Chapter 9

Immunostimulation by Silica Particles and the Development of Autoimmune Dysregulation

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1. Introduction

Immunostimulation by environmental and occupational factors has been shown to cause various human diseases such as allergy and autoimmune diseases [1,2]. For example, solvents such as vinyl chloride have been linked to the development of scleroderma (SSc) [3-5], and previous studies reported the relationship between exposure to solvents and rheumatoid arthritis (RA) [6] or multiple sclerosis [7,8]. Another typical substance is silica. Patients exposed to silica particles were shown to develop not only pulmonary fibrosis, known as silicosis [9,10], one of the typical forms of pneumoconiosis, but also various autoimmune diseases [11,12] such as RA (historically known as Caplan syndrome) [13,14], SSc [15-17], systemic lupus erythematous (SLE) [18,19], and anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis/nephritis [20-22]. Many epidemiological reports have demonstrated that silica exposure is a risk factor of autoimmune diseases [11,12].

The mechanism of silica-induced autoimmune dysregulation has been attributed to silica acting as an adjuvant [23,24]. However, silica particles may directly stimulate circulating peripheral immune cells and cause certain alterations in the cellular or molecular functions of these cells because these particles may be retained in pulmonary lesions as well as the lymph nodes after they are inhaled into the body [9-12]. Therefore, if the direct effects of these particles change the characteristics of immune cells leading to the dysregulation of immune tolerance, clarifying these cellular and molecular mechanisms may be useful in preventing immune

disorders in silicosis patients as well as providing an insight into the etiology of various autoimmune diseases.

We previously investigated the immunological effects of silica using human peripheral blood immune cells derived from healthy donors and silicosis patients [25-27]. In this review, we summarized our findings, in which silica was shown to be an environmental immunostimulator and the chronic activation of immune cells by recurrent and chronic exposure to silica was demonstrated to cause an imbalance in the regulation of T cell responses.

2. Autoantibodies detected in silicosis patients and their relationship with HLA phenotypes

We previously measured autoantibodies in silicosis patients, all of whom were Japanese brickyard workers in Bizen city, Okayama prefecture, Japan. All patients were diagnosed with silicosis based on their radiological findings in accordance with the ILO 2000 guidelines. The amount of free silica inhaled by these patients was estimated to be from 40 to 60%, as determined by their work environment. No subjects exhibited any symptoms of autoimmune diseases such as sclerotic skin, Raynaud's phenomenon, facial erythema, or cancer.

We demonstrated that the overall titer of anti-nuclear antibodies (ANA) was higher in these patients than in healthy volunteers [28]. In addition, silicosis patients without bullous diseases tested positive for anti-desmoglein autoantibodies and the frequencies of HLA-DRB1*04, HLA-DQB1*03, *0303, and *05, and HLA-DPB1*0402 and *0501 alleles were higher in these patients than in a healthy Japanese control population in the literature [29,30]. Moreover, the relationship between the autoantibodies in silicosis patients and HLA phenotypes was also analyzed in silicosis patients with anti-topoisomerase I autoantibodies [30-34], and the results obtained revealed that the allelic frequency of HLA-DQB1*0402 was significantly higher in anti-topoisomerase I positive Japanese silicosis patients than in anti-topoisomerase I negative patients or healthy donors [30-34].

We also assessed autoantibodies against Fas/CD95 [35], the cell death receptor, which plays an important role in the apoptosis of lymphocytes, and caspase-8 [36,37]. This anti-Fas autoantibody, in particular, was shown to induce apoptosis in Fas-expressing cells [35].

Even silicosis patients without any clinical symptoms of autoimmune diseases have various inapparent alterations in self-tolerance depending on individual factors, such as HLA phenotypes. In addition, when both respiratory and immunological factors were analyzed using factor analysis, this immunological progression was not concomitant with the development of respiratory disease [38]. Respiratory and immunological factors were shown to deteriorate to varying degrees in more than half of silicosis patients; however, a subpopulation was classified as a better respiratory and worse immunological group, while the opposite group was also reported [38].

Therefore, we attempted to confirm whether silica particles directly stimulated human immune cells, particularly T cells, with experimental evidence.

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3. Schematic summary of the chronic activation of T cells by silica particles

A summary of the findings obtained and considerations are schematically presented in Figure 1. Silica particles were shown to chronically activate various T cells. Previous studies reported that effector T cells expressed various activation markers such as PD-1 and CD25 and produced many molecular markers for chronic T cell activation such as soluble Fas (sFas), decoy receptor 3 (DcR3), and soluble interleukin (IL)-2 receptor (sIL-2R) [39,40].

On the other hand, silica particles were also shown to activate CD4+CD25+FoxP3+regulatory T cells (Treg). However, this chronic activation caused the enhanced expression of Fas/CD95 on the surface of Treg, which induced early apoptosis [41]. Therefore, Treg may be lost from the peripheral blood, with the resulting imbalance between Treg and effector T cells subsequently leading to autoimmune dysregulation.

Detailed explanations of these findings are presented below.

4. Immune stimulation of effector T cells by silica particles

Freshly isolated peripheral blood mononuclear cells (PBMCs) obtained from healthy donors were incubated with silica particles. The CD69 expression on the membrane surface was examined as a marker, demonstrating gradual activation of T lymphocytes during 10-day incubation [42].

Other activation markers were examined in serum derived from silicosis patients and compared with those from healthy donors [43]. The results showed that sIL-2R levels were slightly higher in silicosis patients than in healthy donors. sIL-2R levels were also examined in serum derived from SSc patients, and correlations between sIL-2R levels and the immunological status of healthy donors, silicosis patients, and SSc patients as 1, 2 and 3 for continuous variables were analyzed. The correlation coefficient was shown to be 0.575 with $p=0.0008$, which indicated that, from the viewpoint of immunological alterations based on serum sIL-2R levels, silicosis patients were located between healthy donors and SSc patients. Elevated sIL-2R levels may be a pathophysiological marker for hematological malignancies such as human T lymphotropic retrovirus type-1 (HTLV-1) associated with adult T cell leukemia (ATL) and hairy cell leukemia, which reflects the increased production of cells leading to an elevation in serum titers [44-47]. Elevated sIL-2R levels have recently been reported in various autoimmune or inflammatory diseases, suggesting that the immune response is activated by chronic stimulation of T cells with an auto-or foreign antigen [48-51]. Therefore, the moderate, chronic activation status of the immune system may play a role in silicosis.

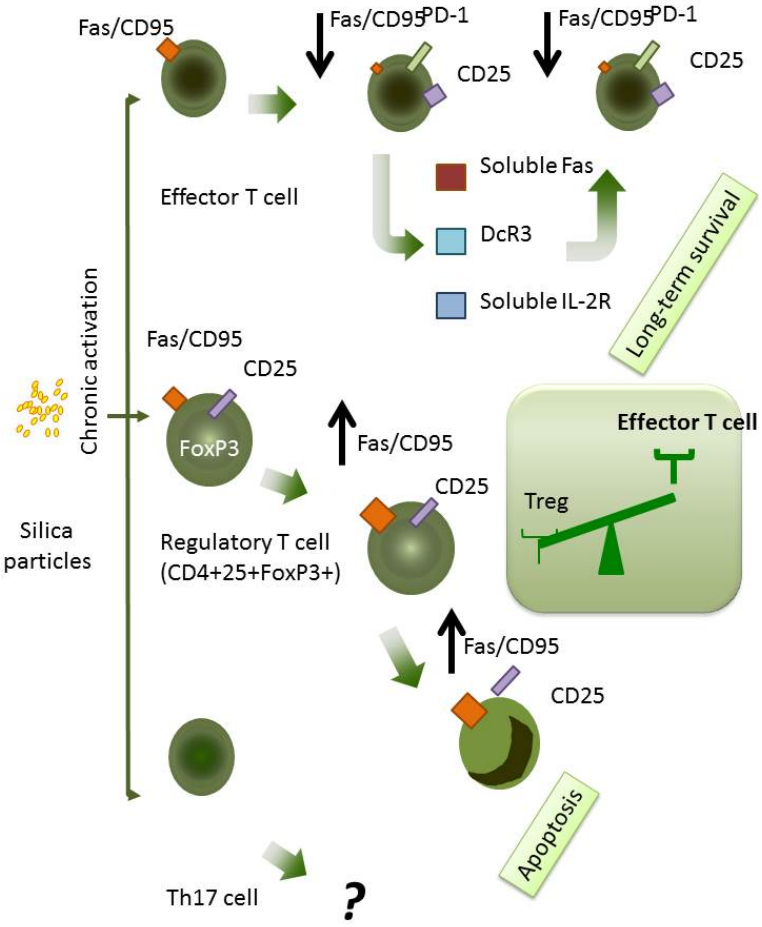

Figure 1. Schematic summary of the chronic activation of effector T cells, regulatory T cells (Treg; CD4+25+FoxP3+), and Th17 caused by exposure to silica particles. The chronic activation of effector T cells caused these cells to express activation surface markers such as CD25 and PD-1 with a decrease in the expression of the Fas/CD95 molecule. Instead of a reduction in membrane Fas, these cells produced soluble Fas, an alternative spliced form of wild-type Fas, and DcR3 as well as the soluble IL-2 receptor (sIL-2R). sFas and DcR3 prevented fas-ligand/Trail-induced apoptosis, leading to longer survival. sIL-2R and DcR3 as well as sFas were markers for the chronic activation of effector T cells. These might be explained by autoantigen-recognizing cells contaminating the long-term surviving cells. Treg enhanced expression of the surface Fas receptor and become prone to Fas-mediated apoptosis as a result of chronic activation. Treg may die quickly in silicosis patients (even when repeatedly recruited from the bone marrow). The overall balance between Treg and reactive T cells moves toward decreased Treg, resulting in the subsequent aberrant regulation of autoimmunity.

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Similar to sIL-2R, DcR3 has been identified as a chronic activation marker for the human immune response [52]. DcR3 was initially discovered in malignant cells such as lung and colon cancers [53], and its role was considered to be that of a protective molecule binding with the TNF-related apoptosis-inducing ligand (TRAIL) or the Fas ligand secreted from tumor-attacking T cells [54]. These functions are similar to the soluble Fas molecule, which is an alternative splicing form of wild-type membrane Fas secreted from lymphocytes due to the absence of a transmembrane domain. Soluble Fas has also been shown to bind to the Fas ligand in extracellular areas, which prevented Fas ligand-inducing and Fas–mediated apoptosis in T cells [55-58].

Elevated DcR3 levels were recently reported in the serum or pathological lesions of patients with various autoimmune diseases such as RA and SLE, and these findings indicated that the production of high levels of DcR3 may reflect chronic activation of the immune system [52,59-62], particularly antigen-recognizing T cells.

We previously demonstrated that the expression of DcR3 mRNA in PBMCs was higher in silicosis patients than in healthy donors [63]. Although the expression of DcR3 mRNA was only examined in whole PBMCs including lymphocytes and monocytes, taken together with recent findings showing elevated DcR3 levels in autoimmune diseases, these results suggest that examining serum DcR3 levels in silicosis patients is of importance. We have started this analysis and will report our findings in the near future.

sFas has been shown to have a similar role to that of lymphocytes. Although its molecular function is to prevent apoptosis, elevations in sFas levels have been reported in serum from patients with various autoimmune diseases [62-64] as well as silicosis patients [67]. Using PBMCs, the sFas and membrane (wild-type) Fas message expression ratio was also shown to be higher in silicosis patients than in healthy donors [68]. Our findings also revealed that peripheral T cells, which produce lower levels of surface membrane Fas, were the producers/expressers of sFas, whereas normal (relatively higher) surface membrane Fas-expressing T cells produced lower levels of sFas [25]. Fas-mediated apoptosis may proceed more easily in the latter fraction due to various stimuli by self-or foreign antigens or the anti-Fas autoantibody, which we discovered in the serum of silicosis patients, and repeated recruitment from the bone marrow. Peripheral T cells derived from silicosis patients were shown to be the dominant sFas producer with a smaller fraction of apoptosis-prone T cells than that from healthy donors [25-27]. The sFas-producing fraction may survive longer and retain a chronically activated status. Thus, this fraction may be stimulated and respond to autoantigens.

Similar to DcR3, the higher expression and production of sFas suggest that the peripheral blood of silicosis patients frequently includes a chronically activated and self-antigen recognizing T cell fraction.

These findings are summarized in Figure 2.

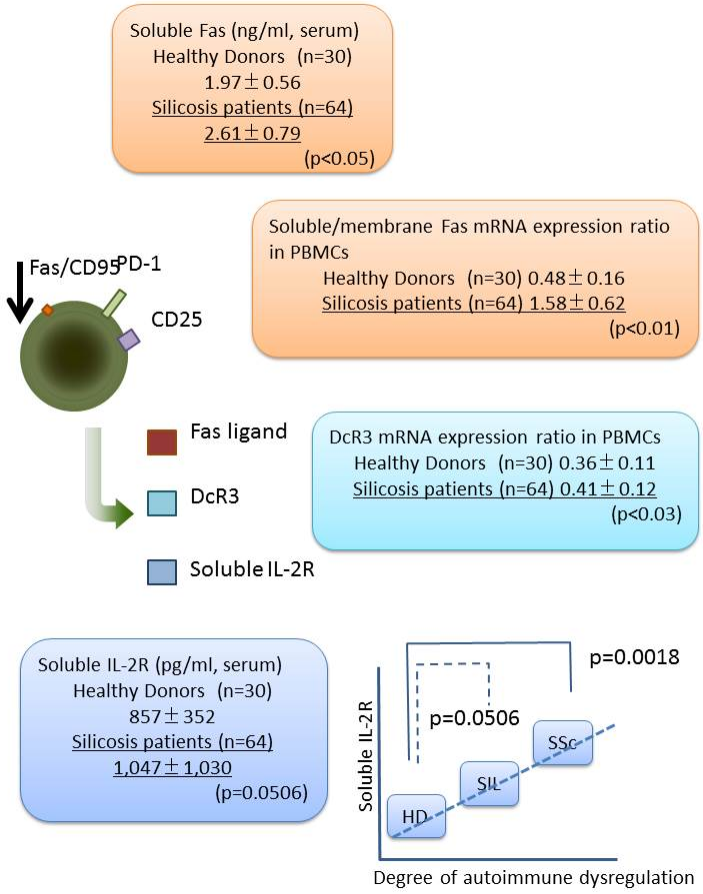

Figure 2. Evidence of chronically-activated effector T cells in silicosis patients. Serum soluble Fas, the soluble/membrane Fas mRNA expression ratio in PBMCs, and DcR3 expression and serum sIL-2R levels were higher in silicosis patients than in healthy donors. In addition, immunological abnormality levels in silicosis patients were determined to be 1, 2, and 3 (normal individuals and silicosis and SSc patients) as continuous variables between healthy donors and SSc patients. Then, the serum sIL-2R levels were positively correlated with these immunological scores. All of these findings indicate that effector T cells chronically and recurrently exposed become activated and their cellular features proceed to autoimmune dysregulation.

5. Immune stimulation of regulatory T cells by silica particle

From the beginning of Treg analysis in silicosis patients, the CD4+CD25+ fraction from PBMCs derived from these patients was shown to be less functional than that from healthy donors [28].

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However, FoxP3-positive cells cannot be used in the experiment, since the use of the collected cells in the subsequent cell biological experiment precludes the permeabilization of the cell membrane because of the staining of nuclear molecules, such as FoxP3. Thus, it is unknown whether the CD4+25+ fraction used in the experiment was pure Treg or a mixture of chronically-activated reactive T cells. In other words, it is unknown whether the reduced functions of the Treg fraction with peripheral CD4+25+ in the silicosis patients was caused by the impurity of the Treg cells or the contamination of chronically-activated CD4+25+reactive T cells.

Therefore, we examined the expression of surface Fas in peripheral CD4+FoxP+ T cells derived from both silicosis patients and healthy donors [41], as shown in Figure 3. The results obtained revealed that the expression of Fas was higher in Treg from silicosis patients than in those from healthy donors. Since when Treg is stimulated, Fas expression was shown to be one of the markers for activated Treg; therefore, Treg may be a self-limited inhibitor for the immune response [69,70] and should be terminated by activation-induced cell death. Taken together, these results indicate that exposure to silica may activate Treg as well as effector T cells and induce the higher production of Fas by Treg.

PBMCs from healthy donors and silicosis patients were incubated with silica particles for four days and the percentage of CD4+FoxP3+ cells was then measured [41]. As shown in Figure 3 and reported previously, the frequency of apoptosis-induced Treg cell death during cultivation with silica particles was higher in PBMCs from silicosis patients than in that from healthy donors.

These results demonstrated that silica activated Treg, which then produced higher levels of surface Fas. Apoptosis then occurred in activated Treg. This cascaded reaction can continue for a long time in silicosis because of recurrent encounters between silica particles and T cells. The early loss of Treg may cause T cell recruitment. However, the overall balance between long-surviving reactive T and Treg cells will move toward predominance of reactive T cells [41].

5. Current issues in immune stimulation by silica

The mechanism by which silica affects Th17 cells has not yet been established. Th17 cells are considered to play an important role in the autoimmune reaction and increases in the Th17 cell population or typical cytokines produced by Th17 cells, including IL-17A, may be related to autoimmune reactions [71,72]. However, the microenvironment surrounding the development of T lymphocytes, defined by cytokine profiles such as IL-6 and TGF-β, may affect the developmental direction of both Treg and Th17 cells. Therefore, importance needs to be placed on investigations of how silica particles cause changes in the cellular and molecular characteristics of Th17 cells, and what the link is between these alterations and autoimmune dysregulation in silicosis patients.

6. Immunological effects of asbestos, a mineral silicate

Asbestos is a mineral silicate, in which the chemical core atom is Si and O, and various metals such as iron, magnesium or calcium have been shown to bind to asbestos to chemically form asbestos fibers [73]. However, the physical properties of silica and asbestos are different. The former is particulate matter while the latter is a fibrous mineral. Although silica has been shown to have an effect on the human immune system, as mentioned above, asbestos fibers may also have immunological effects on human lymphocytes, which may alter human anti-tumor immunity because the most important clinical complication in patients exposed to asbestos is malignant tumors.

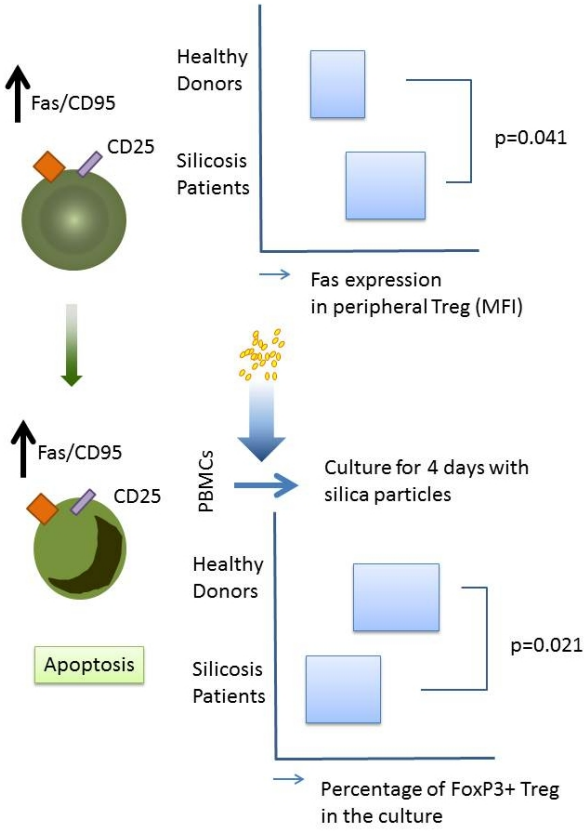

Figure 3. Surface Fas expression in peripheral CD4+FoxP+ T cells (Treg) derived from both silicosis patients and healthy donors. The expression of Fas was higher in the Treg of silicosis patients than in those of healthy donors. PBMCs from healthy donors and silicosis patients were incubated with silica particles for four days and the percentage of CD4+FoxP3+ cells was then measured. The frequency of apoptosis-induced Treg cell death during cultivation with silica particles was higher in PBMCs from silicosis patients than in that from healthy donors.

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Although reports of autoimmune diseases in asbestos-exposed patients are rare [74,75], the main complication noted in these patients is malignancies such as mesothelioma and lung cancer [76,77]. In addition, the incidence of cancer in the larynx, GI-tract, and bladder was shown to be high in asbestos-exposed patients [76,77].

We previously examined the immunological effects of asbestos [78,79], and demonstrated that temporary and relatively high-dose exposure to asbestos caused apoptosis in T cells as well as alveolar epithelial cells and mesothelial cells, whereas low-dose and continuous exposure to asbestos caused the acquisition of resistance to asbestos-caused apoptosis in T cells with the activation of Scr-family kinase, IL-10, l STAT3, and Bcl-2 [80]. The expression of CXCR3, one of the chemokine receptors related to the anti-tumor immunity, as well as IFNγ was also reduced in these cells [81,82]. Asbestos exposure also induced the impaired differentiation and proliferation of CD8+ cytotoxic T cells [83], and the reduced expression of NKp46 activating receptor in NK cells [84,85]. Taken together, these findings indicate that asbestos causes a reduction in anti-tumor immunity.

7. Conclusions

Even if core chemical substances, Si and O_2, are identical, the immunological effects of silica seem to be completely opposite to those of asbestos. Silica is a chronic stimulator of T cells, with chronic exposure leading to autoimmune dysregulation due to the chronic activation of responder T cells as well as Treg, resulting in an imbalance in the regulation of T cell responses. On the other hand, asbestos reduces anti-tumor immunity. Therefore, asbestos is not a stimulator, but can alter the function of immune cells.

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