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RESEARCH ARTICLE

Inflammation inhibitors were remarkably up-regulated in plasma of severe acute respiratory syndrome patients at progressive phase

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Severe acute respiratory syndrome (SARS) is a severe infectious disease that has affected many countries and regions since 2002. A novel member of the coronavirus, SARS-CoV, has been identified as the causative agent. However, the pathogenesis of SARS is still elusive. In this study, we used 2-D DIGE and MS to analyze the protein profiles of plasma from SARS patients, in the search for proteomic alterations associated with the disease progression, which could provide some clues to the pathogenesis. To enrich the low-abundance proteins in human plasma, two highly abundant proteins, albumin and IgG, were first removed. By comparing the plasma proteins of SARS patients with those of a normal control group, several proteins with a significant alteration were found. The up-regulated proteins were identified as alpha-1 acid glycoprotein, haptoglobin, alpha-1 anti-chymotrypsin and fetuin. The down-regulated proteins were apolipoprotein A-I, transferrin and transthyretin. Most of the proteins showed significant changes (up- or down-regulated) in the progressive phase of disease, and there was a trend back to normal level during the convalescent phase. Among these proteins, the alterations of fetuin and anti-chymotrypsin were further confirmed by Western blotting. Since all the up-regulated proteins identified above are well-known inflammation inhibitors, these results strongly suggest that the body starts inflammation inhibition to sustain the inflammatory response balance in the progression of SARS.

Keywords:
DIGE / Inflammation / Plasma / SARS

1 Introduction

In November 2002, a severe disease called severe acute respiratory syndrome (SARS), was found in southern China and spread worldwide in several months. There were more than 8000 probable SARS cases with 774 deaths reported from 26 countries [1]. A novel coronavirus (severe acute respiratory syndrome coronavirus, SARS-CoV) was separated and identified as the pathogen of SARS by serological and RT-PCR methods [2]. Subsequently, many efforts were made to study the mechanism of infection and the structure of the virus itself [3–5]. However, there are few studies on the pathological changes *in vivo* in the affected patients. Plasma is a widely used clinical resource that is closely correlated with disease progression. Analysis of the profile of plasma protein alterations is a promising way to try and elucidate the pathogenesis of SARS.

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Abbreviations: ACT, alpha-1 anti-chymotrypsin; **AGP**, alpha-1 acid glycoprotein; **APP**, acute-phase protein; **Hp**, haptoglobin; **SARS**, severe acute respiratory syndrome; **SARS-CoV**, SARS coronavirus

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With the development of proteomics, many technologies have been employed to study plasma proteomics [6]. In comparative proteomics, the accurate quantitation and the good reproducibility were the basis to provide reliable data. 2-D DIGE is a fluorescence-based technique for protein visualization and quantification, which could compensate the shortage of conventional silver or CBB staining data [7] after 2-DE. Proteins are labeled prior to electrophoresis with fluorescent CyDyes™, and differently labeled samples are then co-separated on the same 2-D gel. In this study, we used DIGE to compare the plasma protein profiles among different SARS phases after the onset of symptoms. We found that some proteins showed interesting changes in the progression phase, which might correspond to the pathological state of the SARS patients.

2 Materials and methods

2.1 Materials

Cy2, Cy3 and Cy5 were from Amersham Biosciences (Little Chalfont, UK); pharmalytes (pH 3–10 NL), dry strips and cover fluid were from Amersham Biosciences (Uppsala, Sweden); DMF was from Aldrich (Dorset, UK); CHAPS, urea, Tris, acrylamide, Bis, TEMED, DTT and iodoacetamide were from Bio-Rad (CA, USA); TFA was from Acros (NJ, USA); trypsin (sequencing grade) was obtained from Promega (WI, USA); ACN (HPLC grade) was purchased from J. T. Baker (NJ, USA)

2.2 Patients and sample preparation

All the SARS plasma specimens were from patients with 'probable' disease admitted to the PLA 309 Hospital in Beijing between May 10 and May 22, 2003, who met the World Health Organization (WHO) case definition (World Health Organization Case Definitions for Surveillance of Severe Acute Respiratory Syndrome (SARS). http://www.who.int/csr/sars/casedefinition/en/). Normal plasma, negative for SARS-CoV, were from healthy donors. The blood collection and plasma procurement protocols conformed to the WHO bio-safety guidelines (World Health Organization biosafety guidelines for handling of SARS specimens. http://www.who.int/csr/sars/biosafety2003_04_25/en/). Individual samples were pooled into three groups based on the clinical course (Table 1). To remove albumin and immunoglobulin (IgG) from human plasma, the samples were treated with an Aurum Serum Mini Kit (Bio-Rad) according to the manufacturer's instruction. The treated samples were measured at 595 nm for protein concentration with a Protein DC kit (Bio-Rad) based on the Lowry method. To evaluate the reproducibility and effect of removal, all the treated samples were separated with 12% SDS-PAGE, 50 μg protein per lane, and stained by SYPRO-Ruby (Bio-Rad).

Table 1. SARS cases

Group	Mean age (±SD)	Numbers of patients (male/female)	Days after symptom onset (±SD)
Normal	32.7 ± 7.1	9 (5/4)	~
(I) Progressive	35.2 ± 9.2	10 (6/4)	17.4 ± 3.1
(II) Convalescent	35.1 ± 13.7	9 (5/4)	28.7 ± 5.2

2.3 Sample preparation for DIGE labeling and analysis

Lyophilized samples (50 μg) were dissolved in lysis buffer (8 M urea, 4% CHAPS, 10 mM Tris, pH 8.5) to give stock solutions with final concentrations of about 5 mg/mL. Cyanine dyes were reconstituted in 99.8% anhydrous DMF and added to labeling reactions at a ratio of 400 pmol CyDye to 50 μg proteins in different groups following the cross-label rule. Prior to IEF, labeled samples to be separated in the same gel were mixed and added to an equal volume of 2 × sample buffer (8 M urea, 4% CHAPS, 130 mM DTT, 2% pharmalytes 3–10), and unlabeled proteins were added up to 500 μg with additional sample dissolved in rehydration buffer (8 M urea, 4% CHAPS, 1% pharmalytes 3–10 NL, 65 mM DTT). 2-DE was performed with Amersham Biosciences (Uppsala, Sweden) IPGphor IEF and Ettan Dalt Six electrophoresis units. Pre-cast IPG strips (18 cm; pH 3–10 NL) were used for the first-dimensional separation with a total focusing time of 120 kV·h at 18°C. Prior to SDS-PAGE, each strip was equilibrated with 10 mL equilibration buffer A (6 M urea, 50 mM Tris-HCl pH 8.8, 30% glycerol, 2% SDS, 10 mg/mL DTT) on a rocking table for 15 min, followed by 10 mL equilibration buffer B (6 M urea, 50 mM Tris-HCl pH 8.8, 30% glycerol, 2% SDS, 25 mg/mL iodoacetamide) for another 15 min. The strips were then loaded and run on 12% acrylamide isocratic Laemmli gels. The running parameter was set as constant power of 5 W per gel at 20°C for 30 min, followed by 10 W per gel at 20°C until the bromophenol blue dye front had run off the bottom of the gels. Labeled proteins were visualized by the Typhoon 9410 imager (Amersham Biosciences, UK). All gels were scanned at 100-nm resolution. Images were cropped to remove areas extraneous to the gel image using ImageQuant V5.2 (Amersham Biosciences, UK) prior to analysis. After scanning, the gel was stained with SYPRO-Ruby (Bio-Rad). The stained gel image was acquired using a Typhoon 9410 and matched with DIGE images by software to pick out the spots of interest. Gel analysis was performed with DeCyder 5.0 (Amersham Biosciences), an analysis software platform designed specifically for 2-D DIGE.

2.4 Protein identification and MS analysis

The spots of interest were picked by SpotCutter (Bio-Rad) according to the analysis using DeCyder software. The picked gel spots were destained with 50% ACN/25 mM

NH_4HCO_3. The gel plugs were lyophilized and immersed in 5 μL 10 ng/mL trypsin solution in 25 mM NH_4HCO_3. The digestion solution was kept at 37°C for 15 h. Tryptic peptide mixtures were first extracted with 100 μL 5% TFA and the same volume of 2.5% TFA/50% ACN. The extracted solutions were lyophilized and used for further identification by MS. Peptide extracts were dissolved in 3 μL matrix solution (5 mg/mL HCCA in 0.5% TFA/ 50% ACN) and 0.6 μL of the solutions were separately spotted onto the ABI-4700 (Applied Bioscience, MA, USA) target plate for MALDI-MS/MS analysis. In the MS mode, the generated ions were accelerated at the source (20 kV) and separated in the first TOF tube. In the MS/MS mode, the parent ion was focused into the gas cell and fragmented using CID. The fragments then were reaccelerated (15 kV) and the *m/z* values were determined in the second TOF tube. The mass spectral data were submitted to search the Swiss-Prot database by GPS Explore software based on MASCOT search engine (Matrix Science, UK). The proteins with identification probabilities of more than 95% given by MASCOT were considered successfully identified. The peptides whose ion score were greater than 29 were listed, as these could give a good match to the fragment ion mass spectrograph.

2.5 Western blot analysis

Samples were run on 12% SDS-polyacrylamide gels and transferred onto PVDF membranes in a trans-blot electrophoresis transfer cell (Bio-Rad). The membranes were blocked overnight at 4–7°C in 20 mM Tris-HCl, 140 mM NaCl, pH 7.5, 0.05% Tween-20 (TBST) containing 5% skim milk. The primary antibodies used were anti-fetuin polyclonal antibody (diluted 1:5000, Rockland, MA, USA) or anti-alpha-1 anti-chymotrypsin (ACT) (diluted 1:1000, Neomarkers, CA, USA). Membranes were incubated at room temperature for 1 h with each primary antibody, and then were washed three times with TBST and incubated with horseradish peroxidase-conjugated secondary antibody (diluted 1:10 000, Santa Cruz Biotechnology, CA, USA) for 1 h at room temperature. Visualization of the immunoreactive proteins was accomplished using ECL reagents (Santa Cruz Biotechnology). All the membranes were exposed on the same X-ray film and scanned by GS-710 scanner (Bio-Rad). A semiquantitative analysis based on OD was performed by QuantitiOne software (Bio-Rad) and ANOVA test analysis.

3 Results

3.1 Sample preparation

According to the reports about the typical clinical course of SARS [10], the definite SARS samples were divided into two groups based on the number of days after symptom onset and the state of illness with chest radiograph diagnosis

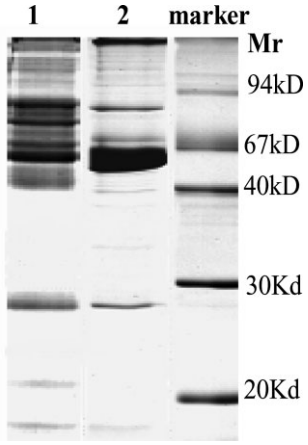

Figure 1. The efficiency evaluation for removal of albumin and IgG from human plasma. (1) Treated plasma, (2) crude plasma

(Table 1). Because albumin and IgG account for 70% proteins in human plasma, they are a bar to 2-DE analysis. Removing the high-abundance proteins from serum samples can increase the visibility of low-abundance proteins and enable precise analysis. We selected to remove the high-abundance proteins before 2-D DIGE, and the effect of depletion was evaluated by SDS-PAGE, and a good efficiency was shown (Fig. 1).

3.2 2-D DIGE analysis

To study the alterations in plasma protein in the SARS patients, comparative proteomic analysis was performed among normal, progressive phase and convalescent phase groups. The 2-DE images of the samples of three groups labeled with different cyanine dyes were obtained by fluorescence scanning (Fig. 2). The 2-D DIGE images were analyzed by DeCyder 5.0 software to objectively estimate the abundance of proteins in each sample and to generate quantitative data. After setting the proper filter threshold in the software, 703 protein spots were auto-detected. Based on a threshold ratio of 1.5, the software detected the protein spots of interest that showed a significant change among the three groups. Compared to normal plasma, 58 protein spots were up-regulated and 30 down-regulated in progressive group; and 36 protein spots were up-regulated and 21 down-regulated in convalescent group. The relative abundance volume ratios of protein spots in each group are shown in Fig. 3 and Table 2. After scanning, the gels were stained with SYPRO-Ruby and matched with the DIGE image by the software. The protein spots with significant change were picked using a SpotCutter and subjected to in-gel digestion for MALDI-TOF/TOF MS analysis. As the cyanine dye was more sensitive than SYPRO-Ruby stain, some spots in DIGE images were invisible in Ruby image. If spots were blind-picked referring to the matched image, these spots usually

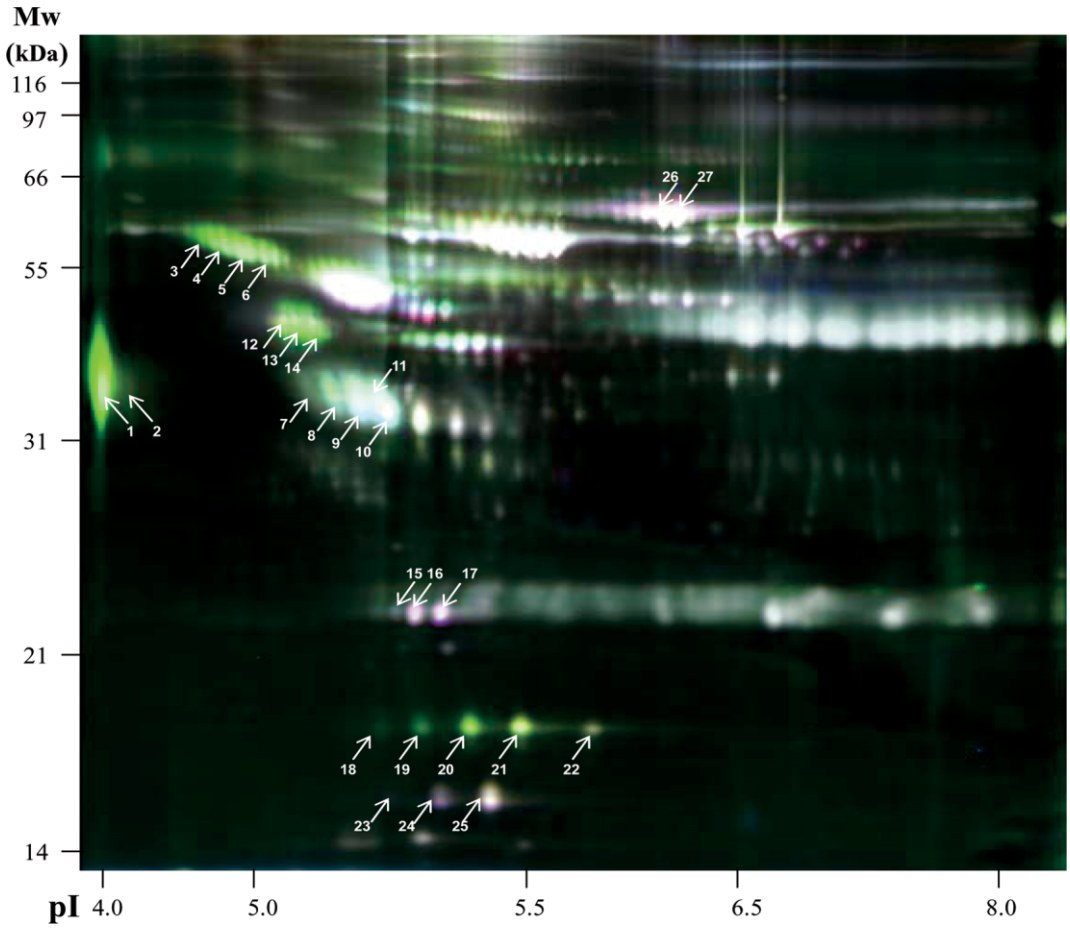

Figure 2. False-colored DIGE gel image of plasma proteins from normal and SARS groups. Cy2 (red) image of proteins from normal plasma; Cy3 (green) image of proteins from plasma with SARS in the progressive phase; Cy5 (blue) image of proteins from plasma with SARS in the convalescent phase. The overlay images showing white spots containing proteins that have equal expression levels in the three samples, green spots containing proteins with a higher expression in the progressive phase of SARS, and purple spots with proteins down-regulated in the progressive phase.

failed to be identified due to the low abundance. All the identified proteins are shown in Table 2. Unexpectedly, most of the proteins have been shown to play a role in inflammation control during the infection. The changes in the identified proteins in different groups can be summarized into three modes (Fig. 3): (A) alpha-1 acid glycoprotein (AGP), ACT, fetuin and haptoglobin (Hp) alpha-2 chain, which were up-regulated in the group with progressive disease compared to the normal group, before their levels tended to return to normal levels in convalescent group; (B) Hp beta chain, which sustained the high level in both progressive and convalescent phase; and (C) apolipoprotein A-1, transthyretin and transferrin, which were down-regulated in progressive group and then rose back to normal levels in convalescent group. It is interesting that most of these proteins showed larger alterations in the progressive phase and then tended to return to normal levels in the convalescence phase. These results strongly suggest that the function of the altered proteins is closely related with the disease progression.

3.3 Variants of AGP, transthyretin and Hp, and their association with SARS pathogenesis

Some variants of the identified proteins appeared in different positions in the 2-D gel due to modifications. Most variants of one protein showed similar changes on the gel. However, the variants of AGP, transthyretin and Hp did not follow this pattern. They had different volume ratios (Fig. 4), *e.g.* both spots 1 and 2 were AGP with different p*I*s in the 2-D gel. Compared with the normal group, the volume ratio of spot 1 in progressive group was 2.5-fold, but the volume ratio of spot 2 was 1.6-fold. This suggests that the variants might play different roles in the SARS progression.

3.4 Validations by Western blot analysis

To validate the results of DIGE, we used Western blot for further confirmation. The pooled samples and each individual sample were loaded with the same quantity to detect the

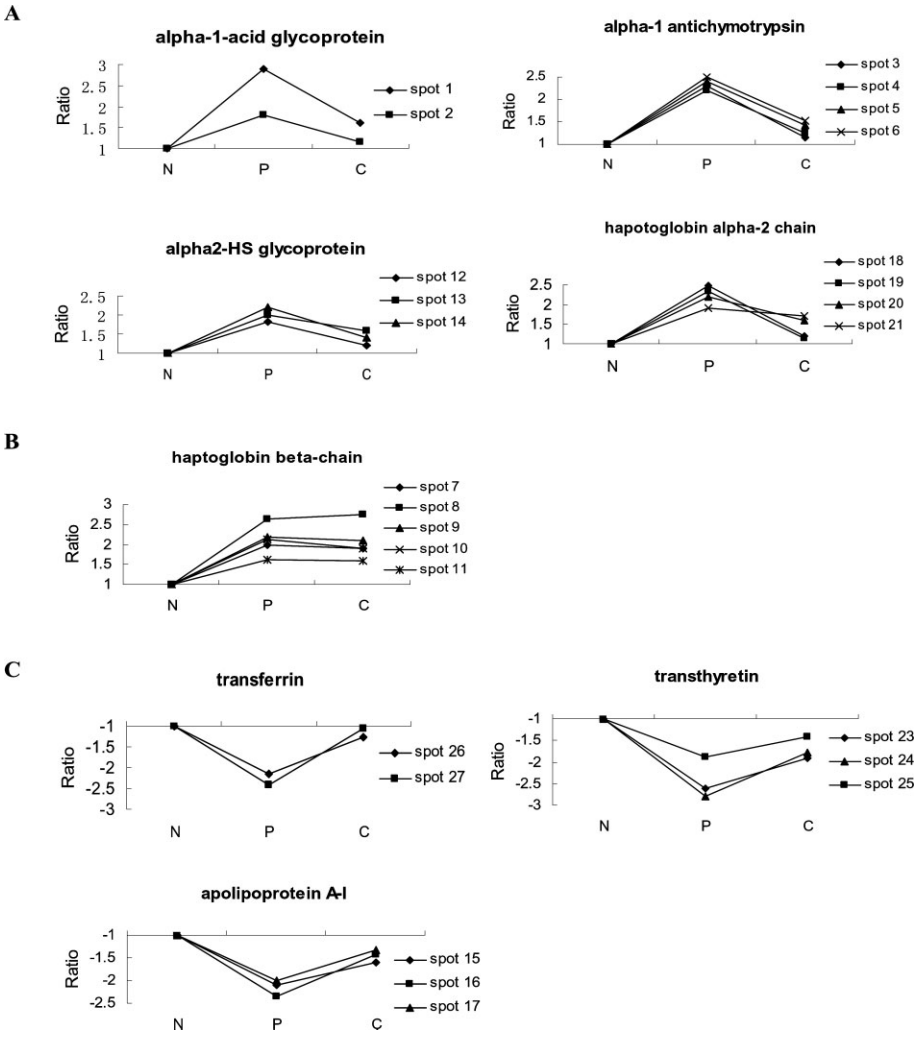

Figure 3. Spots for which the volume ratio was >1.5 based on DeCyder software analysis were identified by MS. In some cases, different spots were identified as the same protein. The relative abundance alterations of identified proteins in different groups can be summarized as three modes: (A) proteins that were up-regulated in progressive group compared to normal group, for which the levels tended to regress to normal levels in convalescent group; (B) protein that sustained the high level in both the progressive and convalescent phases; (C) proteins that were down-regulated in progressive group and then rose back to normal levels in convalescent group. Volume ratio = volume of 'X' group/volume of normal group; N: normal group, P: progressive group, C: convalescent group.

level of target proteins. In general, fetuin is a negative acute reaction protein, which is down-regulated in infection [11]; however, our experiments showed a contradictory result. ACT plays an important role in the physiology of the lungs. Although ACT is considered to be an acute-phase protein (APP), there have been no reports about its up-regulation in the plasma with SARS. Therefore, we investigated these two proteins, fetuin and ACT, using Western blot. The Western blot films were scanned and the OD of each band in the film was evaluated by QuantitiOne software (Fig. 5). The relative levels of fetuin in the three groups were: normal, 30.4 ± 8.5; progressive, 58.1 ± 818.3; convalescent, 48.5 ± 817.1; normal *vs.* progressive, $p < 0.01$. The relative levels of ACT in the three groups were: normal, 35.4 ± 86.6; progressive, 69.7 ± 816.3; convalescent, 47.7 ± 817.4; normal *vs.* progressive, $p < 0.01$. The results validated the significant up-regulation of fetuin and ACT in the progressive phase. Although the differences between the progressive and convalescent groups were not statistically significant, there was a trend to decrease in convalescence.

4 Discussion

SARS is a viral pneumonia that progresses rapidly, and the pathogenesis of which is still poorly understood. In this study, we used the comparative proteomics technology, DIGE, to demonstrate the characteristic alterations of the plasma proteins in the progressive and convalesce phases of SARS. These alterations were strongly suggestive for an anti-inflammatory mechanism in the disease process.

Like other acute respiratory viral infections, SARS-CoV induced the activation of immune system [12, 13]. This activation was often excessive in the lungs, causing bystander tissue damage and airway occlusion [14]. A fine balance of the immune system is very important between the ability to clear the virus and the damage to the delicate architecture of lungs [14]. Some studies have reported an inflammatory pathology of SARS [10, 13, 15], including cytokine dysregulation and immuno-pathological damage. In this study, we found that several plasma proteins changed significantly in the progressive phase of SARS. The up-regulated proteins

Table 2. List of identified proteins[a)]

Spot no.[b)]	P/N ratio[c)]	C/N ratio[c)]	Protein name[d)]	Accession no.[e)]	Theoretical mol. mass (Da)/p*I*	Ion score[f)]	Peptides sequence[g)]
1	1.8	1.1	Alpha1-acid glycoprotein	P02763	23496.8/4.93	104	EQLGEFYEALDCLR
2	2.9	1.6				86	YVGGQEHFAHLLILR
3	2.3	1.1	Alpha-1 anti-chymotrypsin	P01011	47620.5/5.33	74	ITLLSALVETR
4	2.2	1.2				81	LYGSEAFATDFQDSAAAK
5	2.4	1.4				81	AVLDVFEEGTEASAATAVK
6	2.5	1.5					
7	2.0	1.9	Haptoglobin (beta chain)[h)]	P00738	45176.6/6.13	83	YVMLPVADQDQCIR
8	2.6	2.8				79	AVGDKLPECEAVCGKPK
9	2.2	2.1				54	NPANPVQR
10	2.1	1.9					
11	1.6	1.6					
12	1.8	1.2	Alpha2-HS-glycoprotein	P02765	39299.7/5.43	102	HTFMGVVSLGSPSGEVSHPR
13	2.0	1.6				65	EHAVEGDCDFQLLK
14	2.2	1.4					
18	2.5	1.2	Haptoglobin (alpha-2 chain)[h)]	P00738	45176.6/6.13	56	TEGDGVYTLNDKK
19	2.3	1.1				45	GSFPWQAK
20	2.2	1.6				38	VGYVSGWGR
21	1.9	1.7					
22	1.2	1.4					
23	−2.6	−1.9	Transthyretin	P02766	15877/5.52	122	GSPAINVAVHVFR
24	−2.8	−1.8				117	ALGISPFHEHAEVVFTANDSGPR
25	−1.9	−1.4				146	YTIAALLSPYSYSTTAVVTNPK
15	−2.1	−1.6	Apolipoprotein A-I	P02647	30758.9/5.56	55	AHVDALR
16	−2.3	−1.4				62	AKPALEDLR
17	−2.0	−1.3				70	THLAPYSDELR
						89	VSFLSALEEYTK
26	−2.1	−1.3	Transferrin	P02787	76999.6/6.81	64	EGYYGYTGAFR
27	−2.4	−1.0				38	MYLGYEYVTAIR

a) Spots for which the volume ratio was >1.5 based on DeCyder software analysis were identified by MALDI-TOF/TOF MS.
b) Spots referring to Fig. 1.
c) Spot volume ratio in groups. N: normal; P: progressive group; C: convalescent group.
d) Spots in the same line were identified as same protein.
e) Protein ID accessed from Swiss-Prot database by data searching.
f) Ion score of the peptide analyzed by MS/MS.
g) All the spots had high probability results by MASCOT search, and there was at least one listed peptide analyzed by MS/MS in each spot. Parts of the sequence, determined by MS, indisputably confirm the peptide.
h) Protein description from Swiss-2D PAGE reference (http://ca.expasy.org/ch2d/publi/inside1995.html).

were AGP, ACT, alpha-2-HS glycoprotein (fetuin) and Hp; the down-regulated proteins were transferrin, transthyretin and apolipoprotein A-I. The changes in most of these proteins consequently regressed in the convalescent phase. All of the altered proteins are APPs, and known to take part in the inflammation control [16], which implicates the active innate immune responses in infection with SARS-CoV, and suggests that the inflammatory response may play an important role during the disease process. Usually, the virus load falls after 10 days from the onset of symptoms, but the status of patients with SARS still deteriorates [10], which suggested an excessive immunological response. The up-regulated expression of the presented anti-inflammatory proteins in the progressive phase could play a protective role against the excessive inflammatory response to sustain the defense homeostasis during the SARS progression and improvement.

Here we illustrate the biological and physical functions of these proteins in more detail. ACT is a serine proteinase inhibitor (serpin) with target enzymes such as cathepsin G, mast cell chymase and chymotrypsin [17]. During acute inflammation, the equilibrium between blood or tissue proteases and their inhibitors is dramatically disturbed. A massive release of proteases from injured cells and infiltration by

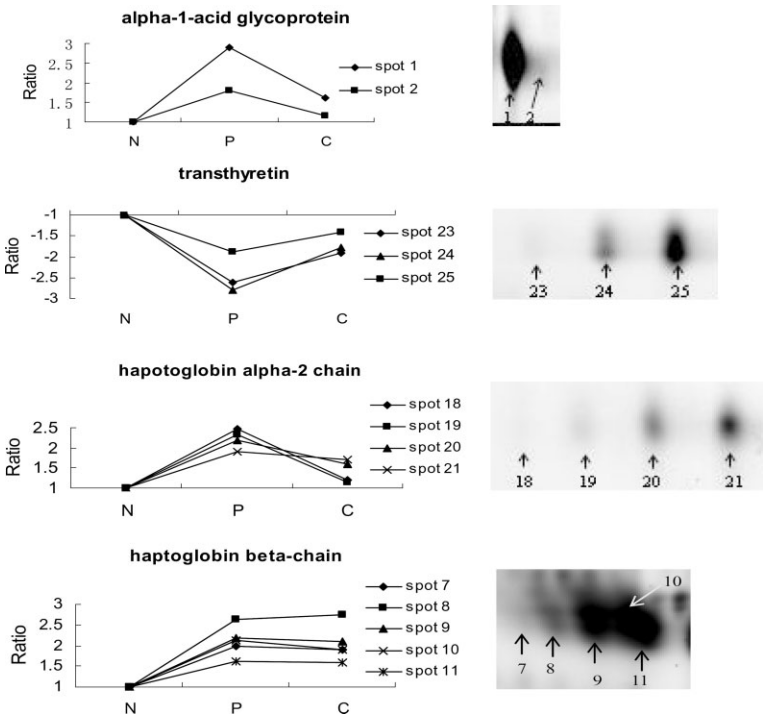

Figure 4. The spots, identified as the same protein but having different locations in the gel, have different volume ratio alterations in the same group during the SARS disease progression.

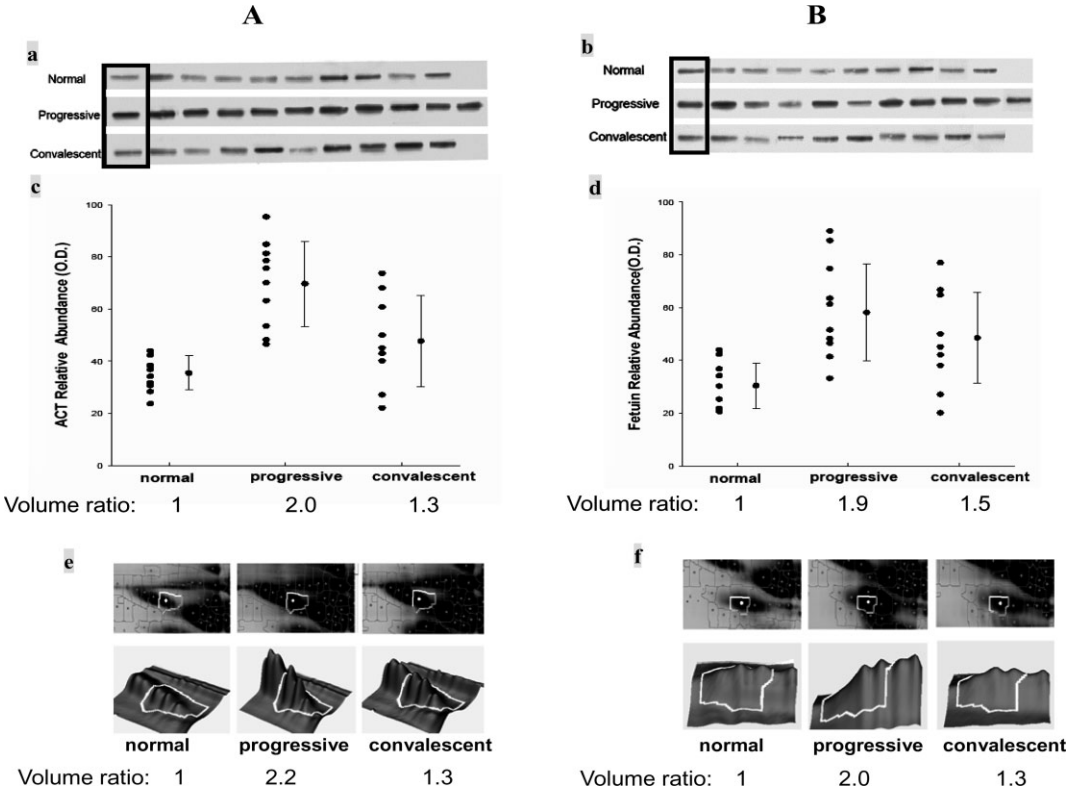

Figure 5. Western blot analysis (a, b) validating ACT (A) and fetuin (B) abundance alterations seen by 2-D DIGE image software in different groups (e, f). Results show good correlation. (a, b) The level of ACT (a) or fetuin (b) in individual samples of each group detected by Western blot. The pooled samples are marked with rectangles and others are individual samples. (c, d) The semiquantitative analysis of Western blot results based on the OD of bands. (e, f) Each brightened spot in the 2-D gel created a corresponding 3-D relative abundance image by DeCyder software and processed by quantitative analysis.

neutrophils and macrophages occur locally, which result in an oxidative or respiratory burst. ACT inhibits the action of proteolytic pathways through forming stable complexes with protease to maintain structural integrity of the lower respiratory tract. ACT has also been thought to act as an anti-inflammatory agent inhibiting neutrophil chemotaxis and superoxide production [18, 19]. Moreover, the ACT-cathepsin G complex can modulate IL-6 secretion by lung fibroblast, and create a positive feedback to control the acute phase reaction [20]. In our study, up-regulated expression of ACT was observed, implying its important role in the inflammation control.

Alpha-2-HS-glycoprotein, also called human fetuin, belongs to the cystatin superfamily of protease inhibitors involved in important functions such as inhibition of insulin receptor tyrosine kinase activity, and regulation of calcium metabolism and osteogenesis [21]. Human fetuin has been demonstrated to be a negative APP in man, and its circulating levels in adults fall significantly during injury and infection [11]. However, we found that it was up-regulated in the SARS patients, which has also been reported by Chen *et al*. [22]. This could be explained by its role as an immunomodulator. Fetuin influences the resolution of inflammation through enhancing the phagocytosis of apoptotic cells by macrophages [23], and antagonizing the deleterious accumulation in lungs, which may cause tissue fibrosis or autoimmunity. Fetuin also opsonizes cationic macrophage-deactivating molecules and suppresses the release of tumor necrosis factor (TNF) from stimulated macrophages to control the magnitude of the inflammatory response, suggesting a positive role in the recovery phase of acute inflammatory responses [24, 25].

AGP plays an important biological function in various immunomodulating effects. AGP inhibits activation, chemotaxis and oxidative metabolism of polymorphonuclear neutrophils [26, 27], and modulates cytokine secretion by monocytes/macrophages [28]. Moreover, AGP has been shown to protect against TNF-α-induced cell killing and complement-induced lung injury [29, 30], and it displays a potent platelet aggregation inhibitory activity *in vivo* [31]. These functions are potentially important for pulmonary inflammatory responses.

Hp sustained a high level of expression in both the progressive and convalescent phases. The best-known biological function of Hp is the capture of hemoglobin to prevent both iron loss and kidney damage during hemolysis [32]. In SARS patients, there was high oxidative stress caused by activated neutrophils in lungs. Plasma Hp is thought to be a major antioxidant protection agent against hemoglobin-driven free radical accumulation, which causes membrane lipid peroxidation and vascular endothelial cell damage [33, 34]. Neutrophil respiratory burst activity can be inhibited by Hp [35]. Moreover, Hp has been identified as one of the serum angiogenic factors, and angiogenesis is important for lung tissue repair and promotes the growth of collateral vessels [36], which could be another reason for sustaining the high level of Hp in convalescence.

All three down-regulated proteins: transthyretin, transferrin and apolipoprotein A-1, belong to the negative APPs [16], which play major roles in the transport of nutrients, hormones and metabolites [37]. It is not clear why their concentrations decreased in SARS plasma. Although in the past it was believed that the lower concentrations of the negative APPs induced by stressed states were due to a reduced rate of synthesis of these proteins [16], some studies have shown that the concentrations of some negative APPs decrease in response to infections and inflammation because of an increased transcapillary escape rate and catabolic rate [38]. The balance between the rates of synthesis and catabolism and loss determine the circulation level of a plasma protein.

Our study also showed that the variants of Hp, AGP and transthyretin in 2-D gels had a different volume ratios in each group. This phenomenon suggests that the proteins with different PTMs took part in different pathways in the disease process, which finally caused different changes in the levels of their variants in the blood circulation. For example, a putative role for the variants of AGP is in the altered carbohydrate composition during immunomodulation [39]. The proteins with different PTMs might be specifically associated with SARS-CoV infection. Additional investigations are needed to reveal the nature of the variants and their significance in SARS. Hp is characterized by molecular heterogeneity with three major phenotypes, and consists of two different polypeptide chains named alpha and beta. The sequence differences between the Hp alleles result in amino acid sequence differences of the corresponding proteins only within the alpha chains, whereas the beta chain sequences are identical in all alleles [32]. The different spots of the alpha chains visible in the 2-D gels have been assigned to genetic Hp subtypes [40]. In our experiments, the pooled samples of each group were from several patients with unknown Hp phenotype, so that the inconsistent volume ratios of Hp alpha-2 chain may due to the different phenotype compositions between groups.

In conclusion, we have shown the quantitative plasma protein profiles in SARS patients, and have found several proteins that change significantly during disease progression. The up-regulated proteins had a relatively high abundance in the plasma, and they all play a positive anti-inflammatory role. The results show that there is some mechanism that sustains the inflammation balance in SARS patients, which is helpful for the proper treatment and therapy of this disease.

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