Reduced Expression of the Survivin Gene in PBMC from Silicosis Patients

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ABSTRACT. To explore the mechanism involved in immunological disorders associated with silicosis, the expression of IAP family genes in peripheral blood mononuclear cells from silicosis patients was examined. Relative gene expression was assayed using the multiplex RT-PCR for the XIAP and survivin genes. The correlation between those expression levels and various clinical parameters was then analyzed. The relative expression level of the survivin gene was reduced in silicosis patients as compared with that of healthy volunteers. The expression level of survivin positively correlated with PCO_2 values. These results support the existence of two populations of T lymphocytes in silicosis patients, one resistant to Fas-mediated apoptosis (a self-recognizing long-surviving fraction) and the other one sensitive to silica-induced apoptosis and repeatedly undergoing death and recruitment.

Key words : silicosis — survivin — apoptosis — immunological disorders

To explore the pathophysiology of immunological disorders associated with silicosis, such as the appearance of autoantibodies and the complications of autoimmune diseases,[1,2] we have been investigating on the Fas-mediated apoptotic pathway,[3-10] because of the abnormalities in Fas and related molecules reported in such human idiopathic autoimmune diseases as systemic lupus erythematosus and rheumatoid arthritis.[11,12] We have reported elevated serum soluble Fas (sFas) levels in silicosis patients,[3] and dominant expression of the genes for sFas and decoy receptor 3 (DcR3),[5,7] which is known to affect membrane Fas-expressing cells to prevent Fas ligand-induced cell death similar to sFas. These results indicated that Fas-mediated apoptosis in peripheral blood mononuclear cells (PBMC) from silicosis patients is suppressed extracellularly by inhibition of Fas-Fas ligand binding.[3-5,7,9]

Recently, inhibitors of apoptosis (IAP) family members have been

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identified as suppressors of apoptosis.[13,14] IAP proteins are characterized by a novel domain of approximately 70 amino acids termed baculoviral IAP repeat (BIR). They function as intracellular physiological caspase inhibitors to prevent apoptosis. Among IAP family genes, the XIAP and survivin genes have been found to be overexpressed in numerous cancer cells and to act as tumor suppressors to prevent the death of cancer cells.[15-21]

In this study, we studied the expression levels of the XIAP and survivin genes in PBMC from silicosis patients to test our hypothesis that two subpopulations of lymphocytes exist in patients with silicosis.

MATERIALS AND METHODS

Subjects

Twenty-four silicosis patients (average age, 68 ± 7 (mean ± SD) years) without any clinical symptoms of autoimmune diseases or any malignancies, and 24 healthy volunteers (64 ± 7 years) were the subjects of this study. All patients were Japanese and workers in a firebrickyard. They gave informed consent according to the guidelines of the Internal Review Boards of Kawasaki Medical School or Kusaka Hospital.

RNA extraction, cDNA synthesis, and multiplex reverse transcriptase-polymerase chain reaction (MP-RT-PCR)

PBMC were isolated from 10 ml of heparinized blood in each case by the Ficoll-Hypaque density centrifugation method (lymhpoprep™, Nycomed Pahrma As, Oslo, Norway). The extraction of total RNA, synthesis of cDNA and MP-RT-PCR were performed as reported previously.[8-12] The primer sequences were as follow: XIAP (F: 5'-TGGCAATATGGAGACTCAGC-3', R: 5'-TGCACTTGGTCACCAATACC-3') and survivin (F: 5'-CTGAGCTGCAGGTTCCTTATC-3', R: 5'-TCTGCCAGACGCTTCCTATC-3'). The analytical details of our MP-RT-PCR method have been described previously[22] and it was employed as indicated in previous reports in this study.[5-9,22]

Statistical analysis

The relative expression level of each gene in the individuals with silicosis and the volunteers was statistically analyzed by the Menn-Whitney test. In addition, the correlation between the relative expression levels of the XIAP and survivin genes and clinical parameters was examined using Pearson's correlation coefficient test. The clinical parameters applied for this analysis were the duration of silica exposure (years), radiological grades (PR: profusion rate according to the ILO guidelines, 1980), subjected dyspnea, PO_2 (torr), PCO_2 (torr), A-aDO_2 (torr), vital capacity (VC) (l), percent VC (%), forced expiratory volume in one second ($FEV_{1.0}$) (l), percent $FEV_{1.0}$ (%), 25% minute volume height (V_{25}/H), peak flow (l-s), the titer of antinuclear antibodies, serum immunoglobulin (Ig) G (mg/dl), membrane Fas expression on the surface of peripheral blood lymphocytes (%), serum sFas (ng/ml), serum soluble Fas ligand (ng/ml), and the soluble/membrane Fas expression ratio as reported previously.[6,10]

RESULTS

MP-RT-PCR and relative expression levels

As shown in Fig 1, the genes for XIAP and survivin were amplified from most of the cDNA derived from silicosis patients and healthy volunteers in our MP-RT-PCR. At a glance, the gene expression of survivin in the silicosis patients seemed to be reduced. To analyze this further, the relative expression levels were calculated as the intensity of RT-PCR products for the XIAP or survivin gene divided by that of the β-actin gene for the same reaction. The lower panels of Fig 1-A and 1-B show a comparison of the relative expression levels of the XIAP and survivin genes, respectively. Although the relative gene expression of XIAP showed no differences between the silicosis patients and healthy volunteers, that of survivin in the silicosis patients was reduced as compared with that in the healthy volunteers. In addition, the each relative expression of XIAP in healthy volunteers was varied with the individual.

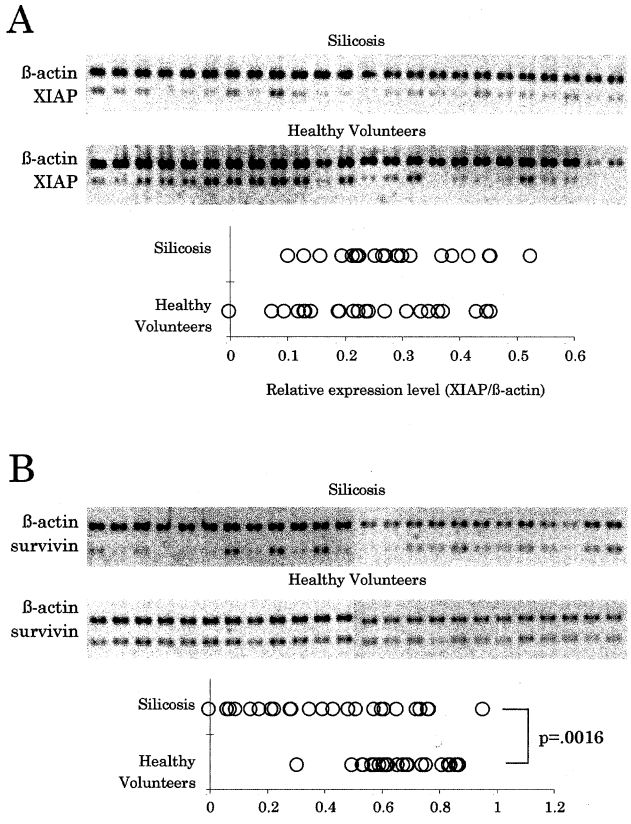

Fig 1. MP-RT-PCR products of the XIAP (panel A) and survivin (B) genes with the β-action gene in PBMC derived from silicosis patients (upper) and healthy volunteers (lower). After visualization of the MP-RT-PCR products on 1.2% agarose gel staiced with ethidium bromide, gel images were obtained in a FAS-II UV-image analyzer (TOYOBO Co. Ltd., Tokyo, Japan), and the densities of products were quantified with Quantity One™ version 2.5 (PDI Inc., Huntingron Station, NY, USA). The relative expression levels of the XIAP and survivin genes were plotted.

Correlation with clinical parameters

There was a positive correlation between the gene expression of survivin and PCO_2 ($r = .436$, $p = .0365$) as shown in Fig 2. No other clinical parameters showed significant correlation with the survivin expression level. In addition, the gene expression of XIAP revealed no correlation with clinical parameters studied.

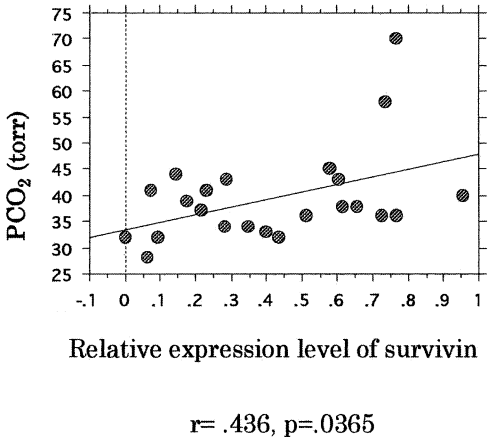

Fig 2. Inverse correlation between the relative expression level of the survivin gene and the PCO_2 value in silicosis patients. Because clinical information was not obtained from one case, analysis was performed in 23 cases.

DISCUSSION

Based on our investigation which demonstrated polyclonal activation of T cells on exposure to asbestos due to superantigenic effects,[23-26] the T cells preventing Fas-mediated apoptosis might include polyclonally activated clones, among which self-recognizing types exist.[9] However, we have also reported reduced gene expression of intracellular inhibitory molecules such as sentrin and I-Flice for Fas-medicated apoptosis, although extracellular inhibitory molecules for the binding of membrane Fas and Fas ligand such as sFas and DcR_3, were elevated in serum and in PBMC.[5,7] These results indicate that there may be another fraction of T cells in the PBMC from silicosis patients which is sensitive to silica-induced apoptosis like healthy T cells and which may die and be recruited again and again due to occupational recurrent exposure to silica compounds.[9] Consistent with these findings, the relative gene expression of survivin, which is another intracellular inhibitor of apoptosis and which blocks caspase activation, was reduced in the PBMC from silicosis patients. These results support our conclusion that two sub-populations of T lymphocytes exist in silicosis.[9] One fraction consists of long-surviving self-recognizing clones induced by chronic and recurrent occupational exposure to silica compounds and characterized by an increase in extracellular inhibitory molecules for Fas-mediated apoptosis. The other is sensitive to silica-induced apoptosis and may die and be recruited. The latter may be represented by enhanced

apoptosis and reduced expression of inhibitors such as survivin, sentrin, and I-Flice (see Fig 3). Based on our investigations, silica compounds seem to act as a superantigen with relation to occurrence of autoimmunity in silicosis patients. Therefore, these two sub-populations of T lymphocytes seem to be classified into helper T cells.

It may be difficult to understand the meaning of the positive correlation between survivin expression and PCO_2. As we previously reported, serum Ig G, serum sFas, serum soluble Fas ligand, and the soluble/membrane Fas expression ratio are the immunological factors among the clinical parameters, and they are independent of other respiratory factors such as the duration of exposure, symptomatic dyspnea, PO_2, PCO_2, and A-aDO_2.[6,10] In addition, there was a population of silicosis patients who exhibit unimpaired respiratory factors but altered immunological factors. These findings indicate that silicosis patients showing reduced survivin expression with normal PCO_2 levels may include an immunological dominant impaired sub-population of individuals. Unfortunately, we could not conduct a factor analysis due to the small number of patients studied. It may necessary to characterize the patients with a reduced survivin expression.

Recently, there have been several reports concerning the involvement of the Fas-Fas ligand apoptotic pathway in the development of pulmonary fibrosis.[27,28] Although we have been focusing on dysregulation of this pathway in PBMC in the relationship between silica-exposure and immunological impairment found in silicosis patients, this dysregulation may affect the developing pulmonary lesion in silicosis. If there are two fractions of T-cells in silicosis patients as mentioned above and shown in Fig 3, patients with un-reduced expression of the survivin gene may possess a lower number of fraction which is sensitive to silica induced apoptosis and may die and be recruited. In addition, since these patients showed higher and impaired PCO_2 levels, it may be possible to consider that the other fraction, long-term surviving self-recognizing clones, is involved in the

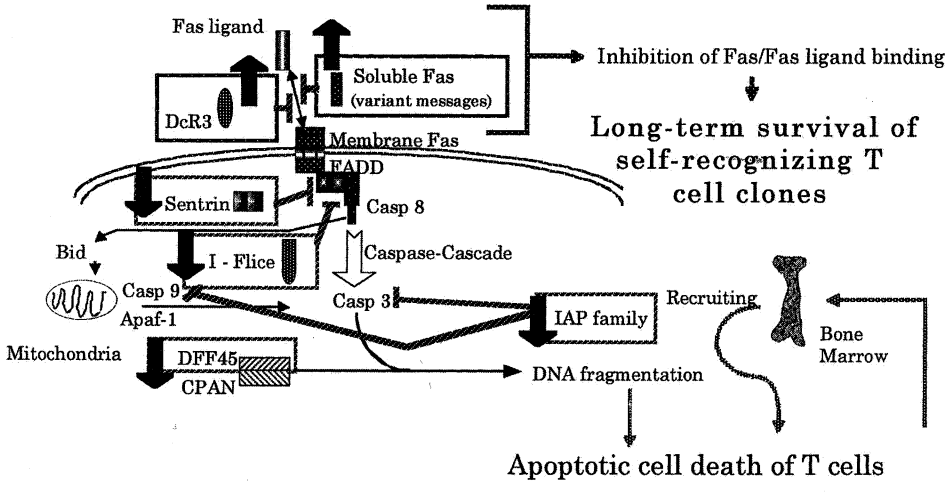

Fig 3. Schematic model of dysregulation of the Fas-mediated apoptosis pathways in silicosis patients modified from a previous report.[9]

development of pulmonary lesions. Recently, Broaddus discussed the relationship between apoptosis and asbestos-induced disease.[29] Although apoptosis in the lung or pleura has been mentioned as a marker for the injurious effects of asbestos, fibrosis may result when asbestos-induced apoptosis is excessive or persistent. To clarify these hypotheses, further studies are required to examine the expressions and roles of the molecules related to Fas-mediated apoptosis in circulating and lung-oriented immunological cells.

Cellular and molecular characterization of the supposed two fractions of T lymphocytes is required to understand the pathophysiology of the immunological disturbance caused by silica compounds and to protect workers from immunological disorders induced by work-related substances.

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