysosome ATPase 21KDV0C and prosapsin were >2-fold downregulated at 24 h after SARS-CoV infection. These data demonstrated that although the genes of lysosome-related enzymes were differentially regulated after SARS-CoV infection, most of them were downregulated. The results indicate that SARS-CoV-infected monocytes lost the ability to degrade ingested proteins.

TABLE 3. CYTOKINE GENE EXPRESSION

		Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
Group/Gene	GeneBank accession number	Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
TNF superfamily													
TNFRSF21	NM614452	1.00	1.00	1.00	1.00	1.00	1.00	2.23	2.16	2.30	1.11	1.06	1.17
TNFAIP1	NM021137	1.05	1.10	1.00	1.00	1.00	1.00	1.93	2.55	1.32	0.85	0.81	0.88
TRAF4	NM004295	0.72	0.68	0.77	1.11	1.80	0.41	0.67	0.49	0.86	0.96	0.99	0.92
TRAIL	NM003810	0.94	0.99	0.88	1.05	1.00	1.10	0.64	0.52	0.75	0.76	0.73	0.79
TGFB-related													
TGF- β 2	NM003242	1.06	1.00	1.12	0.87	1.13	0.61	0.65	0.48	0.82	0.93	1.01	0.85
Endoglin	NM000118	0.65	0.63	0.66	1.09	1.25	0.94	0.27	0.26	0.29	0.72	0.76	0.68
Interleukin-related													
IL-6R	NM181359	0.83	0.79	0.88	1.17	1.00	1.34	0.66	0.46	0.86	1.12	1.16	1.08
IL-13	NM002188	0.76	0.84	0.69	1.33	1.52	1.14	0.55	0.36	0.74	1.01	0.95	1.08
IFN- γ -related													
ADRM1	NM007002	1.14	1.21	1.06	0.79	1.00	0.57	0.55	0.62	0.48	0.88	0.86	0.90

A and B represent duplicate samples.

Figures in bold face underlined type indicates <0.67 (>1.5-fold) downregulation.

TNFRSF21, tumor necrosis factor receptor superfamily member 21; TNFAIP1, TNF- α inducible protein 1; TGF- β 2, transforming growth factor- β 2; ADRM1, TH1-related IFN- γ inducible adhesion regulating molecule 1; TRAIL, TNF ligand family 10; TNFAF4, TNF receptor-associated factor 4.

TABLE 4. LYSOSOME GENE EXPRESSION

Gene	GeneBank accession number	Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
ATPaseV01	<u>NM005177</u>	1.21	1.41	1.00	1.00	1.00	1.00	2.45	1.78	3.12	1.23	1.45	1.00
G-6-S	<u>NM002076</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.55	1.55	1.56	1.17	1.17	1.16
GlcNAcp	<u>NM032520</u>	0.60	0.54	0.66	1.23	1.00	1.45	0.66	0.62	0.69	0.86	0.87	0.85
PPTE2	<u>NM005155</u>	0.75	0.69	0.81	1.00	1.00	1.00	0.66	0.57	0.75	1.02	1.02	1.03
Mannosidase	<u>NM022077</u>	0.80	0.81	0.78	0.80	0.73	0.87	0.66	0.48	0.85	0.84	0.86	0.82
ATPase14KDV1F	<u>NM004231</u>	0.87	0.86	0.88	0.72	0.81	0.63	0.67	0.40	0.97	0.93	0.96	0.90
ATPase protein1	<u>NM001183</u>	0.62	0.63	0.62	0.99	1.00	0.97	0.60	0.57	0.62	0.81	0.84	0.78
ATPase16KDV0C	<u>NM001694</u>	0.98	1.13	0.84	0.81	1.04	0.57	0.62	0.33	0.90	0.96	0.98	0.93
AP2M1	<u>NM004068</u>	0.93	0.91	0.95	1.00	0.83	1.16	0.53	0.34	0.73	0.91	0.96	0.86
LAMP5	<u>NM006762</u>	1.16	1.13	1.19	0.92	0.92	0.91	0.52	0.23	0.81	1.02	1.01	1.02
ATPase21KDV0C	<u>NM004047</u>	1.01	1.02	0.99	0.67	0.85	0.49	0.46	0.29	0.64	0.76	0.79	0.73
Prosaposin	<u>NM002778</u>	0.72	0.74	0.70	1.34	1.52	1.17	0.49	0.22	0.75	1.00	0.97	1.03

A and B represent duplicate samples.

Figures in bold face type indicates >1.5-fold upregulation; those in bold face underlined type indicates <0.67 (>1.5-fold) downregulation.

GlcNAcp, GlcNAc phosphotransferase; G-6-S, glucosamine-6-sulfatase; PPTE2, palmitoyl-protein thioesterase 2; LAMP5, lysosomal-associated protein; AP2M1, mu-1 subunit.

The expression of genes of the cathepsin and proteasome family are downregulated

The cathepsins and proteasomes are directly involved in antigen processing in the MHC class II and I pathways, respectively (14,21). While CoV-229E infection of monocytes did not result in much change in the cathepsin and proteasome genes, SARS-CoV infection induced downregulation of all of them (Table 5). It was most apparent 24 h after infection, that cathepsin A, as well as proteasomes β 2, 26S ATPase 4 (26S/A4), and β 3, were all downregulated >1.5-fold; cathepsin H was downregulated >2-fold. These results show that the MHC class II and I antigen processing pathways are inhibited in SARS-CoV-infected monocytes.

The expression of chaperon and MHC-related genes are differentially regulated

To further understand the regulation of genes that are directly involved in antigen processing/presentation, chaperon and MHC-related genes were analyzed. Table 6 shows that CoV-229E-induced downregulation of SEC61B and upregulation of the calreticulin gene at 6 h after infection. The expression of other chaperon and MHC-related genes remained unchanged at either 6 or 24 h. SARS-CoV infection, however, induced upregulation of cyclophilin G and downregulation of cyclophilin C and FK506 binding protein 10 at 6 h. By 24 h after SARS-CoV infection, though cyclophilins D and G and heat shock factor 1 were upregulated,

TABLE 5. CATHEPSIN/PROTEASOME EXPRESSION

Group/Gene	GeneBank accession number	Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
Cathepsins													
Cathepsin A	<u>NM000308</u>	0.87	0.75	0.99	1.22	1.00	1.43	0.59	0.40	0.78	0.88	0.92	0.84
Cathepsin S	<u>NM004079</u>	0.53	0.59	0.47	1.52	1.00	2.04	0.54	0.42	0.66	1.07	1.11	1.03
Cathepsin H	<u>NM004390</u>	0.65	0.62	0.68	1.00	1.00	1.00	0.45	0.56	0.33	1.00	0.93	1.07
Proteasomes													
Proteasome β 2 (PSMB2)	<u>NM002794</u>	1.07	1.15	1.00	0.96	1.00	0.92	0.63	0.57	0.69	1.05	0.96	1.14
Proteasome 26S/A4	<u>NM006503</u>	1.00	0.96	1.05	1.07	1.39	0.75	0.54	0.62	0.47	0.82	0.85	0.80
Proteasome β 3 (PSMB3)	<u>NM002795</u>	1.10	0.96	1.24	0.75	0.45	1.04	0.52	0.36	0.67	0.85	0.86	0.84

A and B represent duplicate samples.

Figures in bold face type indicates >1.5-fold upregulation; those in bold face underlined type indicates <0.67 (>1.5-fold) downregulation.

TABLE 6. CHAPERON/HLA EXPRESSION

Gene	GeneBank accession number	Fold change											
		6h						24h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
Cyclophilin D	<u>NM005038</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.95	2.04	1.85	1.11	1.10	1.12
Heat shock factor 1	<u>NM005526</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.69	1.91	1.48	0.99	0.96	1.01
Cyclophilin G	<u>NM004792</u>	2.44	2.20	2.68	1.00	1.00	1.00	1.66	1.59	1.73	1.39	1.45	1.32
HLA-B-associated 2	<u>NM004638</u>	0.98	0.99	0.97	0.78	1.00	0.55	0.63	0.71	0.56	1.46	1.52	1.41
FK506 binding protein 8	<u>NM012181</u>	0.69	0.58	0.81	0.85	0.65	1.05	0.64	0.51	0.76	0.87	0.91	0.84
Activator of HSP90KD	<u>NM012111</u>	1.02	1.04	1.00	1.00	1.00	1.00	0.63	0.71	0.54	0.98	0.88	1.08
HLA complex group9	<u>NM005844</u>	1.12	1.03	1.21	1.02	1.00	1.04	0.58	0.49	0.66	0.84	0.90	0.78
SEC61 complex Beta	<u>NM006808</u>	0.80	0.73	0.88	0.62	0.74	0.51	0.61	0.38	0.84	0.87	0.93	0.80
Cyclophilin C	<u>NM000943</u>	0.58	0.57	0.59	1.07	0.57	1.57	0.62	0.32	0.92	0.91	0.91	0.91
Valosin-containing protein	<u>NM007126</u>	1.18	1.25	1.11	1.06	1.09	1.02	0.53	0.36	0.71	0.93	0.90	0.95
Heat shock protein 10KD1	<u>NM002157</u>	0.86	0.84	0.88	1.25	0.37	2.13	0.51	0.37	0.66	0.87	0.88	0.87
FK506 binding protein 10	<u>NM021939</u>	0.66	0.68	0.63	1.03	1.23	0.83	0.48	0.36	0.61	0.89	0.88	0.91
Calreticulin	<u>NM004343</u>	1.23	1.36	1.10	1.96	2.45	1.48	0.43	0.23	0.62	1.27	1.23	1.31
Cyclophilin B	<u>NM000942</u>	0.69	0.71	0.67	1.12	0.54	1.71	0.36	0.12	0.59	1.03	1.04	1.01

A and B represent duplicate samples.

Figures in bold face type indicates >1.5-fold upregulation; those in bold face underlined type indicates <0.67 (>1.5-fold) downregulation.

most chaperon and MHC-related genes were down-regulated. The downregulated genes included chaperonin (valosin-containing protein), HLA genes (HLA-B-associated transcript 2 and HLA complex group 9), FK506 binding proteins (FK506 BP8 and FK506 BP10), heat shock protein (activator of HSP90KD), cyclophilins C and B, and the MHC transporters SEC61B and calreticulin. The expression profiles of the cathepsins, proteasomes, chaperons, and MHC-related molecules together strongly indicate that antigen processing and presentation pathways are dysfunctional in SARS-CoV-infected monocytes.

The expression of chemokine receptors and related genes are differentially regulated

Given that recruitment of leukocytes to the site of inflammation is orchestrated by chemokines (6), and ARDS is characterized by heavy infiltration of monocytes, neutrophils, and fibroblasts to the lungs (60), we next examined the expression profile of chemokine-related genes. While all chemokine-related genes except MIP-1 α /CCL3 (which was downregulated at both 6 and 24 h) in CoV-229E-infected cells remained unchanged, many of them were up- or down-regulated in SARS-CoV-infected cells (Table 7). In fact, as early as 6 h after SARS-CoV infection, the downregulation of MIP-1 α /CCL3, prostaglandin D synthase (PGDS), RANTES/CCL5, and LTXC4 synthase genes was already obvious. At 24 h after infection, the genes that were upregulated included CXCL8/IL-8, hyaluronidase 3, platelet-derived growth factor receptor α (PDGFR α), and leukotriene A4 (LTXA4) hydroxylase. The expression of CXCL8/IL-8 genes was >2-fold higher than controls. Others, like prostaglandin E synthase

(PGEs), chemokine-like factor (CLF), MIP-1 α /CCL3, RANTES/CCL5, and LTXC4 synthase, were downregulated. Among them, the expression of CCL3/MIP-1, RANTES/CCL5, and LTXC4 synthase genes were >2-fold lower than controls. The upregulated genes (CXCL8/IL-8, hyaluronidase 3, PDGFR receptor α , and LTXA hydroxylase) are known to recruit monocytes, neutrophils, and fibroblasts, which may explain the complication of ARDS in SARS-CoV infection.

The expression of fibrosis-related genes is differentially regulated

Pulmonary fibrosis resulting from lung injury is a serious complication of ARDS and SARS. Thus we examined whether fibrosis-related gene expression is changed after SARS infection. The data in Table 8 show that while CoV-229E infection did not change the gene expression profile except for tissue inhibitor of metalloproteinase 1 (TIMP1) at 6 h, SARS-CoV infection induced up- and downregulation of fibrosis-related genes. The other genes that were upregulated at 24 h include matrix metalloproteinase 28 (MMP28) and A disintegrin and metalloproteinase domain 19 (ADAM 19). On the other hand, other fibrosis-related genes were down-regulated, and these include protein S, MMP2, collagens XVIII α 1 and I α 2, spondin 2 (SPON2), procollagenlysin-2-oxoglutarate 5-dioxygenase 3 (PLOD3), A disintegrin and metalloproteinase with thrombospondin type 1 motif 4 (ADAMTS4), TIMP3, and TIMP1. The genes that were downregulated by >2-fold were collagens XVIII α 1 and I α 2, and TIMPs 1 and 3. The regulation of fibrosis-related genes

TABLE 7. CHEMOKINE GENE EXPRESSION

Gene	GeneBank accession number	Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
Interleukin-8	<u>NM000584</u>	0.80	0.60	1.00	8.50	16.91	0.08	2.55	2.28	2.83	0.91	0.90	0.92
C/EBP delta (CEBPD)	<u>NM005195</u>	1.18	1.05	1.32	0.82	1.21	0.44	2.04	1.30	2.77	0.86	0.79	0.82
Hyaluronidase 3	<u>NM003549</u>	1.29	1.27	1.30	1.00	1.00	1.00	1.80	1.35	2.25	0.79	0.77	0.81
Complement 3	<u>NM000064</u>	1.09	1.18	1.00	1.00	1.00	1.00	1.66	1.75	1.57	0.99	0.93	1.05
Platelet-derived growth factor receptor α	<u>NM006206</u>	1.03	1.00	1.06	1.00	1.00	1.00	1.65	1.68	1.62	1.27	1.29	1.24
Leukotriene A4 hydroxylase	<u>NM000895</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.68	1.37	1.99	1.08	1.07	1.08
CD14	<u>NM000591</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.58	1.62	1.54	1.14	1.24	1.04
Prostaglandin E synthase	<u>NM004878</u>	1.10	0.86	1.34	1.00	1.00	1.00	0.58	0.59	0.57	1.15	1.18	1.13
Chemokine-like factor	<u>NM016326</u>	0.83	0.65	1.00	1.00	1.00	1.00	0.58	0.72	0.44	1.16	1.25	1.08
ANPEP	<u>NM001150</u>	0.75	0.78	0.71	0.85	1	0.69	0.57	0.47	0.68	1.08	1.13	1.04
CCL3	<u>NM002983</u>	0.19	0.20	0.18	1.35	1.00	1.69	0.49	0.52	0.45	0.39	0.42	0.37
Prostaglandin D synthase	<u>NM014485</u>	0.49	0.44	0.55	0.98	1.00	0.95	0.54	0.28	0.80	0.82	0.85	0.78
CCL5	<u>NM002985</u>	0.35	0.33	0.38	0.73	0.87	0.59	0.28	0.26	0.29	0.94	1.02	0.86
Leukotriene C4 synthase	<u>NM000897</u>	0.32	0.28	0.37	1.30	1.00	1.59	0.28	0.25	0.30	0.69	0.74	0.64

A and B represent duplicate samples.

Figures in bold face type indicates >1.5-fold upregulation; those in bold face underlined type indicates <0.67 (>1.5-fold) downregulation.

may help to explain the pathogenesis of ARDS and pulmonary fibrosis in SARS-CoV infection.

The expression of oxygen stress-related genes are upregulated

Change of oxygen tension is a source of stress. While CoV-229E infection did not induce oxygen stress-related genes, SARS-CoV infection induced the upregulation of hypoxia upregulated 1 gene as early as 6 h, and sustained it until 24 h

(Table 9). Oxidative responsive 1 gene upregulation was observed at 24, but not at 6 h after infection. These results demonstrate that SARS-CoV infection of monocytes creates a hypoxic environment, which induces the expression of hypoxia-related genes.

Discussion

Monocytes play a key role in mediating the inflammatory response in the lungs of SARS patients (36). Thus, the

TABLE 8. FIBROSIS GENE EXPRESSION

Gene	GeneBank accession number	Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
SERPINB2	<u>NM002575</u>	1.12	1.25	1.00	1.00	1.00	1.00	2.69	1.29	4.08	1.08	1.02	1.13
MMP28	<u>NM024302</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.75	1.99	1.51	0.77	0.77	0.78
ADAM19	<u>NM023038</u>	1.00	1.00	1.00	1.00	1.00	1.00	1.65	1.62	1.67	1.07	1.13	1.01
Protein S	<u>NM000313</u>	0.58	0.62	0.55	1.00	1.00	1.00	0.66	0.60	0.71	1.24	1.26	1.23
MMP2	<u>NM004530</u>	0.94	0.88	1.00	1.00	1.00	1.00	0.64	0.55	0.74	0.91	0.88	0.94
Spondin 2	<u>NM012445</u>	0.82	0.84	0.79	1.04	0.70	1.38	0.63	0.43	0.82	0.93	0.94	0.93
PLOD3	<u>NM001084</u>	0.73	0.64	0.81	1.07	0.68	1.46	0.61	0.78	0.44	0.71	0.74	0.67
ADAMTS4	<u>NM005099</u>	0.80	0.59	1.00	1.00	1.00	1.00	0.56	0.62	0.50	1.10	1.19	1.01
Collagen XVIII $\alpha 1$	<u>NM030582</u>	0.76	0.75	0.77	0.81	0.89	0.74	0.48	0.31	0.66	0.90	0.93	0.87
TIMP3	<u>NM000362</u>	1.03	0.93	1.13	1.16	1.26	1.06	0.45	0.26	0.63	1.09	1.13	1.06
Collagen I α 2	<u>NM000089</u>	0.57	0.57	0.57	0.98	0.91	1.04	0.40	0.25	0.55	0.97	0.93	1.01
TIMP1	<u>NM003254</u>	0.75	0.69	0.81	0.67	0.82	0.51	0.38	0.15	0.62	0.98	1.04	0.91

A and B represent duplicate samples.

Figures in bold face type indicates >1.5-fold upregulation; those in bold face underlined type indicates <0.67 (>1.5-fold) downregulation.

MMP, matrix metalloproteinase; ADAM19, A disintegrin and metalloproteinase domain 19; SERPINB2, plasminogen activator inhibitor type 2; ADAMTS4, A disintegrin and metalloproteinase with thrombospondin type 1 motif 4; TIMP, tissue inhibitor of metalloproteinase; PLOD3, procollagenlysin-2-oxoglutarate 5-dioxygenase 3.

TABLE 9. HYPOXIA GENE EXPRESSION

Gene	GeneBank accession number	Fold change											
		6 h						24 h					
		SARS-CoV			CoV-229E			SARS-CoV			CoV-229E		
		Mean	A	B	Mean	A	B	Mean	A	B	Mean	A	B
Oxidative responsive 1	NM005109	1.00	1.00	1.00	0.87	1.00	0.73	1.64	1.83	1.45	1.04	0.97	1.12
Hypoxia upregulated 1	NM006389	1.52	1.49	1.55	0.78	0.90	0.67	1.65	1.29	2.01	0.99	1.09	0.88

A and B represent duplicate samples.

Figures in bold face type indicates >1.5-fold upregulation.

interaction between SARS-CoV and monocytes is important to the outcome of the infection. How SARS-CoV regulates gene expression has been a subject of interest. The PBMC and monocyte gene expression profiles were analyzed. A microarray analysis of PBMCs of patients recovering from SARS revealed that SARS-CoV infection causes a general suppression of gene expression, and induces the upregulation of several specific ESTs and eosinophil-derived neurotoxins (31). A different study showed that in contrast to CoV-229E and influenza (H1N1) infections, interferon- α/β signaling fails to occur in macrophages after SARS-CoV infection (9). The results of our study showed that most of the IFN- α/β -inducible, lysosome-related, cathepsin/proteasome, and MHC/chaperon genes are downregulated, while the genes for which products play suppressive roles (e.g., IRAK-M) are upregulated. Together these results indicate that SARS-CoV infection causes macrophage dysfunction.

Interferon- α/β play a key role in the host defense against virus infection. Type 1 interferons suppress cellular protein expression to prevent virus replication, and also trigger host immunity against virus infection (35). It is reported that SARS-CoV infection of primary human monocyte-derived macrophages, in contrast to CoV-229E and influenza infections, induces very low or no IFN- β response (9). Our results also showed that while CoV-229E induced upregulation of RNA-activated protein kinase EIF2AK, and no change in IFN- α , ISF3g, IFN exonuclease, SARS-CoV infection downregulated the expression of all of the above genes and a host of other genes. Since type I interferons also play important roles in innate immunity, downregulation of their expression not only impairs the antiviral immune response, it also affects the innate immune response, such as the expression of cathepsin/proteasome- and lysosome-related genes that are critical to antigen presentation functions. Therefore, downregulation or null expression of IFN- α/β -related genes is significant as a pathogenic mechanism of SARS-CoV.

TLR7 is among the few genes that are upregulated by SARS-CoV (Table 2). TLR7 was recently shown to appear on the endosomal membrane, and is an intracellular receptor for single-stranded RNA (ssRNA) (19,32). It can distinguish cellular from viral ssRNAs because the cellular RNAs contain a greater number of modified nucleotide bases (m5C, m6A, m5U, and s2U) (26). It has not been explored whether intracellular TLR7 binds the nucleic acid of SARS-CoV and triggers downstream signals. Our data showing that SARS-

CoV induces the upregulation of TLR7 and the negative signal regulator IRAK-M, and the downregulation of the TLR downstream signal molecules MyD88, TRIF1, and SIT-PEC, as well as most of the IFN- α/β -inducible genes, demonstrate that even if TLR7 binds to SARS-CoV nucleic acids, its downstream signaling is inhibited (48,63). TRIF can activate IRF3 to initiate type 1 interferon expression. The downregulation of TRIF in SARS-CoV-infected monocytes and upregulation in CoV-229E-infected monocytes, may explain why the interferon response fails in SARS infection. MyD88-IRAK-NF- κ B signaling is also inactivated after SARS-CoV infection. However, TLR2, TLR4, and TLR5 are upregulated in SARS-CoV-infected monocytes. These three TLRs are strong antibacterial Th-17 immune response initiators. In addition, MyD88-independent CD14 signaling is upregulated after SARS-CoV infection. Monocytic CD14 signaling is usually activated in the LPS reaction to bacteria. This means that SARS-CoV fails to initiate an antiviral interferon response, and may induce ineffective antibacterial immunity. It has been shown that *Mycobacterium tuberculosis* binding to DC-SIGN on dendritic cells inhibits TLR signaling, and thus inhibits dendritic cell maturation (58). It is therefore also possible that SARS-CoV binding to DC-SIGN on THP-1-DC-SIGN monocytes inhibits TLR signals, as has been demonstrated in *M. tuberculosis*.

Cytokines are important immune mediators. SARS-CoV upregulates TNFRSF 21 >2.2 fold, and TNFAIP1 >1.9 fold (Table 3). All the TGF- β -related genes are downregulated. TGF- β is the key mediator of regulatory T cells. Thus, the Treg immune response is unlikely to be upregulated in SARS infection. These findings are consistent with several previous studies measuring cytokine responses in SARS-CoV-infected culture cells or SARS patients (44,52,66). In addition, IL-13 and IFN- γ inducible ADRM1 are downregulated. Thus, traditional Th-2 and Th-1 immune responses are unlikely upregulated in SARS infection. Since IL-1, IL-6, CCL2, and IP-10 were not included in the gene chip, even though they were shown to be important in the pathogenesis of SARS in several previous studies, their expression could not be evaluated in this study (7,30).

Lysosomal enzymes responsible for digesting vesicular proteins in the lysosome are important to the functions of monocytes and macrophages (55). SARS-CoV infection downregulates most lysosomal enzymes, including GlcNAcp, PPTE2, mannosidase, and ATPase (14KDV1F, protein 1, and 21KDV0C) (Table 4). Being professional

antigen-presenting cells, monocytes/macrophages process antigens through the activities of cathepsins and proteasomes, and present antigens via chaperon/MHC molecules (38). It is interesting that SARS-CoV infection downregulates all the cathepsin and proteasome genes, and most of the chaperon/MHC genes (Tables 4, 5, and 6), and that most of these downregulated genes in the proteasome family are directly or indirectly interrelated (Fig. 2), seemingly rendering monocytes/macrophages unable to process and present antigens. Thus it appears that by downregulating the genes that are important to antigen processing and presentation, SARS-CoV suppresses the primary functions of monocytes/macrophages. Since interferon- α/β upregulates MHC-related genes, the downregulation of interferon- α/β genes could be partially responsible for the downregulation of MHC-related genes (47). It is our speculation that by suppressing the antigen-processing and presentation functions of monocytes/macrophages, SARS-CoV delays specific T-cell activation and thus delays its own clearance.

The clinical picture of SARS is characterized by pulmonary cellular infiltration and lung consolidation (50). We have demonstrated infiltration of neutrophils, macrophages, and T lymphocytes in the lungs of SARS-CoV-infected patients during the early phase of infection (64), and CXCL8/IL-8 was produced by SARS-CoV-infected monocytes. The results of microarray analysis showed that CXCL8/IL-8 was upregulated >2-fold (Table 7). Pathway network analysis showed that many genes that upregulate CXCL8/IL-8 were upregulated after SARS-CoV infection (Fig. 2). These include C/EBP delta, CD14, and complement C3 (11,42,59,62). A previous study demonstrated that C/EBP delta can substitute for IL-17 to induce neutrophil activation and accumulation (45). Interestingly, alanyl aminopeptidase (ANPEP, Fig. 2), which is known to suppress IL-8 expression (25), was downregulated after SARS-CoV infection. We have previously shown using RNase protection and protein chip assays that CCL5/RANTES and CCL3/MIP-1 β were upregulated upon infection of THP-1-DC-SIGN cells at day 1 after infection (64). However, possibly due to the difference in the sensitivity of the methodologies, this was not the case by oligonucleotide-based microarray analysis. Taken together, SARS-CoV infection induces the upregulation of genes that upregulate and the downregulation of the gene that suppresses CXCL8/IL-8, showing that inducing neutrophil migration and activation is an important event in SARS-CoV infection.

The anti-inflammatory high-molecular-mass agent hyaluronan is known to be a protective factor against acute lung injury (24). Hyaluronidase degrades hyaluronan to hyaluronan fragments (53). In patients with acute lung injury numbers of hyaluronan fragments are increased (24). Hyaluronan fragments mediate macrophage and neutrophil migration, and increase the phagocytic function of neutrophils (17) and macrophage CXCL8/IL-8 production (33). Interestingly, SARS-CoV infection induces the upregulation of hyaluronidase 3 (Table 7). We speculate that through the activity of hyaluronidase 3 expression, more hyaluronan fragments are produced, and contribute to the increase in neutrophil and monocyte infiltration and function.

The relationship between leukotriene and lung fibrosis has been demonstrated in mice deficient in 5-lipoxygenase. Knock-out mice that are deficient in leukotriene are protected from lung fibrosis induced by bleomycin (41). No increase of inflammatory

cells in the lungs was noted in the knockout mice, in contrast to wild-type mice, which have abundant leukocytes. LTxA4 hydroxylase catalyzes the production of leukotriene B4 from leukotriene A4, while leukotriene C4 (LTXC4) synthases mediate the production of leukotriene C4 from leukotriene A4 (29,34). Our results showing downregulation of LTXC4 synthase and upregulation of LTxA4 hydroxylase (Table 7) indicate that there is increased leukotriene B4 accumulation after SARS-CoV infection. Therefore, leukotriene B4, a potent chemoattractant for neutrophils (39), could also account for the increase in neutrophil migration to the lungs.

PGE is commonly used as a treatment for ARDS (57). PGE₂ inhibits fibroblast proliferation, collagen synthesis, and fibroblast chemotaxis (4). In cyclo-oxygenase-2-deficient mice, the severity of intratracheal bleomycin-induced lung fibrosis is increased due to a reduction in PGE₂ (20). It is worth noting that both prostaglandin E and prostaglandin D synthetases (PGES and PGDS) are downregulated in SARS-CoV-infected cells (Table 7), implying that reduced PGE₂ levels in SARS patients may contribute to lung fibrosis. PGD₂ and PGE₂ are important Th-2 immune mediators. Thus Th-2 immunity is likely suppressed in SARS infection. PDGF is a major fibroblast mitogen (61). It also serves as a chemoattractant for neutrophils, monocytes, and fibroblasts (49). Therefore upregulation of PDGF receptor α by SARS-CoV (Table 7) through PDGF signaling would result in fibroblast proliferation, neutrophil and monocyte infiltration, and worsened lung fibrosis (1).

Lung fibrosis is a sequela of severe acute pulmonary disease (3). It is thus of interest to understand how SARS-CoV affects the expression of fibrosis-related genes. A previous study revealed that collagen III is upregulated in ARDS, and increased levels are related to poor prognosis (10). Collagen I α 2 and collagen XVIII α 1 are downregulated by SARS-CoV infection (Table 8). Studies have also shown that TIMP deficiency or a TIMP/MMP imbalance contributes to pulmonary fibrosis (18,27,46). Fibrinolytic proteins are protective against lung fibrosis (28). SARS-CoV infection upregulates proteinases, including MMP28, ADAM19, and SERPINB2, and downregulates TIMP1, TIMP3, and fibrinolytic protein (protein S) (Table 8). Our data showing the up- and downregulation of fibrosis-related genes provide the molecular basis of the clinical presentation of pulmonary fibrosis in SARS.

Conclusion

In summary, our study of the gene profiles in macrophages after SARS-CoV infection shows that the expression of (1) IFN- α/β -inducible and cathepsin/proteasome genes are downregulated; (2) hypoxia/hyperoxia-related genes are upregulated; and (3) TLR/TLR-signaling, cytokine/cytokine receptor-related, chemokine/chemokine receptor-related, lysosome-related, MHC/chaperon-related, and fibrosis-related genes are differentially regulated. These results demonstrate that SARS-CoV infection regulates immune-related and fibrotic gene expression in monocytes/macrophages, implying that the dysregulation of these genes is important in the pathogenesis of SARS.

Author Disclosure Statement

No competing financial interests exist.

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