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An Interferon-γ-Related Cytokine Storm in SARS Patients

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Fourteen cytokines or chemokines were analyzed on 88 RT-PCR-confirmed severe acute respiratory syndrome (SARS) patients. IFN-γ, IL-18, TGF-β, IL-6, IP-10, MCP-1, MIG, and IL-8, but not of TNF-α, IL-2, IL-4, IL-10, IL-13, or TNFRI, were highly elevated in the acute phase sera of Taiwan SARS patients. IFN-γ was significantly higher in the Ab(+) group than in the Ab(−) group. IFN-γ, IL-18, MCP-1, MIG, and IP-10 were already elevated at early days post fever onset. Furthermore, levels of IL-18, IP-10, MIG, and MCP-1 were significantly higher in the death group than in the survival group. For the survival group, IFN-γ and MCP-1 were inversely associated with circulating lymphocytes count and monocytes count, but positively associated with circulating neutrophils count. It is concluded that an interferon-γ-related cytokine storm was induced post SARS coronavirus infection, and this cytokine storm might be involved in the immunopathological damage in SARS patients. ***J. Med. Virol. 75:185–194, 2005.***

KEY WORDS: SARS; SARS-CoV; cytokine; interferon-gamma; enterovirus 71; pulmonary edema; dengue hemorrhagic fever

INTRODUCTION

Severe acute respiratory syndrome (SARS) is a new emerging disease that has affected many countries including Taiwan. A novel virus, the SARS-associated coronavirus (SARS-CoV), has been identified as the causal agent [Ksiazek et al., 2003; Peiris et al., 2003a,b]. High fever, myalgia, dry cough, and lymphopenia were the most characteristic symptoms seen in patients with this new illness. In around 30% of the cases, patients also developed an atypical form of pneumonia, with acute respiratory distress as a result of acute lung damage, characterized by infiltrates on chest radiography. Amongst the changes observed in the lungs of SARS patients were epithelial cells proliferation and desquamation, hyaline membranes formation along alveolar walls and cells infiltration (lymphocytes, neutrophils, and monocytes) during the early stage of the disease, while increased fibrosis and multinucleated epithelial giant cells formation were seen at a later stage. These changes seem to indicate the existence of a two-phase development of the lung injury, acute phase diffuse alveolar damage followed by a more organized diffuse alveolar damage. Based on such observations, the existence of an abnormally excessive inflammatory response in the lungs has been hypothesized to explain the development of an acute lung injury. Indeed, patients still manifested lung injury at a time when the viral load was falling, in support of the immune nature of the lung damage.

The laboratory findings of SARS were lymphopenia, thrombocytopenia, and elevated lactate dehydrogenase and creatine kinase levels. Several factors including advanced age, male sex, a high peak lactate dehydrogenase level, a high peak creatine kinase level, and a high initial absolute neutrophil count were significant predictive factors for intense care unit admission and death. The mortality of SARS is reported to be around 10%–15%. Acute viral infection might produce damage to host cells by direct cytopathy or by indirect immunopathological damage. In the early stage, cytopathy is

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accompanied by viral amplification, such that antiviral drugs may be important for treatment. In the later stage, when an adaptive immune response is mounted, viral clearance can be accompanied by severe inflammatory damage, especially with high viral burden. How the lung is damaged is not clearly understood. The intensive inflammation developed in the lung will generate cytokines to activate lung epithelial cells to undergo pathological changes [Nicholls et al., 2003]. Is there a cytokine storm involved in the pathological changes of the lung? In this study, an IFN-γ-related cytokine and chemokine storm was found to be indeed present in SARS patient and its level was associated with some clinical manifestations.

MATERIALS AND METHODS

Patients

From March 14, 2003 to August 15, 2003, a total of 346 cases were defined as probable SARS cases based on the medical records reviewed retrospectively plus laboratory data and epidemiological data by the SARS Advisory Committee in Center for Disease Control (CDC)-Taiwan and SARS expert committees in National Health Insurance Bureau-Taiwan. Among these patients, 88 RT-PCR confirmed samples of acute phase sera and convalescent sera were analyzed for their serum cytokine levels. The epidemiological characteristics of age and gender and clinical information such as symptoms, underlying diseases, outcomes including death and hospital length-of-stay, as well as laboratory findings of hematological, biochemical, and arterial blood gas data were collected from medical chart records, information of disease investigation, web-based data in our reporting system, and laboratories in CDC-Taiwan.

Analysis of SARS-CoV Infection

SARS-CoV analysis was done primarily in the CDC-Taiwan Central Laboratory. SARS-CoV RT-PCR was used to define the infection. The primers were used according to the CDC-US recommendation. The ELISA, immunofluorescence assay, and neutralization test were done for serological identification of SARS-CoV infection [Wu et al., 2004b]. The acute and convalescent sera were tested in parallel for SARS-CoV. The sensitivity and specificity of these assays were described previously, and our 88 samples were selected from these pool [Wu et al., 2004a]. A combination of ELISA, immunofluorescence, and neutralization test was used to define the antibody to SARS-CoV. The cases that had serum antibody against SARS-CoV were classified as Ab positive group while those who had no sera antibody were classified as Ab negative group.

Quantitation of Cytokine Level

The cytokines levels were determined by either BD Human Th1/Th2 Cytokine or Chemokine Bead Array (CBA) Kit. The BD Human Th1/Th2 Cytokine CBA Kit (BD PharMingen, San Diego, CA) was used to measure IL-2, IL-4, IL-5, IL-10, TNF-α, and IFN-γ protein levels by flow cytometry in a particle-based immunoassay. This kit allowed simultaneous measurement of six cytokines from 50 μl of sample. The use of 50 μl of sample is very critical for precious patient serum samples, and we have routinely used the CBA kit to quantitate the cytokines level on patients with various diseases [Wang et al., 2003; Hung et al., 2004]. The assays were performed according to the instructions of the manufacturer. The limits of detection of these immunoassays were 2.6 pg/ml of IL-2, 2.6 pg/ml of IL-4, 2.4 pg/ml of IL-5, 2.8 pg/ml of IL-10, 2.8 pg/ml of TNF-α, and 7.1 pg/ml of IFN-γ. The BD Human Chemokine CBA Kit was used to measure IP-10, MIG, MCP-1, RANTES, and IL-8 protein levels by flow cytometry in a particle-based immunoassay. This kit allowed simultaneous measurement of five chemokines from 50 μl of sample. The limits of detection of these immunoassays were 0.2 pg/ml of IL-8, 1.0 pg/ml of RANTES, 2.5 pg/ml of MIG, 2.7 pg/ml of MCP-1, and 2.8 pg/ml of IP-10. TGF-β, sTNFRI, and TNF-α were detected with ELISA kits from Biosource (Camarillo, CA). The detection limits were 50 pg/ml of sTNFRI, 15.6 pg/ml of TGF-β, and 1.7 pg/ml of TNF-α. IL-18 and IL-13 were detected wit ELISA kits from MBL Medical & Biological Laboratories Co. (Nagoya, Japan) and R&D Systems, Inc. (Minneapolis, MN), respectively. The detection limits were 12.5 pg/ml of IL-18 and 32 pg/ml of IL-13.

Statistical Analysis

The cytokine, clinical, and laboratory data between antibody positive and antibody negative group or death and survival group was compared by student's *t*-test with the SigmaPlot 8.0 software (SPSS, Inc., Chicago, IL). $P < 0.05$ was taken to be significant.

RESULTS

Cytokines and Chemokines Levels Are Highly Elevated in SARS Patients

Using the CBA Th1/Th2 cytokines, chemokines kits and ELISA, 14 cytokines or chemokines, including IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, IL-13, IL-18, TGF-β, IP-10, MCP-1, MIG, RANTES, and IL-8, as well as TNFRI, were analyzed. As shown in Figure 1, in a total 88 RT-PCR-confirmed SARS patients, high levels of IFN-γ, IL-18, TGF-β, and IL-6 were found in the sera of SARS patients. The chemokines IP-10, MCP-1, MIG, and IL-8 were also elevated. The mean values of IFN-γ, IL-18, TGF-β, IL-6, IP-10, MCP-1, MIG, and IL-8 were 456.1, 638.6, 768.9, 245.7, 6775.3, 755.7, 1229.8, and 325.6 pg/ml, respectively. The serum levels of cytokines varied a great deal between different individuals, but they were highly elevated in the acute phase post SARS-CoV infection, and were significantly different from normal healthy controls. All of these cytokines had returned to normal levels at the convalescent phase (30 days or later post disease onset) (Table I). In

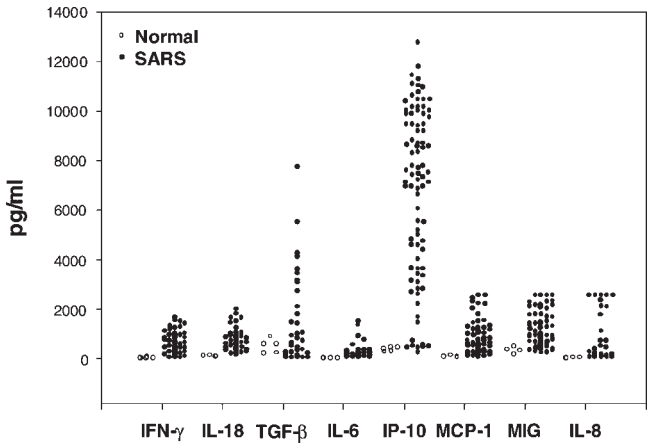

Fig. 1. Cytokine IFN-γ, IL-18, TGF-γ, IL-6, IP-10, MCP-1, MIG, and IL-8 levels in severe acute respiratory syndrome (SARS) patients. The serum cytokine levels of 88 RT-PCR-confirmed SARS patients were presented in dot plot format.

contrast, cytokines TNF-α, IL-2, IL-4, IL-10, IL-13, or TNFRI were very low or undetectable. Among the detected cytokines, IFN-γ and its related chemokines were especially prominent. Only 18% (16/88) of patients had no detectable IFN-γ in the serum, while 50% of the patients had IFN-γ level higher than 500 pg/ml, and 20% higher than 1,000 pg/ml. The IFN-γ-stimulated chemokines such as IP-10, MCP-1, and MIG were also highly elevated.

The Th1/Th2 cytokines profile of SARS was then compared with that of other viral diseases, namely enterovirus 71 (EV71)-induced pulmonary edema and dengue virus-induced dengue hemorrhagic fever/dengue shock syndrome. The amount of IFN-γ observed in the SARS patients was four to sixfold higher than that of EV71-induced pulmonary edema (PE) or dengue virus-induced dengue hemorrhagic fever (DHF) [Wang et al., 2003; Hung et al., 2004]. The mean values of IFN-γ in pulmonary edema patients and dengue hemorrhagic fever patients were 68 and 111 pg/ml, respectively (Fig. 2a). Surprisingly, IL-10 level was very low as 82% (72/88) of patient had serum IL-10 level lower than 20 pg/ml. This is in contrast to pulmonary edema and dengue hemorrhagic fever where the mean concentration of IL-10 in pulmonary edema and dengue hemorrhagic fever were 123.6 and 81.7 pg/ml, respectively. The multiple cytokines were detected simultaneously by the CBA kit, therefore, the Th1-prone IFN-γ of each individual was plotted to his/her Th2-prone IL-10. A reciprocal relationship between IFN-γ and IL-10 was found for all individuals (Fig. 2f). The IL-10-reciprocal relationship was also found for IL-18, MCP-1, IP-10, and MIG (data not shown). This pattern was different from that of EV71-induced pulmonary edema or dengue virus-induced dengue hemorrhagic fever. In these two diseases, a mix of IFN-γ and IL-10 is present simultaneously. While, with regard to other cytokines such as IL-6, IL-18, and IL-8, there were all present in SARS, pulmonary edema, and dengue hemorrhagic fever (Fig. 2b,d,e). But for IL-13, only 8 % (5/66) of SARS patients had detectable level higher than detection limit, 32 pg/ml (Fig. 2c). On the contrary, IL-13 was markedly elevated in pulmonary edema patients, and it plays an important role in the pulmonary edema post EV71 infection.

Comparison of Cytokines in Ab(−) and Ab(+) Groups

To understand the cytokines profile in the developmental process of SARS post SARS-CoV infection, the 88 RT-PCR-confirmed cases were divided into two categories based on their antibody status. As shown in Table II, the mean age of 51 patients in Ab(−) group was significantly higher than that of 37 patients in Ab(+) group. A larger percentage of males than of females were found in the Ab(−) group. On the other hand, a larger percentage of females than males were found in the Ab(+) group. The mortality was also significantly higher in the Ab(−) than in the Ab(+) group. Duration of stay in hospital reflects the mortality rate, therefore, it was lower in the Ab(−) than in the Ab(+) group. Laboratory data on blood urine nitrogen, white blood count, and complete blood count were not different between these two groups. IL-6, IL-18, IL-10, TGF-β, IP-10, MIG, MCP-1, and IL-8 were also not significantly different between these two groups: they were all highly elevated. The only difference was the IFN-γ level: the serum IFN-γ level in the Ab(+) group was significantly higher than that in the Ab(−) group (Table II). To understand the kinetic changes of these cytokines in the early stage of acute

TABLE I. Cytokine Levels in SARS Patients

	SARS					Normal		
pg/ml	Acute	n	Convalescent	n	*P*		n	*P*
IFN-γ	456.1 ± 510.7	88	4.0 ± 14.3	71	<0.001	3.3 ± 10.5	10	<0.05
IL-6	245.7 ± 770.2	88	7.5 ± 30.4	71	<0.05	0.4 ± 0.9	10	0.319
IL-18	638.6 ± 392.8	66	ND			78.4 ± 17.9	8	<0.001
IL-10	107.0 ± 591.7	88	1.3 ± 3.83	71	0.132	0.2 ± 0.6	10	0.571
TGF-β	768.9 ± 1514.2	66	ND			461.1 ± 283.1	5	0.653
IP-10	6775.3 ± 3399.5	88	ND			339.1 ± 78.4	5	<0.001
MIG	1229.8 ± 791.6	88	ND			307.8 ± 117.5	5	<0.05
MCP-1	755.7 ± 689.8	88	ND			48.5 ± 35.6	5	<0.05
IL-8	325.6 ± 697.6	88	ND			6.3 ± 1.1	5	0.311

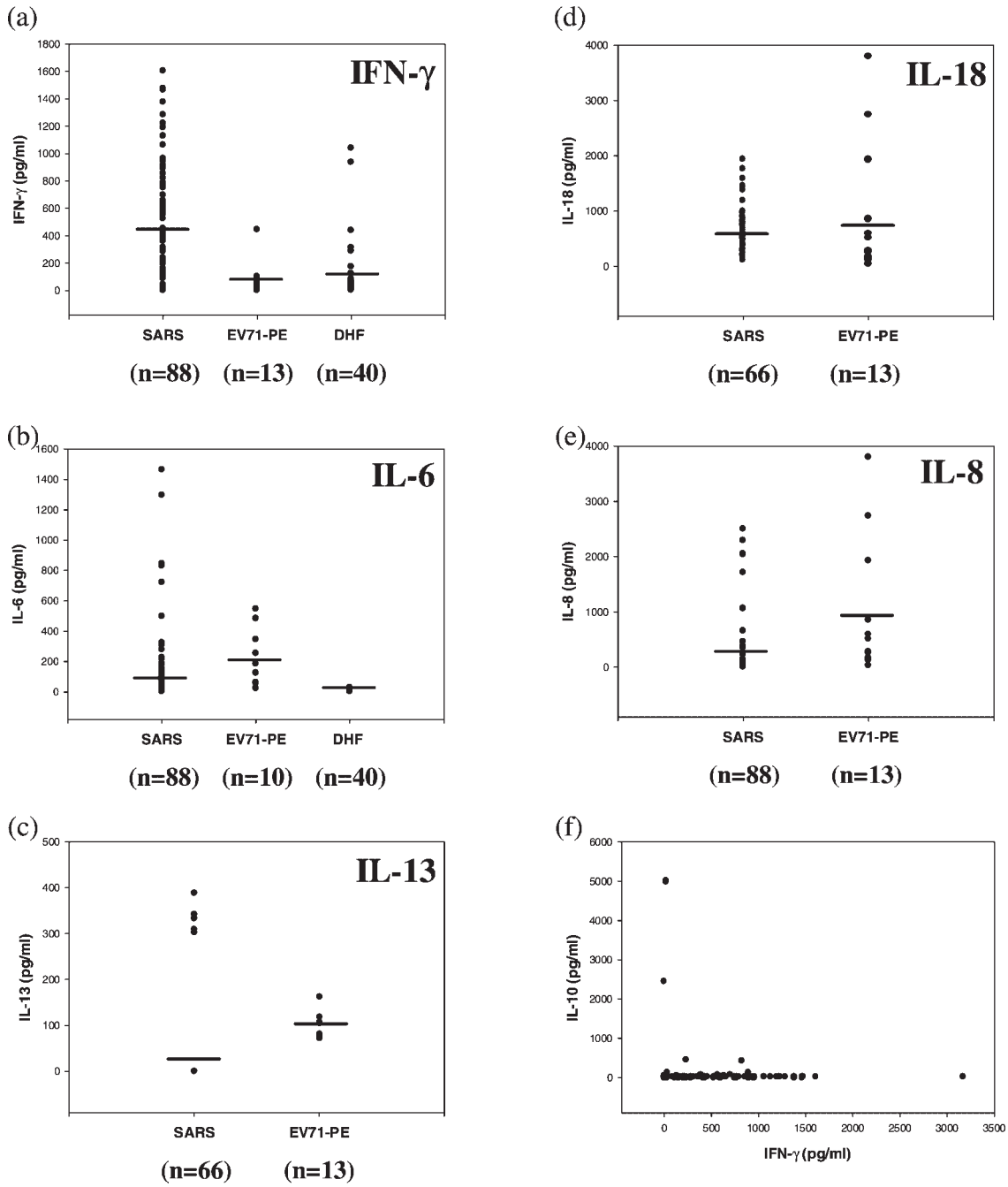

Fig. 2. Comparison of IFN-γ, IL-18, IL-6, IL-8, and IL-13 between SARS, EV71-induced pulmonary edema, and dengue virus-induced dengue hemorrhagic fever. The serum cytokines levels from SARS, pulmonary edema, and dengue hemorrhagic fever patients were compared for IFN-γ (**a**), IL-6 (**b**), IL-13 (**c**), IL-8 (**d**), and IL-18 (**e**). A reciprocal relationship of IFN-γ and IL-10 was shown for SARS patients (**f**). The bar represents the mean value of each cytokine. N is the patient serum samples determined.

infection, the levels of IFN-γ, IP-10, MCP-1, MIG, IL-18, IL-8, IL-6, and TGF-β in Ab(−) group were plotted against the days post fever onset (Fig. 3). IFN-γ and its related chemokines MCP-1, MIG, and IP-10 were already highly elevated at early days. IFN-γ, MIG, IP-10, and MCP-1 seemed to appear the earliest, and peaked at days 1–4 post fever onset. IL-8 and IL-18 increased later, and peaked at days 4–6 post fever onset. IL-6 and TGF-β also appeared later. To further delineate the relationship between IFN-γ and its related cytokines, the linear regressions between IFN-γ or IL-18 and IP-10, MCP-1 or MIG were analyzed. A good association was found for IL-18 and IP-10, MCP-1, or MIG. The correlation coefficient and r^2 for IL-18 and IP-10, MCP-1 or MIG were 0.568/0.344, 0.688/0.473, and 0.745/0.555, respectively (Fig. 4). The association was better for IL-18

TABLE II. Comparison of Cytokines in Ab(−) and Ab(+) Groups of SARS Patients

	PCR(+)Ab(−)	n	PCR(+)Ab(+)	n	*P*-value
Age*	50.8 ± 19.4	51	37.3 ± 13.9	37	<0.001
Death*	54.9%	51	10.8%	37	<0.001
Duration of stay-in-hospital*	14.8 ± 18.5	49	23.2 ± 13.1	37	<0.05
IFN-γ* (pg/ml)	357.2 ± 367.0	51	592.4 ± 640.5	37	<0.05
IL-6 (pg/ml)	358.2 ± 993.2	51	90.6 ± 143.8	37	0.108
IL-18 (pg/ml)	668.6 ± 470.4	37	600.3 ± 266.4	29	0.487
IL-10 (pg/ml)	127.0 ± 701.2	51	79.4 ± 402.4	37	0.711
TGF-β (pg/ml)	602.1 ± 3581.6	51	981.7 ± 1630.0	29	0.315
IP-10 (pg/ml)	6588.7 ± 3581.6	51	7032.5 ± 3161.3	37	0.540
MIG (pg/ml)	1319.1 ± 848.3	51	1106.8 ± 698.6	37	0.216
MCP-1 (pg/ml)	738.4 ± 716.9	51	779.6 ± 659.5	37	0.780
IL-8 (pg/ml)	422.0 ± 806.2	51	192.7 ± 491.8	37	0.120
Blood urea nitrogen (mg/dl)	17.0 ± 11.4	33	12.8 ± 9.6	17	0.104
White cell count (/mm^3)	6973.8 ± 2968.9	45	6504.5 ± 3968.0	31	0.557
Neutrophil (/mm^3)	75.3 ± 16.1	32	76.5 ± 18.1	22	0.804
Lymphocyte (/mm^3)	15.1 ± 8.9	34	13.5 ± 7.0	27	0.442
Platelet (/mm^3)	172.3 ± 70.8	43	153.2 ± 64.2	31	0.228
Monocyte (/mm^3)	5.7 ± 3.6	27	5.2 ± 2.6	19	0.577
Gender					
Male*	62.7%	32	27.0%	10	<0.001
Female	37.3%	19	63.0%	27	

*$P < 0.05$.

than for IFN-γ. On the contrary, a reciprocal relationship existed between IL-10 and IFN-γ and other related chemokines because no or low IL-10 was produced. These cytokine patterns on Ab(+) group were not so obvious as those on Ab(−) group (data not shown). It seems that IL-18, IFN-γ, IP-10, MCP-1, and MIG are activated together during the early phase of SARS-CoV acute infection.

Association of IFN-γ, IL-18, TGF-β, IL-6, IP-10, MCP-1, MIG, and IL-8 With Clinical Manifestation of SARS

Since SARS-CoV infection can be fatal, and intensive inflammatory response is involved in the disease process, we compared the cytokines between death and survival cases of SARS patients. As shown in Table III, the mean age of the death group was older than that of survival group. The death group also had higher blood urine nitrogen or asparate aminotransferase value than the survival group. The SARS-CoV-infected patients with underlying diseases were more susceptible to death. Their cytokines IL-18, IP-10, MIG, and MCP-1 levels were significantly higher than in the survival group. IL-8 seemed also higher in the death group than in the survival group, but the *P* value (0.091) was not statistically significant. IP-10, MIG, and MCP-1 are chemokines for activated T cells while IL-8 is a chemokine for neutrophils. This suggests that the extensive inflammation is probably responsible for the inflammatory response in the lung of SARS fatal cases. For the survival group, when MCP-1 and IFN-γ were plotted to the circulating lymphocytes count and monocytes count of the SARS patients, an inverse association was found. For MCP-1 and lymphocyte and monocyte counts, the correlation coefficient and r^2 were −0.438/0.192 and −0.378/0.143, respectively (Fig. 5). The inverse association was also found for IFN-γ and circulating lymphocyte counts (−0.366/0.134). However, the pattern was different for neutrophils, as a positive association occurred between MCP-1 and circulating neutrophils count (0.432/0.145). As for other parameters, a positive association existed between TGF-β and the duration of stay in hospital in the survival group (Fig. 5e). TGF-β was only detected in 48 % of the patients, and it appeared late post infection. But when generated, its serum level was positively associated with the duration of stay in hospital for the survival group (correlation coefficient, 0.539/r^2, 0.291). These cytokine patterns of death group were not so obvious as those of survival group (data not shown). Based on these data, it is concluded that there is an interferon-γ-related cytokine storm after SARS-CoV infection, and these cytokines are probably involved in the inflammatory response and damage of the host.

DISCUSSION

During the SARS outbreak in Taiwan, all the sera from either suspected or probable cases were sent to CDC-Taiwan officially for final diagnosis of SARS-CoV infection. Therefore, in this study, the sera analyzed were the first-time point collected after hospitalization, no immunomodulators such as steroids, intravenous immunoglobulin, or ribavirin were given to patients before blood collection, and only the RT-PCR-confirmed cases were used. This is a cross-section study, as only the first-time point before treatment in acute phase stage was analyzed. The sera collected at the convalescent phase were also determined for their cytokine levels, but all of them had returned to basal levels. Sequential serum samples were not available for examining the

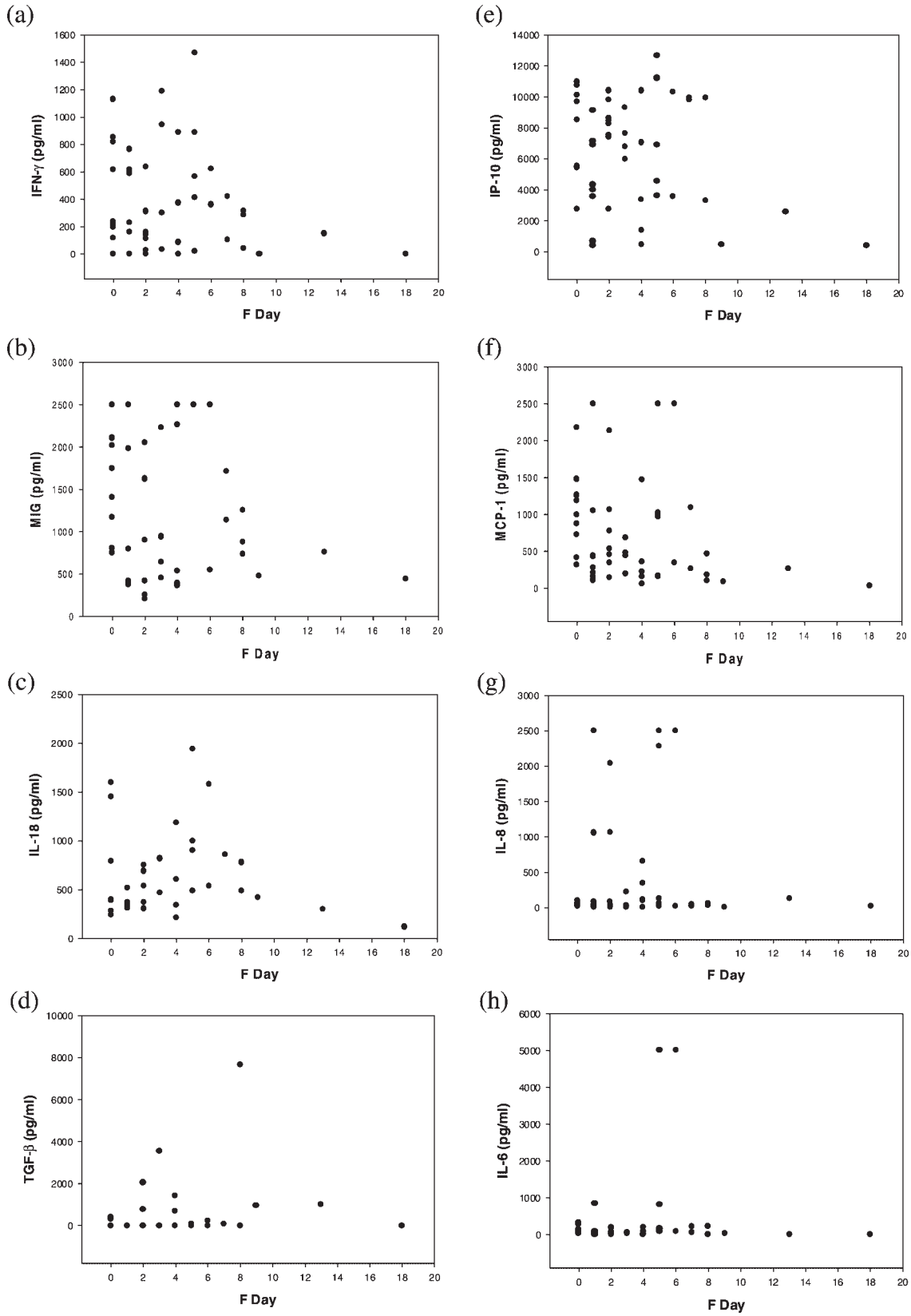

Fig. 3. Kinetic changes of IFN-γ, IP-10, MCP-1, MIG, IL-18, IL-8, IL-6, and TGF-β in Ab(−) SARS patients. Serum cytokine level from 51 Ab(−) group of SARS patients was plot against the fever day of that individual.

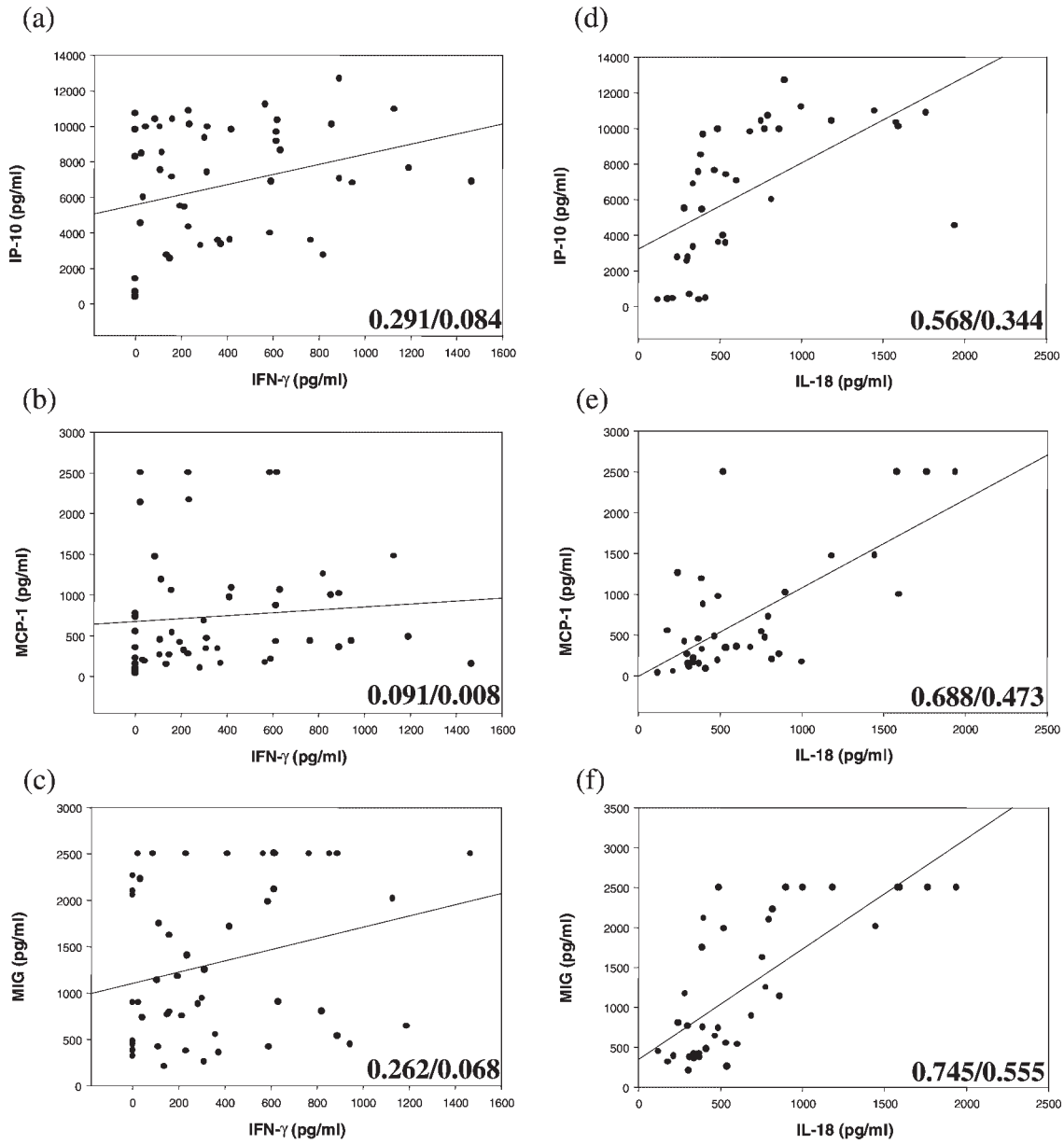

Fig. 4. Association between IFN-γ/IL-18 and IP-10/MCP-1/MIG in Ab(−) SARS patients. Serum chemokine (IP-10, MCP-1, and MIG) level from 51 Ab(−) group of SARS patients was plot against the IFN-γ or IL-18 of that individual. Linear regression and association correlation were analyzed. The former number represents the correlation coefficient while the latter is r^2.

kinetic changes of cytokines in individual disease progression. But when the patients were pooled and plotted against the day post fever onset, we found a high elevation of IFN-γ and its related cytokine or chemokine. The serum levels of IL-18, IFN-γ, IP-10, MCP-1, MIG, and IL-8 were already very high even at the day of fever onset. The strong bias production toward IFN-γ is different from EV71-induced pulmonary edema and dengue virus-induced dengue hemorrhagic fever: a reciprocal relationship existed for individual IFN-γ and IL-10. The over-production of inflammatory IFN-γ and chemokines might be resulting from the lack of IL-10-mediated down-regulation of the immune responses to SARS-CoV infection.

IFN-γ is an inflammatory cytokine, and possesses many biological activities [Barber, 2000; Liu et al., 2002]. It can enhance the major histocompatibility complex expression, activate macrophage function, stimulate chemokine production, induce apoptosis, arrest cell cycle, and enhance Fas expression. IFN-γ is primarily produced by NK cells, Th1 cells, and macrophages. Its production can be up-regulated by IL-12 and/or IL-18. The chemokines IP-10, MCP-1, and MIG were found to be markedly elevated in the acute stage of SARS patients. The IP-10 levels are extremely high and it seems to be a more reliable marker for viral infection, which have been reported for respiratory syncytial virus [Miller et al., 2004], influenza virus [Ishiguro et al.,

TABLE III. Comparison of Cytokines in Death and Survival Groups of SARS Patients

	Death	n	Survival	n	*P*-value
Age*	58.2 ± 17.2	32	37.7 ± 14.9	56	<0.001
Duration of stay-in-hospital*	11.9 ± 11.89	32	22.3 ± 18.15	54	<0.001
IFN-γ (pg/ml)	357.1 ± 371.86	32	512.7 ± 570.67	56	0.171
Il-6 (pg/ml)	387.2 ± 911.82	32	164.8 ± 671.80	56	0.194
IL-18* (pg/ml)	821.5 ± 497.33	24	534.1 ± 274.04	42	<0.001
IL-10 (pg/ml)	266.5 ± 967.34	32	15.9 ± 56.77	56	0.055
TGF-β (pg/ml)	1642.7 ± 2002.01	14	1684.6 ± 1777.59	18	0.951
IP-10* (pg/ml)	8319.2 ± 2966.44	32	5893.1 ± 3337.82	56	<0.001
MIG* (pg/ml)	1590.9 ± 800.25	32	1023.5 ± 714.92	56	<0.001
MCP-1* (pg/ml)	955.6 ± 734.49	32	641.4 ± 641.86	56	<0.05
IL-8 (pg/ml)	491.8 ± 828.47	32	230.6 ± 597.98	56	0.091
Blood urea nitrogen* (mg/dl)	21.9 ± 12.92	23	10.2 ± 4.05	27	<0.001
AST* (IU/L)	95.9 ± 149.82	21	35.0 ± 22.43	40	<0.05
ALT (IU/L)	43.9 ± 52.04	20	27.9 ± 18.88	27	0.148
White cell count (/mm^3)	7127.2 ± 4000.18	29	6054.3 ± 3375.54	51	0.205
Neutrophil (/mm^3)	71.6 ± 23.98	21	76.2 ± 16.36	34	0.401
Lymphocyte (/mm^3)	12.1 ± 7.06	21	15.5 ± 8.50	40	0.118
Platelet (/mm^3)	169.8 ± 76.06	28	161.2 ± 63.83	46	0.604
Monocyte (/mm^3)	5.5 ± 2.67	18	5.5 ± 3.50	28	0.940
Gender					
Male	59.4%	32	29.1%	79	0.100
Female	40.6%		70.9%		
Any underlying disease*	54.8%	57	15.9%	31	<0.001

AST, serum aspartate aminotransferase; ALT, serum alanine aminotransferase.
*$P < 0.05$.

2004] as well as SARS-CoV [Cinatl et al., 2004]. The IP-10 can be directly induced either by viral-infected epithelial cells or through the IFN-γ stimulation. Therefore, the correlation between levels of IFN-γ and IP-10 is not very strong. A stronger association existed for IL-18 and IP-10 and other chemokine MCP-1 and MIG. However, the SARS-CoV-induced chemokine production in the lung without the concomitant production of inflammatory cytokine probably would not cause lung pathological changes in SARS-CoV-infected mice [Glass et al., 2004]. The detection of another chemokine, RANTES, was above the upper-limit of the CBA kit (>2,500 pg/ml) for both the normal healthy control and SARS patients. Unfortunately the sera were not diluted to determine the exact concentration because of the limited amount of the patient sera. But IFN-γ can stimulate lung epithelial cell line to produce these chemokines including RANTES (unpublished observation). Therefore, RANTES is supposed to be also elevated in SARS infection. These chemokines can recruit activated T cells into the lung. There were several reports on the cytokine levels in SARS patients: pro-inflammatory cytokines TNF-α, IL-1, and IL-6 were increased in acute stage of SARS-CoV infection [Pang et al., 2003; Ng et al., 2004; Wong et al., 2004; Zhang et al., 2004]. In our study, neither TNF-α nor TNFRI were detectable. This might be related to the timing of the samples collected and the use of corticosteroid, intravenous immunoglobulin, or ribavirin in treated SARS patients, which would modulate the cytokine production. All the sera tested are from the first-time point collection after hospitalization, patients have not been treated with these immunomodulators. Lung pathological study has shown intense lymphocytic infiltration in SARS patients. IFN-γ is already highly induced at the early Ab(−) stage, and it was even higher in the late Ab(+) stage. Although IFN-α or β were reported to inhibit in vitro replication of SARS-CoV or protect type I pneumocytes against SARS-CoV infection in macaques [Haagmans et al., 2004; Tan et al., 2004], interferons, especially IFN-γ, is a double-edged sword. The bias IFN-γ and its related chemokine production might be responsible for the abnormal inflammatory response in SARS patients. An IFN-γ-related cytokine storm was induced post SARS-CoV infection.

The antibody response to SARS-CoV antigen was delayed after SARS-CoV infection. It is not detectable until 2–3 weeks later [Peiris et al., 2003b]. The Ab(−) group represents the early stage of acute SARS-CoV infection as shown by early timing of sera collected after fever onset in Figure 3. The elders with weak immune response were also included in this category. Based on this antibody distinction, the factors of age, gender, mortality, duration of stay-in-hospital were significantly different between these two groups. Older males had high mortality while young females seemed to have a strong immune response and higher IFN-γ production. This is consistent with the risk factor analysis between survival and death groups. The IP-10, MIG, and MCP-1 levels were higher in death than in survival groups. It seems that male, older patients who have higher chemokine production or with underlying diseases were more susceptible to death post SARS-CoV infection. A SARS in the lung was caused by intense chemokine-induced activated T cell-mediated inflammation and might be fatal. IL-8-mediated neutrophil inflammation is probably also involved.

For the surviving SARS patients, the inversed relationship between MCP-1 or IFN-γ and lymphocyte and monocyte count is interesting. Lymphopenia is

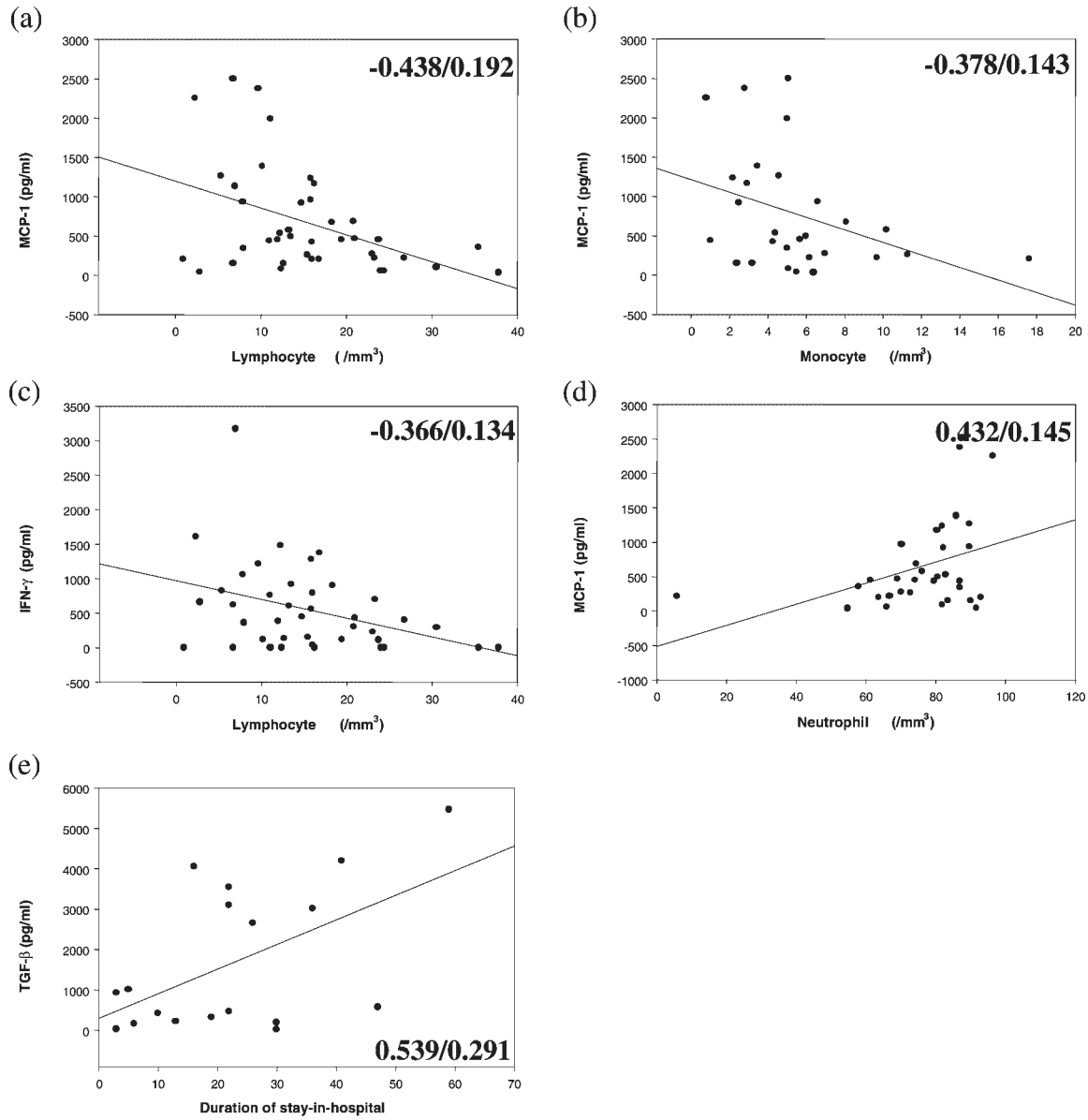

Fig. 5. Association between MCP-1, IFN-γ, or TGF-β and clinical manifestations of the SARS surviving patients. Serum chemokine MCP-1 or IFN-γ level from 56 surviving SARS patients was plot against the circulating lymphocyte counts, monocyte counts, or neutrophil counts of that individual. In the analysis of TGF-β with duration of stay-in-hospital, only patients (n = 18) that have detectable TGF-β were determined. Linear regression and association correlation were analyzed. The former number represents the correlation coefficient while the latter is r^2.

common in SARS patients. How lymphopenia occur is not clearly understood. IFN-γ was reported to induce apoptosis of activated T cells [Bernabei et al., 2001; Sobek et al., 2002]. Two possibilities are reasoned: sequestration into the lung of β-chemokine-recruited lymphocytes and IFN-γ-induced apoptosis. But for neutrophils, a positive association was found for MCP-1 and neutrophils count. The neutrophils α-chemokine IL-8 was also elevated, and once the neutrophils are recruited into the lung, they will enhance more lung damage.

TGF-β is found to be increased after SARS-CoV infection, 48% (32/66) of patients having detectable serum TGF-β at the late stage. The TGF-β level is not significantly different between death and survival group. But when this TGF-β level was plotted to the clinical data of the patient, it was interesting to find a positive association between TGF-β amount and his or her duration of stay-in-hospital for the individuals who survived. Fibrosis is a sequelae post SARS-CoV infection in some individual. TGF-β can induce proliferation of fibroblasts [Krein and Winston, 2002; Dhainaut et al., 2003], therefore, high production of TGF-β might be involved in the development of fibrosis.

Dexamethasone and intravenous immunoglobulin were used to treat SARS patients with some beneficial

effect, although their effectiveness has been debated recently [So et al., 2003]. Dexamethasone is known to inhibit the cytokine production and delayed chemokine-recruited inflammation while the immunoglobulin can modulate the cytokine over-action and inhibit the lymphocyte or macrophage activation. If the IFN-γ cytokine storm is induced and is responsible for the immunological damage of the host, it is reasoned that their beneficial effects were probably due to the interference with the IFN-γ cytokine storm. It was reported that IFN-γ alone and in combination with activation of the Fas pathway induced apoptosis in A549 lung epithelial cells, and this IFN-γ and IFN-γ plus anti-Fas-induced apoptosis could be blocked by dexamethasone [Wen et al., 1997].

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