MINIREVIEW

Alterations of Fas and Fas-Related Molecules in Patients with Silicosis

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Persons with silicosis have not only respiratory disorders but also autoimmune diseases. To clarify the mechanisms involved in the dysregulation of autoimmunity found in patients with silicosis, we have been focusing on Fas and Fas-related molecules in the Fas-mediated apoptotic pathway, because Fas is one of the most important molecules regulating autoimmunity involving T cells. Our findings showed that patients with silicosis exhibited elevated serum soluble Fas levels, an increased relative expression of the soluble *fas* and *dcr3* genes in peripheral blood mononuclear cells, high levels of other variant messages of the *fas* transcript, relatively decreased expression of genes encoding several physiological inhibitors (such as survivin and toso), and dominancy of lower-membrane Fas expressers in lymphocytes, which transcribe soluble *fas* dominantly, compared with soluble *fas* transcription in healthy donors. These findings are consistent with known features regarding immunological factors, such as serum immunoglobulin G levels and the titer of anti-nuclear autoantibodies in silicosis. In addition, anti-caspase 8 autoantibody and anti-Fas autoantibody were detected in serum specimens from patients with silicosis, and a functional assay showed that anti-Fas antibody stimulated Fas-mediated apoptosis. We hypothesize that there are two subpopulations of silicosis lymphocytes. One is a long-term surviving fraction that includes self-recognizing clones showing lower levels of membrane Fas and inhibition of Fas/Fas ligand binding in extracellular spaces. The other subpopulation exhibits apoptosis caused by silica and silicates, is recruited from bone marrow, shows higher levels of membrane Fas, and is sensitive to anti-Fas autoantibody. Further investigation should be performed to confirm the effects of silica and silicates on the human immune system. Exp Biol Med 231:522–533, 2006

Key words: silicosis; Fas; autoimmunity; apoptosis

Introduction

Patients with silicosis are known to not only have pulmonary disorders but also immunological complications such as rheumatic arthritis (also known as Caplan syndrome), systemic sclerosis (SSc), and systemic lupus erythematosus (SLE) (1–10). These immunological abnormalities have been found in patients with silicosis who were exposed to natural crystalline silica (SiO_2) and in patients who received plastic surgery with implants containing silicone ($[SiO_2–O–]_n$) (11–15). These epidemiological findings indicate that crystalline silica causes dysregulation and/or a disturbance of the human immune system, particularly autoimmunity. In addition, asbestos, which is

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categorized as a silicate (mineralogical complexes containing metals, such as iron and magnesium, and includes chrysotile, crocidolite, and amosite), is known to cause malignant tumors, such as alveolar cancers and mesotheliomas, in addition to asbestosis (16–25). The occurrence of these malignancies may be considered the result of a decline in immunity due to exposure to asbestos.

Silica and silicates may disturb immune functions such as autoimmunity and tumor immunity. Silica-induced disorders of autoimmunity have been recognized to be due to adjuvant-type effects of silica (26–33). However, more-precise analyses are needed, given recent immunomolecular biological findings.

The discovery of Fas has led to a remarkable improvement in our understanding of apoptosis and its signal transduction (34–39). Abnormal regulation of apoptosis, particularly involving the Fas/Fas ligand (FasL) pathway, has been considered to play a role in the pathogenesis of autoimmune diseases (40–43). Mutations of the *fas* gene and the *fas ligand* gene, which lead to defects in apoptosis, have been found in autoimmune strains of mice (i.e., *lpr* mice and *gld* mice, respectively) and in human autoimmune lymphoproliferative syndrome (ALPS) in childhood (44–47). During the past several years, we have been investigating immunological aspects of silicosis focusing on the Fas-mediated apoptotic pathway. In this review, we describe the results of experiments involving clinical samples from patients with silicosis and discuss possible mechanisms underlying the dysregulation of autoimmunity in such patients.

Patients

All patients with silicosis in the study were employees at the brickyards in Bizen, Okayama, Japan. The percentage of free silica inhaled by these patients has been estimated to be 40%–60%. Each patient received a diagnosis of pneumoconiosis, according to records of the International Labor Office (ILO) published in 2000 (48). They showed no clinical symptoms of autoimmune diseases, including sclerotic skin, Raynoud phenomenon, facial erythema, or arthralgia, and had no malignancies. Specimens were obtained only in cases in which informed consent had been obtained.

Fas and Fas-Related Molecules

Fas (CD95), which is mainly expressed on the cell membrane of lymphocytes, usually exists as membrane-type Fas and forms a Fas-trimer after binding with Fas ligand (34, 35). The signal-transducing death domain located in the intracellular domain of Fas then recruits FADD and procaspase-8 to form the active death-inducing signaling complex (DISC) (49–51). Thereafter, activated caspase-8 triggers a caspase-cascade involving the activation of CAD/CPAN/DFF40 (by removing its inhibitor, ICAD/DFF45), DNA fragmentation, and, finally, apoptotic cell death (52, 53).

The most typical alternatively spliced variant of the wild-type *fas* gene transcript is known as soluble *fas*. Because this variant transcript lacks 63 bp of the transmembrane domain, its product (soluble Fas) can be secreted from cells to suppress membrane Fas-mediated apoptosis by blocking the binding between membrane Fas and Fas ligand in the extracellular region (34, 35). If there is high level of soluble Fas in the extracellular regions, lymphocytes in these regions may avoid apoptosis and survive longer. Actually, several studies have shown elevated serum levels of soluble Fas in patients with autoimmune diseases (54–57).

Therefore, we compared the cellular and molecular changes of the levels of the following Fas and Fas-related molecules between patients with silicosis and healthy donors:

1. Serum level of soluble Fas (58).
2. Serum level of the soluble Fas ligand (59). Although the Fas ligand is usually localized at the cell membrane on natural killer cells, activated T cells, and cytotoxic T cells, it is sometimes cleaved by matrix-metalloproteinase-like enzymes and secreted into extracellular spaces (60–61).
3. Percentage of Fas-positive lymphocytes (membrane Fas expression) (58).
4. Mean fluorescent intensity (MFI) of membrane Fas.
5. Wild-type and soluble *fas* expression in Fas[High] and Fas[Low] fractions of lymphocytes from patients with silicosis.
6. Relative ratios of expression of wild-type to (i) soluble *fas* gene (62), (ii) various genes related to Fas-mediated apoptosis, such as the gene encoding decoy receptor 3 (dcr3) (63), the apoptosis-accelerating genes *caspase-8*, *-3*, and *-9* and *cpan*, and (iii) the intracellular poptosis-inhibitory encoding genes *xiap*, *survivin*, *dff45*, *toso*, *I-flice*, and *sentrin* in peripheral blood mononuclear cells (PBMCs) (64, 65).

DcR3 was initially discovered as a protein secreted from lung and colon cancer cells that prevents the Fas ligand from targeting them, and is also expressed on cytotoxic T cells and natural killer cells (66). Thus, DcR3 functions similarly to soluble Fas to inhibit membrane Fas-mediated apoptosis.

7. Detection of alternatively spliced variants of *fas* and mutational screening of *fas* and *fas ligand* genes (67).

As shown in Table 1, serum soluble Fas levels were significantly higher in patients with silicosis than healthy donors (58), whereas levels of Fas ligand did not differ between the two groups (59). Although there was no difference in the proportion of membrane Fas-positive cells between patients with silicosis and healthy donors (58), the MFI of membrane Fas was significantly lower in the patients than in donors, having shown positive patterns of membrane Fas in two healthy donors and two patients with silicosis (Fig 1A, B). These data indicate that the membrane

Table 1. Changes in Fas and Fas-Related Molecules in Patients with Silicosis

	Healthy patients ($N = 30$)	Patients with silicosis ($N = 64$)	P*
Soluble Fas (ng/ml)	1.97 ± 0.56	2.61 ± 0.79	<0.005
Soluble Fas ligand (ng/ml)	0.16 ± 0.07	0.16 ± 0.07	NS
Membrane Fas (lymphocytes) (%)	62.11 ± 10.15	56.14 ± 15.91	NS
Membrane Fas, mean fluorescent intensity (lymphocytes)	48.08 ± 8.81	39.92 ± 9.23	<0.0001

* NS, not statistically significant ($P \geq 0.05$).

Fas in the membrane of lymphocytes from patients with silicosis is distributed relatively sparsely compared with that in lymphocytes from healthy donors.

The membrane Fas-positive lymphocytes were divided into Fas[High] and Fas[Low] groups, as indicated in Figure 1B. The lymphocytes in the Fas[Low] group may be the secretors of soluble Fas. To test this possibility, the membrane Fas[High] and Fas[Low] fraction in $CD4^+$ lymphocytes derived from 19 patients with silicosis and 1 healthy donor were sorted using FACSAria flow cytometry (BD Biosciences, San Jose, CA), and wild-type *fas* and soluble *fas* gene expression were assayed as demonstrated schematically in Figure 1C by means of real-time reverse-transcriptase–polymerase chain reaction (rt–RT-PCR) with SYBR-G. The primer set for the wild-type was designed to hybridize to sequences deleted in soluble *fas*. The primer set for the soluble type was designed to bind to a site produced by the deletion of the 63 bp fragment. The relative expression of the wild-type and soluble *fas* genes was calculated as $1/2(B - A)$, where A is the number of PCR cycles required to reach a certain intensity of fluorescence for the *gapdh* product and B is number of PCR cycles required to reach the same fluorescent intensity for the target gene product (wild-type or soluble type) derived from the same sample; *gapdh* expression was 1.

The products of rt–RT-PCR were confirmed to be successfully amplified by standard agarose gel electrophoresis and staining with ethidium bromide, as shown in Figure 1C. Thereafter, the Fas expression index was calculated as indicated in Figure 1D. If this index exceeds 1, it is assumed that the wild-type *fas* expression is greater in the Fas[High] fraction than in the Fas[Low] fraction obtained from the same patients. This means that the Fas[Low] fraction is the dominant secretor for soluble Fas. As the results in Figure 1E indicate, all but 2 of the 19 patients with silicosis and a healthy donor had a Fas expression index ranging from approximately 1–20. The reason why we did not examine more healthy donors was that we assumed that the expression of *fas* in the Fas[Low] and Fas[High] groups was similar and that the important factor affecting the immunological pathophysiology in silicosis was the increase in the Fas[Low] fraction and the relative decrease in the Fas[High] fraction. Taken together, our findings imply that the elevated level of soluble Fas in the serum of patients with silicosis depended on an excess proportion of Fas[Low] in $CD4^+$ lymphocytes.

The relative ratio of expression of wild-type (i.e., membrane) to soluble *fas* (s/mFER) was assayed previously by using total RNA derived from patients with silicosis and healthy donors and primer sets bracketing the sequence deleted in soluble *fas*. This reaction yielded two products; one derived from membrane *fas*, and the other (63 bp shorter) derived from soluble *fas* (Fig. 2A). Consequently, it was easy to determine the relative expression ratio of the two products, and the s/mFER was found to be markedly higher in the patients with silicosis (Fig. 2B) than in healthy donors, and resembled the serum level of soluble Fas in both groups (Fig. 2C and Table 1) (62).

In addition, the relative gene expression of Fas-related molecules in PBMCs was examined using multiplex RT-PCR by amplifying the control *β-actin* gene along with the target gene in the same tube and comparing the signal intensity of the target with the control in each sample for *dcr3* and *survivin* in patients with silicosis and healthy donors (Fig. 2D). Among the various genes related to Fas-mediated apoptosis, the relative expression of *dcr3* was greater in the patients with silicosis, compared with healthy donors (63), whereas the expression levels of *survivin*, *i-flice*, *sentrin*, and *icad* were significantly lower in the patients with silicosis, as we reported previously (64, 65).

The detection of alternatively spliced variants other than typical soluble *fas* was achieved using primers bracketing the full-length *fas* coding sequences for cDNA. This RT-PCR yielded various shorter products in PBMCs from patients with silicosis than in PBMCs from healthy donors. Some of these shorter products were cloned and sequenced. As a result, four alternatively spliced variants, together with typical soluble *fas*, were found; and all were assumed to possess signal peptides but not transmembrane or signal-transducing domains (67). These results indicate that the variant messages function similarly to soluble Fas and DcR3 to disrupt the binding of membrane Fas and Fas ligand in extracellular spaces. However, there were no mutations causing an amino acid substitution in the coding regions of *fas* or *fas ligand* in cDNA from patients with silicosis (data not shown) (67).

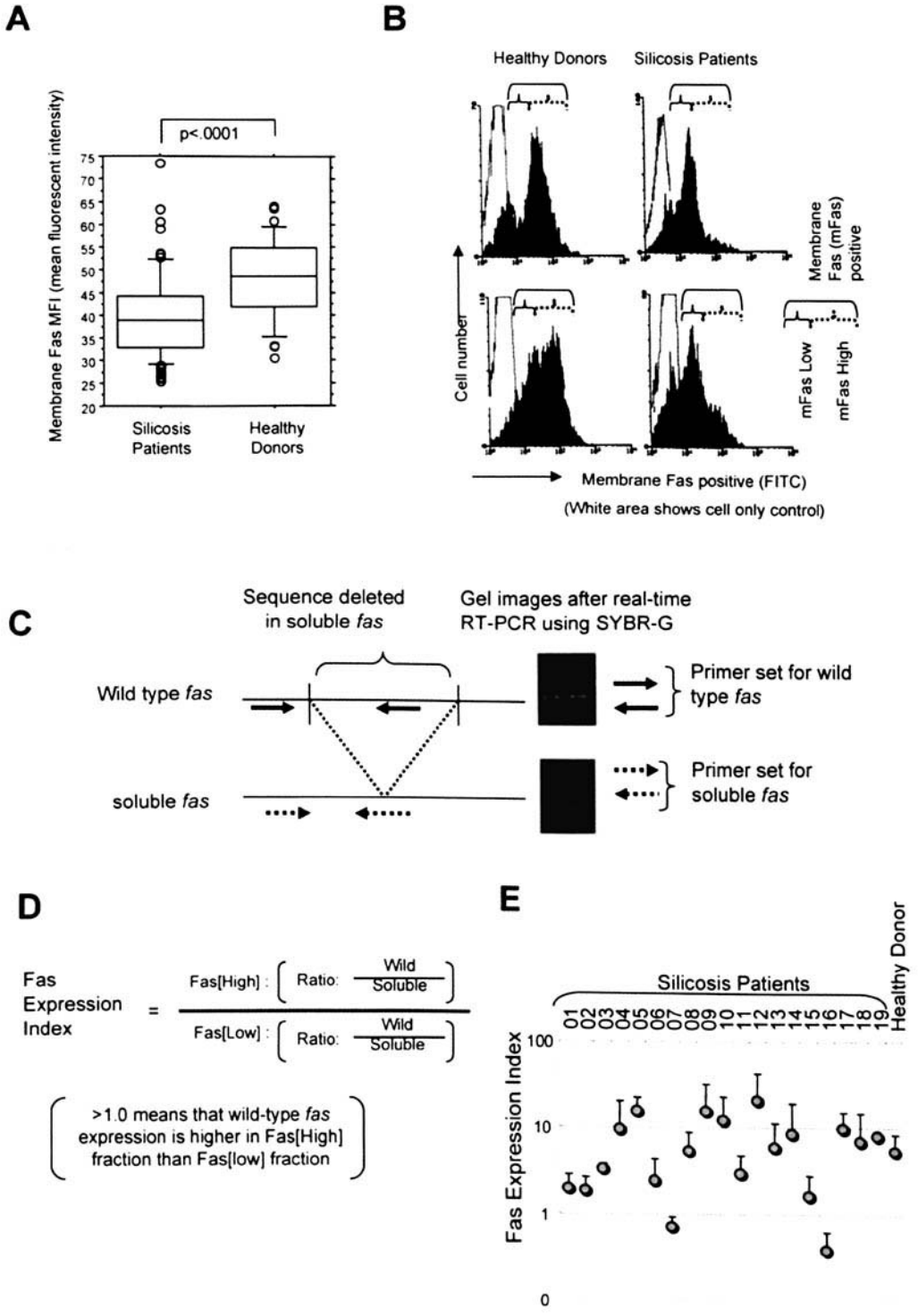

Figure 1. (A) The distribution of mean fluorescent intensity (MFI) of mFas from healthy donors and patients with silicosis, demonstrated by a percentile graph. The mFas MFI was significantly lower in the patients with silicosis than in the healthy donors. (B) The cell membrane Fas receptor (mFas) in peripheral blood $CD4^+$ T lymphocytes was stained with FITC-labeled anti-Fas antibody and analyzed by flow cytometry. Representative examples from two healthy donors (left) and two patients with silicosis are shown. In addition, positive mFas fractions can be divided into two populations, mFas high and mFas low. (C) Schematic demonstration of primer sets for the amplification of wild-type and soluble *fas* using real-time RT-PCR with the SYBR-G method from RNA extracted from $CD4^+$ T cells in Fas[High] and Fas[Low] fractions from patients with silicosis. (D) If the Fas expression index exceeds 1.0, the Fas[High] fraction was assumed to be a secretor of wild-type *fas* and the Fas[Low] fraction a secretor of soluble *fas*. (E) The Fas expression index in 19 patients with silicosis and 1 healthy donor.

Table 2. Selection of Common Factors

Parameters[a]	Factor loading after VARIMAX rotation and contribution score	
	Factor 1	Factor 2
Duration of exposure (years)	0.3721[b]	−0.0527
Symptomatic dyspnea (numbering)	−0.4846[c]	−0.2358
PR (x-ray classification) (numbering)	−0.0184	−0.0287
PO_2 (torr)	0.9698[c]	0.0853
PCO_2 (torr)	−0.3861[c]	−0.1730
A-aDO_2 (torr)	−0.6991[c]	0.0586
Ig G (mg/dl)	−0.0614	0.6546[c]
ANA titer (numbering)	−0.0919	0.3832[b]
mFas (%)	−0.1961	0.0940
sFas (μg/ml)	0.1855	0.6627[c]
sFasL (μg/ml)	0.2511	0.4012[c]
s/m FER (ratio)	−0.1021	0.3511[b]
Contribution Score	17.5878	11.7286

[a] PR, profusion rate; ANA, antinuclear antibody; mFas, membrane Fas expression in peripheral blood lymphocytes analyzed by Flow cytometer; sFas, serum soluble Fas level analyzed by ELISA; sFasL, serum soluble Fas ligand level analyzed by ELISA; s/m FER, soluble/membrane Fas gene expression ratio analyzed by RT-PCR.
[b] Parameters that had a factor loading of between ±0.3 and ±0.4, These parameters also contributed to the extraction of factors but less than those with [c].
[c] Parameters that showed a factor loading greater than ±0.4, which means they contributed significantly to the extraction of factors positively or negatively.

Clinical Evaluation of Alterations of Fas and Fas-Related Molecules in Patients with Silicosis

As shown in Table 2, a factor analysis was used to compare abnormalities of Fas and Fas-related molecules in patients with silicosis and to distinguish the relationships between these abnormalities and respiratory clinical characteristics, such as radiological classification, duration of exposure (occupational history), symptomatic dyspnea, and blood gas analysis (68). The main applications of factor analyses are to reduce the number of variables and to detect structure in the relationships between variables (i.e., to classify variables). Thus, we used this approach to extract the immunological characteristics of interest from the set of all of the clinical and experimental characteristics, including abnormalities in Fas, described above for patients with silicosis. Factor analysis indicated that Fas-related characteristics, such as serum levels of soluble Fas and fas ligand, membrane Fas expression, and s/m FER, were associated with the serum level of immunoglobulin G and titer of antinuclear antibodies (ANA), but not with the respiratory factors mentioned above. Although these Fas and Fas-related characteristics were considered to be linked to the dysregulation of autoimmunity in patients with silicosis, this had not been confirmed before by means of factor analysis, although several reports showed that Fas played a role in the development of pulmonary fibrosis (69–73).

In addition, when the scores for Factor 1 (respiratory factor; a higher score is associated with less-severe conditions) and Factor 2 (immunological factor; a higher score is associated with more-severe conditions) in individual patients with silicosis were plotted (Fig. 3A), there were several individuals (two with higher scores for both factors and for several others) whose score was in the upper right quarter, which indicates a better respiratory score and a worse immunological score. Thus, a small percentage of individuals exposed to silica may tend to have immunological rather than respiratory abnormalities. This possibility may depend on certain individual factors, such as HLA type (74). Patients whose scores are in the bottom half of the graph had better immunological findings, with patients showing a relatively slight respiratory disturbance plotted on the right side, and patients with moderate-to-severe respiratory conditions plotted on the left side. However, none of the patients with scores in the bottom half of the graph had severe immunological disorders, and their conditions might not be complicated with autoimmune diseases in the future.

The membrane Fas MFI (mFasMFI) shown in Figure 1A and Table 1, which was not used for the factor analysis, differed among the three groups (Fig. 3B). Patients with a higher mFasMFI demonstrated a lower titer of ANA, whereas patients with an ANA titer higher than 40 showed a lower MFI. This indicated that a lower mFasMFI, which may be synonymous with dominance of the Fas[Low] fraction in lymphocytes and excess expression/secretion of soluble Fas, is closely related to autoimmune-related disturbances.

The central role of Fas ligand cell signaling in inflammatory lung diseases was reported (75–77). Our factor analysis and findings concerning the relationship between alterations of Fas and Fas-related molecules and immunological disturbances in patients with silicosis do not exclude an important role for Fas ligand in the development of pulmonary fibrosis and inflammatory changes. The role of Fas ligand is not only central to the onset of fibrosis in the

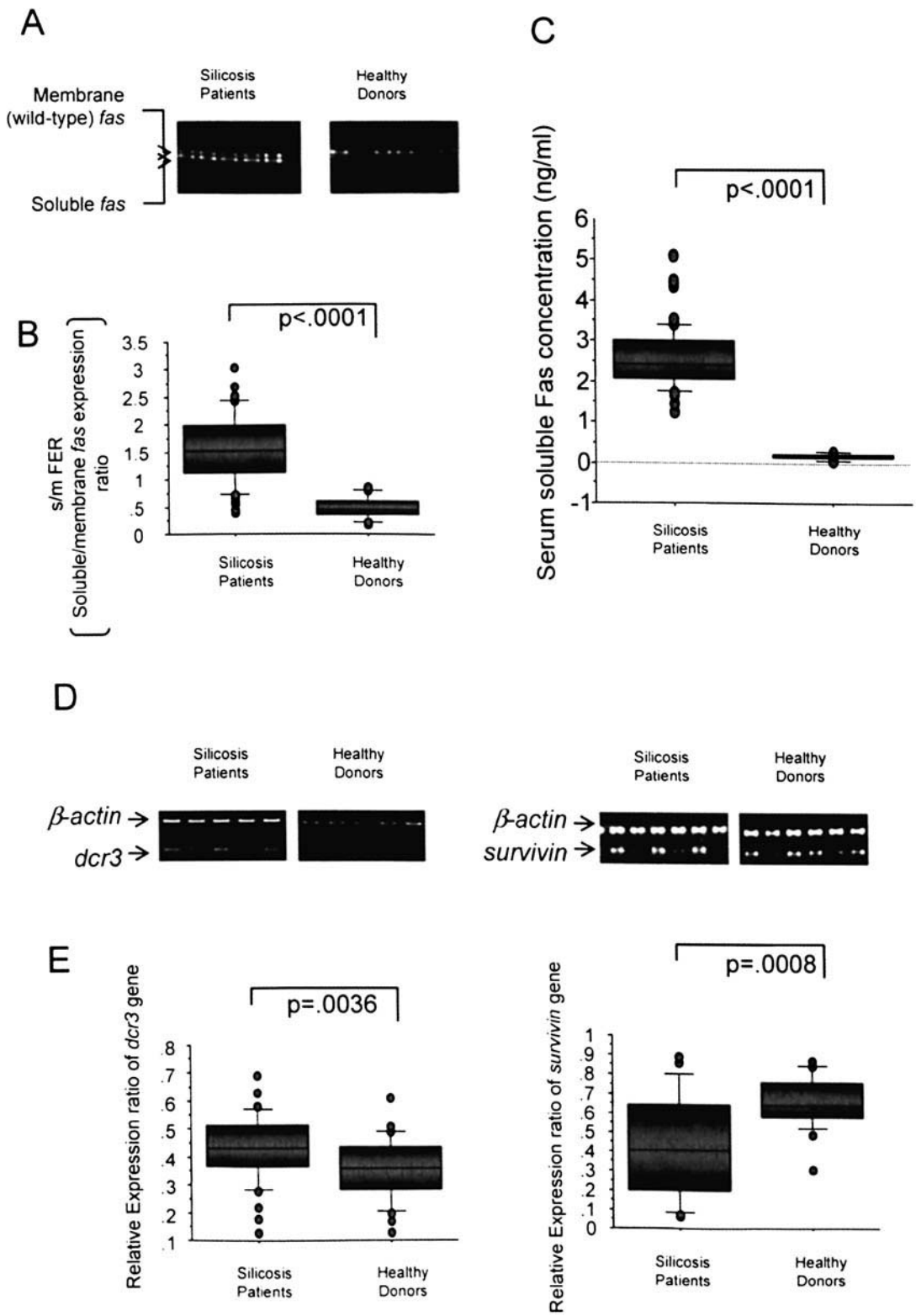

Figure 2. (A) Representative gel images after multiplex RT-PCR for detecting wild-type and soluble *fas* in patients with silicosis (left) and healthy donors (right) (62). (B) s/mFER was calculated as the densitometric intensity of the soluble *fas* product divided by that of the wild-type/membrane *fas* product from results of the same RT-PCR shown in Figure 4A. Patients with silicosis had a higher s/mFER than did healthy donors. (C) Serum soluble Fas levels, as shown in Table 1, were higher in patients with silicosis than in healthy donors. (D) Representative gel images after multiplex RT-PCR for examining the relative expression of the *dcr3* (upper) and *survivin* (lower) genes in patients with silicosis (left) and healthy donors (right). The primer sets for the control, *β-actin* gene, and target gene were used in a single RT-PCR with an optimal ratio. The relative expression ratio for the target gene was calculated as the densitometric intensity of the target gene product divided by that of the *β-actin* product from the same reaction. (E) A comparison of the relative expression ratios for *dcr3* and *survivin* between patients with silicosis and healthy donors, respectively. That of *dcr3* was higher, whereas that of *survivin* was lower, in patients with silicosis than healthy donors.

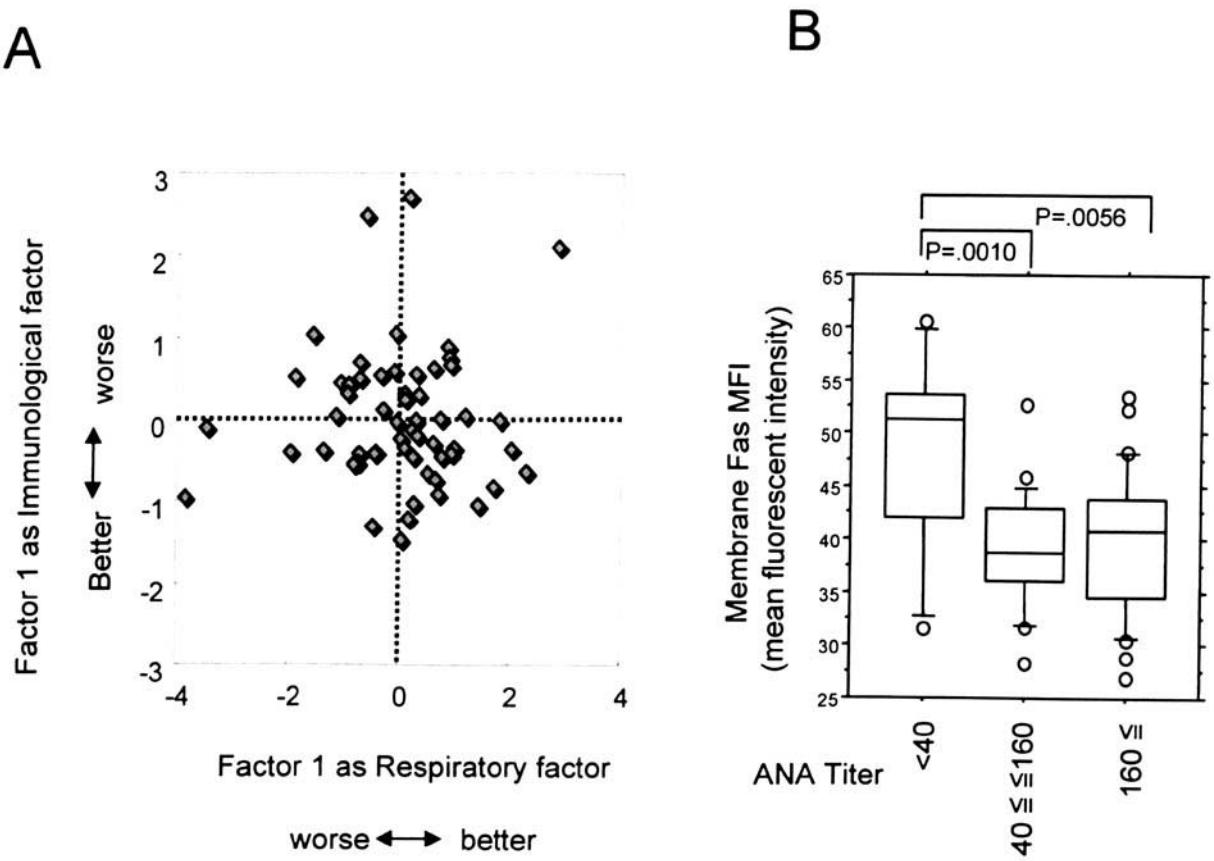

Figure 3. (A) The values for Factor 1 as a respiratory factor and Factor 2 as an immunological factor in individual patients with silicosis, calculated as shown in Table 1 by means of the factor analysis method. (B) A comparison of membrane Fas MFI (Fig. 1B) among three groups (titers for anti-nuclear antibody; <40, ≤40, <160, and ≤160).

pulmonary region, but is also important in the disruption of autoimmunity by Fas and Fas-related molecules generally.

Autoantibodies Against Fas and Casphase 8

Patients with silicosis are known to possess various autoantibodies even before receipt of a clinical diagnosis of autoimmune disease (1–10). The various autoantibodies examined in our laboratory included anti-topoisomerase I autoantibody (78–80), which is known to be specific to SSc, and anti-desmoglein antibody (81), which is unique to bullous diseases. In addition, of the molecules related to Fas-mediated apoptosis, anti-caspase 8 autoantibody (82) and anti-Fas autoantibody (83) were detected in patients with silicosis. The immunoblot in Figure 4A shows a positive band indicative of the presence of a synthetic Fas polypeptide in the serum of a patient with silicosis (the stronger band corresponds to the patient with silicosis, rather than the healthy donor). Figure 4B shows that 23.1% of patients with silicosis tested positive for the anti-Fas autoantibody. Subsequent investigation revealed that epitope spreading (i.e., that the response to autoantigens was more diverse as the response persisted) occurred for both anti-caspase 8 and anti-Fas autoantibodies (82, 83). These results indicate the diversity of immunological abnormalities found in patients with silicosis.

Because the signal peptide in the Fas molecule was detected as one of the epitopes for anti-Fas autoantibodies found in patients with silicosis, the ability of anti-Fas autoantibody to bind Fas and induce Fas-mediated apoptosis was analyzed (83). If anti-Fas autoantibody acts to stimulate membrane Fas to trigger apoptosis, the Fas-expressing lymphocytes in these patients will die quickly after encountering the autoantibody.

For this study, two human myeloma cell lines, KMS-12PE and KMS-12BM, established in our laboratory as sister lines derived from pleural effusion (12PE) and bone marrow (12BM), were used to assay the function of anti-Fas autoantibody, because one marked cellular difference between these two lines is the expression of membrane Fas (Fig. 4C) (84, 85). The Fas receptor was functional because CH11 antibody, which is known to stimulate Fas and cause Fas-mediated apoptosis in Fas-expressing cells, caused growth suppression of KMS-12PE, but not of KMS-12BM (Fig. 4D). Similarly, serum from the patient with silicosis (Sil-70) who showed the highest titer of anti-Fas autoantibody caused growth suppression of KMS-12PE but not of KMS-12BM, whereas serum from a healthy donor

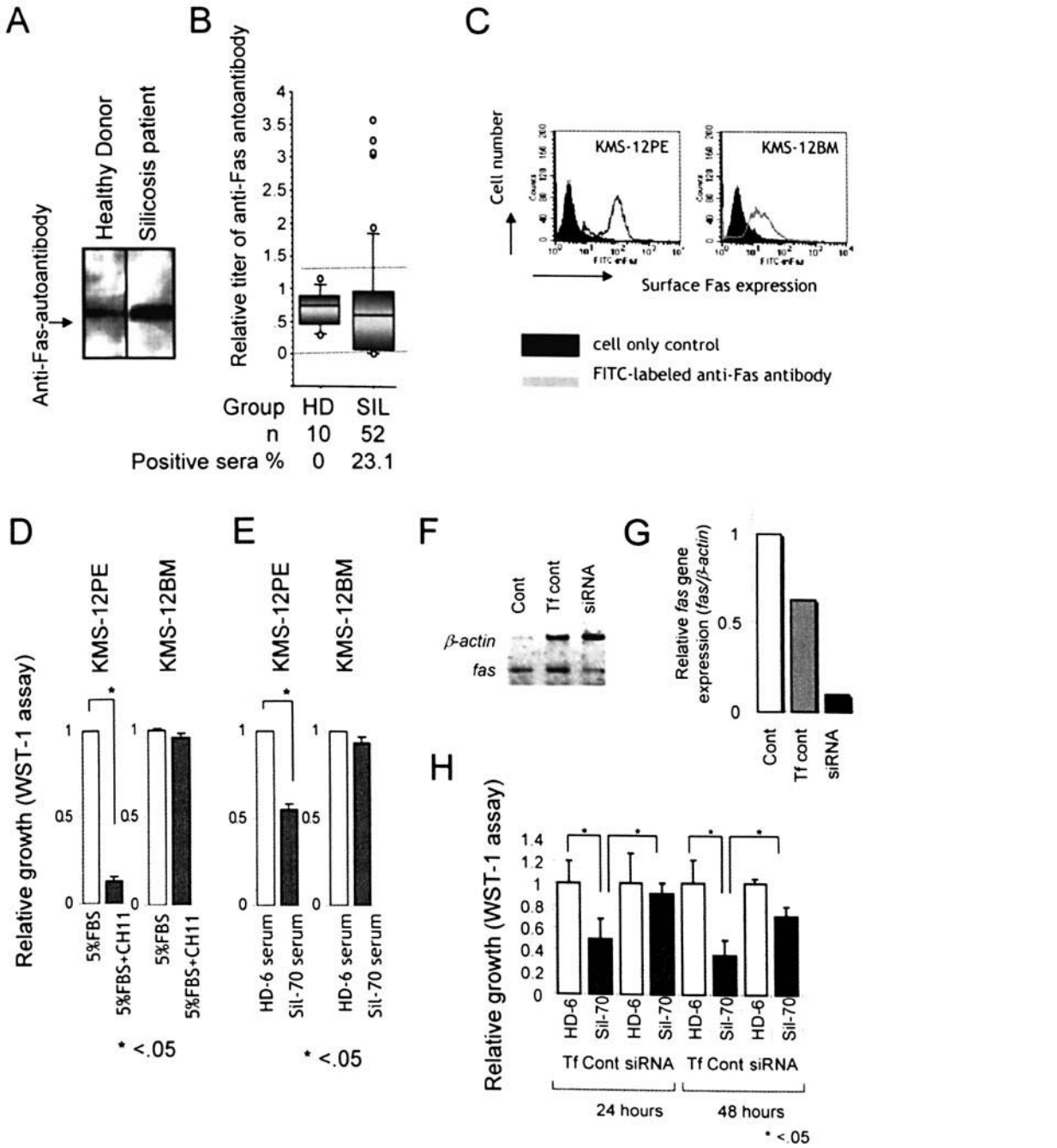

Figure 4. (A) A synthetic polypeptide corresponding to part of Fas was transferred onto a PVDF membrane and reacted with serum specimens from healthy donors (left) and a patient with silicosis (right) to detect anti-Fas autoantibody. (B) The cutoff was determined as reported previously (83) and 23.1% of patients with silicosis were positive for the autoantibody. (C) Membrane Fas expression in KMS-12PE (left) and KMS-12BM (right) human myeloma cells. The hatched line indicates the control and the solid line shows cells stained with FITC-labeled anti-Fas monoclonal antibody. (D) The growth of KMS-12PE and KMS-12BM cells cultured with RPMI1640 medium plus 5% FBS with or without (control) Fas-stimulating, Fas-mediated apoptosis-inducing CH11 antibody (100 ng/ml) analyzed by WST-1 assay with the control being 1.0. Although the growth of the Fas-expresser KMS-12PE was inhibited by CH11, KMS-12BM showed no change. (E) KMS-12PE and KMS-12BM cell lines were cultured with serum from HD-6 or Sil-70 (serum from Sil-70 contained a large amount of anti-Fas autoantibody that was analyzed by Western blotting and with the ProteinChip System previously). Sil-70's serum inhibited the growth of KMS-12PE, a Fas-expresser, but not KMS-12BM, a nonexpresser. (F) The gel image of multiplex-RT-PCR for the *fas* gene in KMS-12PE cells cultured 24 hrs in RPMI1640 medium supplemented with 5% FBS with or without (Cont) mock (Tf cont) or siRNA for *fas* transfection. (G) The relative *fas* gene expression level was analyzed from the gel image shown in (A). Although Tf cont showed a slight reduction, the siRNA sample demonstrated a marked decrease in *fas* gene expression. (H) After the harvesting of RNA samples, each group of cells was resuspended with 5% serum from HD-6 or Sil-70. After 24 and 48 hrs, the WST-1 assay was performed. At both time points, silencing of the *fas* gene significantly reversed the Sil-70 serum-induced growth inhibition.

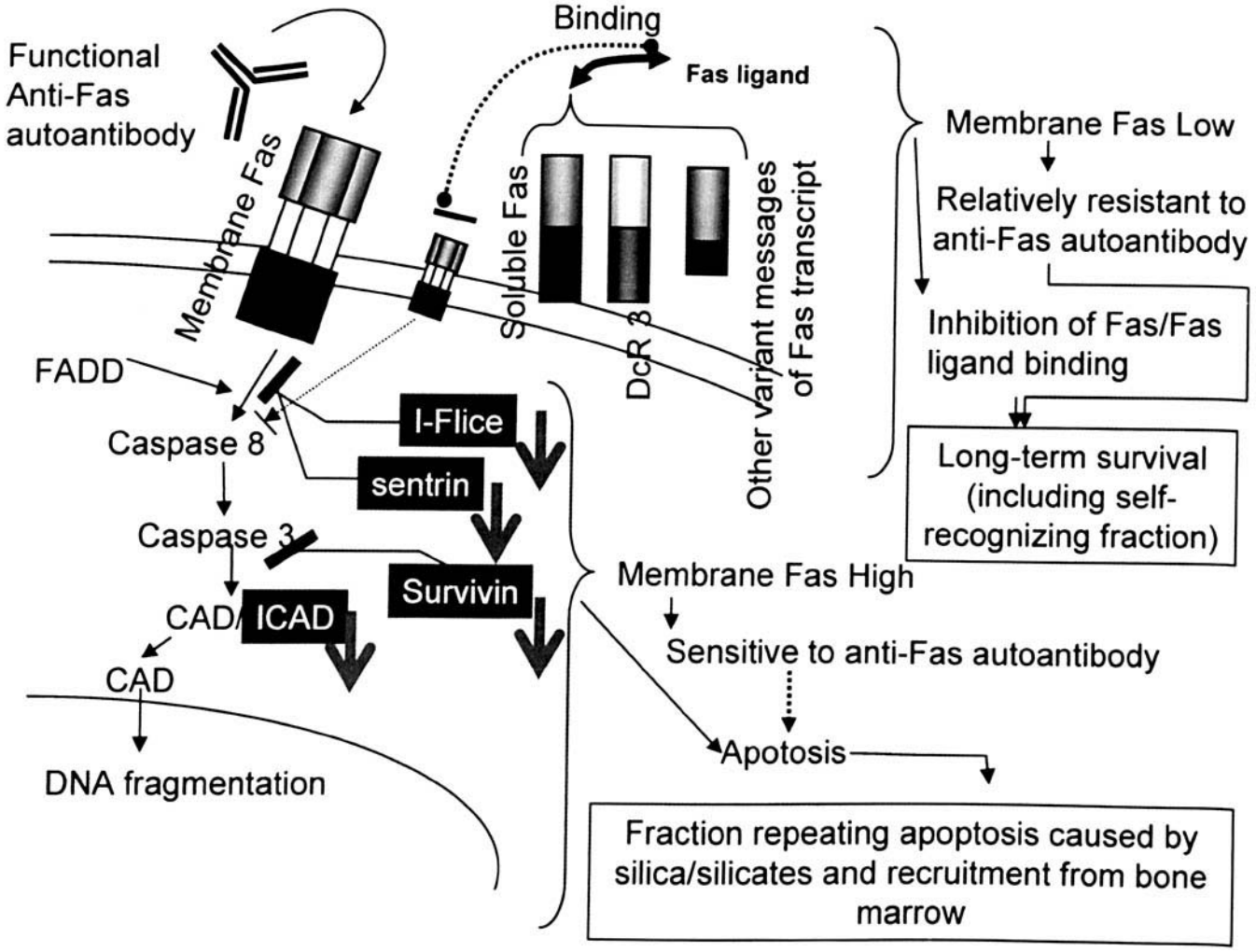

Figure 5. Schematic model of the dysregulation of Fas and Fas-related molecules found in patients with silicosis. Two groups may exist among lymphocytes from these patients: a fraction surviving long term that includes self-recognizing clones and a fraction repeatedly undergoing apoptosis caused by silica/silicates and recruited from bone marrow.

(HD-6) did not (Fig. 4E). Thereafter, to ensure the participation of Fas, the *fas* gene was silenced by siRNA transfection in KMS-12PE cells. As evident from the image of the actual gel after multiplex RT-PCR, siRNA-transfected cells exhibited a remarkable suppression of *fas* expression, compared with control and mock-transfected cells (Fig. 4F). The results of densitometric analysis of the gel image are shown in Fig. 4G. These mock- or siRNA-transfected cells were subsequently cultured with serum derived from a healthy donor (HD-6) or from a patient with silicosis (Sil-70) for an additional 24 and 48 hrs. siRNA-transfected cells recovered from the growth inhibition induced by serum from Sil-70 at both time points (Fig. 4H). These results indicate that *fas* played a critical role in the Sil-70–induced growth inhibition found in KMS-12PE but not in KMS-12BM cells (83). Taken together, these findings imply that the anti-Fas antibody found in patients with silicosis functions similarly to CH11 and stimulates membrane Fas to cause cells to undergo apoptosis.

Possible Fas-Related Pathophysiology in Patients with Silicosis

In this review, we have described various abnormalities in Fas and Fas-related molecules in patients with silicosis. But what happens in immunocompetent cells, particularly T lymphocytes, in patients with silicosis?

The findings about the levels of factors in extracellular spaces, such as soluble Fas, DcR3, and the products from various alternatively spliced *fas* variants, indicate that apoptosis mediated by membrane Fas seems to be interfered with and reduced. However, because there was reduced expression of intracellular molecules associated with anti-Fas–mediated apoptosis, such as the *i-flice*, *sentrin* and *survivin* gene products, compared with expression in healthy donors, it seemed likely that Fas-mediated apoptosis would be enhanced in the lymphocytes from patients with silicosis. In addition, the anti-Fas autoantibody found in serum from patients with silicosis may contribute to the enhanced apoptosis of lymphocytes, because of the Fas-stimulating function of this antibody. Relative to healthy donors, in

whom apoptosis of lymphocytes is assumed to be neither enhanced nor reduced, it seemed that the two groups of lymphocytes would show enhanced and reduced Fas-mediated apoptosis in the patients with silicosis.

Thus, there are two populations of $CD4^+$ lymphocytes, Fas[High] and Fas[Low], in patients with silicosis. The Fas[Low] population may be relatively small in membrane Fas, because of an excess of alternative splicing of soluble *fas* and other variant messages. Therefore, these cells may be resistant to functional anti-Fas autoantibody, because membrane Fas is relatively scant. Consequently, it is speculated that there is a proportion of $CD4^+$ T lymphocytes in patients with silicosis that express low levels of membrane Fas, secrete higher levels of soluble Fas, DcR3, and spliced variants, and are resistant to anti-Fas autoantibody-induced apoptosis (Fig. 5). Because the patients with lower mFasMFI had a higher titer of ANA (Fig. 3B), the self-recognizing clones in patients with silicosis may be included in this population of Fas, because these clones may survive longer and show resistance to apoptosis.

It is possible that Fas-mediated apoptosis occurs to a certain degree in the lymphocytes of patients with silicosis, because of the relative decrease in the levels of intracellular inhibitors of Fas-mediated apoptosis. This may be explained by the presence of a different group of lymphocytes in patients with silicosis that are strongly positive for membrane Fas, sensitive to anti-Fas autoantibody, and undergoing apoptosis. However, this group may be recruited from bone marrow after reaching the final stage of cell death. These newcomers would not have encountered silica and would be sensitive to silica- and silicate-induced apoptosis. As a result, cells in this group would be continuously undergoing renewal and apoptosis.

Unfortunately, the series of patients in whom the anti-Fas autoantibody and serum soluble Fas levels were examined were not identical. Consequently, we could not compare these two parameters. According to our hypothesis, the patients with higher levels of anti-Fas autoantibody may be the ones who show relatively lower serum soluble Fas levels. However, during years of lower exposure to silica, the Fas[High] fraction in these patients will be reduced by their own anti-Fas autoantibody, and during these events, for example, the DNA-binding activity of silica may affect the transcription of Fas messenger and make lymphocytes able to express a high level of soluble Fas and lead them to become resistant to Fas-mediated apoptosis. These events may cause the long-term survival of self-recognizing T cell clones. These are issues that we need to address quickly.

Finally, it should be mentioned that it is difficult to explain why silicosis leads to Fas/Fas-ligand abnormalities. Given the frequent occurrence of alternative splicing but not nucleotide substitution, silica may have stronger activity to bind some DNA region that results in interference with splicing but not induction of nucleotide mutations. This seems to correspond to the relatively frequent occurrence of autoimmune disorders, rather than cancer, in patients with silicosis. However, more investigations are needed to clarify the effects of silica on the molecular immunological aspects of apoptosis.

Conclusion

There are many abnormalities involving Fas and Fas-related molecules in patients with silicosis. These results indicate a dysregulation of autoimmunity in these patients. In the near future, use of newly developed molecular biological tools, such as DNA arrays, transcriptome analysis, and proteosome analysis should make profiling of the Fas[High] and Fas[Low] fractions possible. In addition, an *in vitro* model for chronic, recurrent, and low-dose exposure to silica and silicates should be established in specific cell lines by means of tissue culture methods, because it is assumed that low-dose and long-term exposure to silica and/or silicates induces biological or genetic changes in human lymphocytes, causing the reduction and perturbation of autoimmunity and/or tumor immunity.

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