Alteration of Immune Cells in Silicosis: Roles in Development of Autoimmunity and Lung Fibrosis

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Abstract

In addition to lung fibrosis, silicosis (SIL) patients often suffer from complicated autoimmune disorders such as rheumatoid arthritis, systemic sclerosis and anti-neutrophil cytoplasmic antigen-related vasculitis/nephritis. Thus, chronic and recurrent exposure to silica particles located in the lung and lymph nodes can result in alterations in the function of immune cells, which can lead to the dysregulation of autoimmunity in addition to the development of lung fibrosis. Regarding B cells which produce various antibodies, in SIL many autoantibodies are often detected in autoimmune diseases, and specifically autoantibodies against apoptosis-related molecules. Responder T helper (rTh) cells which respond to foreign and auto-antigens have been reported to survive longer and have apoptosis inhibited. Additionally, regulatory T (Treg) cells seem to proceed to early apoptosis. This imbalance between rTh and Treg cells may make SIL patients prone to autoimmune disorders. Although the role of dendritic cells (DCs) including alveolar macrophages and T helper 17 (Th17) cells in the dysregulation of immune tolerance in SIL remains poorly understood, these cells play a role in pulmonary inflammation and the development of fibrosis via specific receptor and signaling molecules. Further studies are required to delineate the roles of DCs and Th17 cells in the disturbance of autoimmunity found in SIL, and investigation of the immunological alterations that lead to autoimmune dysregulation may assist in the recognition, prevention, and treatment of complicated autoimmune diseases found in SIL.

ABBREVIATIONS

SIL: Silicosis; DC: Dendritic Cell; AM: Alveolar Macrophage; RA: Rheumatoid Arthritis; SLE: Systemic Lupus Erythematosus; Ssc: Systemic Sclerosis; ANCA: Anti-Neutrophil Cytoplasmic Antibody; rTh: Responder T Helper Cell; Treg: Regulatory T Cell; CD: Cluster Of Differentiation; Foxp3: Forkhead Box P3; Th17: T Helper 17 Cell; Ig: Immunoglobulin; ANA: Anti-Nucleus Antibody; HV: Healthy Volunteer; APC: Antigen-Presenting Cell; PBMC: Peripheral Blood Mononuclear Cell; PD-1: Programmed Cell Death-1; Sil-2R: Soluble IL-2 Receptor; Sfas: Soluble Fas; PR: Profusion Rate; FEV1.0: Percentage of Forced Expiratory Volume In 1 Second; FVC: Forced Vital Capacity; V25/H: PFR At 25% FVC/Height V25/H; %VC: Percent Volume Capacity; Dec3: Decoy Receptor 3; NALP: NACHT, LRR and PYD Domains-Containing Protein; MARCO: Macrophage Receptor With Collagenous Structure; ROS: Reactive Oxygen Species; TNFRSF9: Tumor Necrosis Factor Receptor Superfamily Member 9; Myd88: Myeloid Differentiation Primary Response Gene 88; Nfkb: Nuclear Factor-Kb

INTRODUCTION

Silicosis (SIL) represents a typical form of pneumoconiosis and is caused by chronic and recurrent occupational exposure to silica particles [1-3]. Inhaled silica particles are recognized as foreign bodies and a danger signal by dendritic cells (DCs) and alveolar macrophages (AMs) in the pulmonary region, which leads to chronic inflammation and the development of fibrosis by various functional alterations in DCs as well as other immune cells such as T helper cells [4-6]. Although clinical subtypes of SIL are divided as acute, subacute and chronic, simple SIL is characterized by the presence of small nodules found in the upper and middle lobes of the lung [1-3]. These nodules sometimes develop to more than 1 cm in diameter. The inhaled silica particles remain in the lung and related lymph nodes. Thus, circulating immune cells may recurrently encounter these particles and are caused various alterations.

Pulmonary complications such as lung tuberculosis, chronic bronchitis and airflow limitation, non-tuberculosis mycobacterium infection, fungal lung infection, compensatory emphysema, pneumothorax and lung cancer are well known [7-10]. As a result of these complications, SIL patients suffer from shortness of breath, coughs, fever, and cyanosis. In addition to these pulmonary complications, SIL often reveals various symptoms of autoimmune diseases [4,11,12]. Complicated...
rheumatoid arthritis (RA) is well-known and referred to as Caplan’s syndrome [13-15]. Many epidemiological studies have been reported regarding complicated autoimmune diseases found in SIL, such as systemic lupus erythematosus (SLE) [16-18], systemic sclerosis (SSc) [19-21], and anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitis/nephritis [23-25]. Although the causative mechanism responsible for the silica-induced dysregulation of autoimmunity was thought to involve an adjuvant effect of silica particles to present relatively small molecules as self-antigens for antigen-presenting cells (APCs) [26,27], recent investigations have revealed direct effects of silica particles on immune cells such as CD helper T cells and regulatory T (Treg) cells, defined as CD (cluster of differentiation) 4 and CD25 positive with forkhead box P3 (FoxP3; a transcription factor) positive [28-30].

In this review, the pathophysiological alteration of the immune status found in SIL and causative mechanisms involved in the development of disorders of autoimmunity are summarized, as well as the relations of immune cells such as DC and T helper 17 (Th17) cells in the development of pulmonary inflammations and fibrosis.

**B CELLS**

B cells produce various antibodies (immunoglobulins: Ig). Although the direct cellular and molecular alterations of B cells caused by direct exposure to silica particles remain to be delineated, it is supposed that the detection of various autoantibodies present in SIL is an important clue when considering alterations of humoral immunity in SIL.

Many epidemiological studies have identified the presence of general and specific autoantibodies such as anti-nuclear antibody (ANA), anti-Scl-70 (antibody for topoisomerase I) and anti-CENP-B (antibody for centromere protein B) antibodies specific for SSc and ANCA in SIL [31-33].

Regarding autoantibodies for SSc, anti-Scl-70 was detected in Japanese SIL patients who did not reveal any symptoms of autoimmune diseases with higher frequencies compared with healthy volunteers (HV) [34-36]. Regarding anti-Scl-70 autoantibody specificity, the allelic frequency of HLA-DQB1*0402 was investigated and results showed a significantly higher frequency in anti-Scl-70-positive SIL (28.6%) than in anti-Scl-70-negative SIL or HV. Additionally, HLA-DQB1*0301, DQB1*0601 and DPB1*1801 alleles were more frequently detected in positive SIL than in SIL without anti-Scl-70 or in HV (although no significant difference was observed) [34-36]. In addition to anti-Scl-70 antibody, anti-CENP-B autoantibody is also recognized as specific to SSc. Although anti-CENP-B is clinically more common in the limited form than in the diffuse form, and anti-Scl70 is more common in the diffuse form [37,38], the detection of these antibodies in SIL is important. Investigation of the relationship between anti-CENP-B titers and other immunological parameters such as cytokines, apoptosis-related molecules, immunoglobulins, and molecules related to the T cell activation may provide a better understanding of immunological modifications in SIL.

For example, the titer index (Log10) of anti-CENP-B autoantibody in SIL was higher than that in HV from the Japanese population, and that of SSc was higher than those of HV and SIL patients [39]. This titer index was positively correlated with an assumed immune status of 1 for HV, 2 for SIL, and 3 for SSc. The correlation study of the titer indices of anti-CENP-B and Scl-70 autoantibodies showed a significant positive correlation. Although the titer index of the anti-CENP-B autoantibody showed no significant correlation with other parameters examined, the titer index of the anti-Scl-70 autoantibody showed a significant positive correlation with the ANA titer index and serum IgA. Additionally, although factor analysis revealed that the titer index of the anti-CENP-B autoantibody formed the same factor with the anti-Scl-70 autoantibody, IgG value and age in SIL cases, another extracted factor indicated that the IgA value and anti-Scl-70 antibody were positively related, although anti-CENP-B showed the opposite pattern in the results from the factor analysis. These findings indicated that the titer index of anti-CENP-B autoantibody may be a biomarker of dysregulation in SIL cases [39].

ANCA positivity in SIL has been reported in many case reports and epidemiological studies [23-25]. Although the pathophysiological role of ANCA positivity has not been extensively investigated, monitoring the high frequency of ANCA-positive SIL may be important in SIL [23-25].

Other particular auto-antibodies have been reported in Japanese cases of SIL. The anti-desmoglein autoantibody was found and this antibody is specific to pemphigus, an autoimmune bullous disease. Thirteen-percent of SIL cases were found to be positive by immunofluorescence. Additionally, use of ELISA showed that 11% of cases were positive against the desmoglein 1 antigen, two SIL cases against the desmoglein 3 antigen, and two SIL cases against both desmoglein 1 and desmoglein 3 [40]. This report indicated that dermatological follow-up may be important in the treatment of SIL.

Regarding molecules related to cell apoptosis, anti-Fas and anti-caspase-8 auto antibodies were found in SIL [41-43]. Fas, known as CD95, is a key molecule involved in cellular apoptosis, especially in immune cells. Approximately one-fourth of Japanese SIL cases were positive for anti-Fas. Interestingly, this anti-Fas was functional, in that it could induce cellular apoptosis. The sera of the highest titer of anti-Fas in SIL caused cellular growth inhibition due to apoptosis in the Fas-presenting human myeloma cell line KMS-12-BM derived from pleural effusion of a Japanese myeloma patient. However, it did not induce growth inhibition in the KMS-12-BM cell line, a sister cell line of KMS-12-PE, derived from bone marrow myeloma cells obtained from the same patient, but did show the presence of scant Fas molecules on the cell surface [41,44]. This investigation suggested that certain immune cells prone to Fas-mediated apoptosis readily proceed to apoptosis and that the cellular function of these cells may be lost in anti-Fas-positive SIL. This may be related to the dysregulation of autoimmunity in SIL.

The anti-caspase-8 autoantibody is also of interest since caspase-8 is closely related to Fas-mediated apoptosis [42,43]. Although functional analyses were not performed with this
autoantibody, it was detectable in SIL, SSc and SLE patients [42,43]. Using epitope mapping and employing 12 amino acid polypeptides with the SPOTs system, a minimum of four epitopes and a maximum of 13 were found, which implied that epitope spreading was in progress. The study showed that the unique catalytic cysteine residues were included within the epitopes, comprising active-site cysteine Cys287, and Cys360 located in the penta-peptide motif QACQG. These analyses may provide a better clue to the relationship between altered cellular and molecular mechanisms of apoptosis in lymphocytes and irregular immune tolerances in SIL [42,43].

Taken together, B cells in SIL produce various auto-antibodies that may result from cellular signals derived from rTh as shown in Figure (1), DC and APC cells, in addition to certain alterations of antigen recognition in B cells.

RESPONDER T HELPER (RTH) CELLS

Silica particles chronically activate rTh and Treg cells. Regarding rTh cells, CD69 expression, the earliest marker for T cell activation, was induced under in vitro culture conditions when peripheral blood mononuclear cells (PBMCs) were cultured with silica particles [45]. Additionally, the molecule programmed cell death-1 (PD-1) was highly expressed in CD4+ T cells derived from SIL patients, but not from HV [46]. Since these surface markers are considered to be T cell activation markers, silica exposure caused chronic activation of circulating rTh cells in SIL.

Moreover, soluble IL-2 receptor (sIL-2R) in the serum of SIL patients tended to be higher than in HV, and increased gradually in the order HV to SIL to SSc [47]. A correlation assay, multiple regression stepwise and factor analyses using sIL-2R levels and various immunological parameters including percentage of CD4+CD25+ and CD8+CD25+ cells in PBMCs, serum IgG, serum soluble Fas (sFas), serum IL-2, titer of ANA, titers of anti-Scl-70 and CENP-B autoantibodies as well as respiratory and exposure parameters including age, exposure years, profusion rate (PR; according to the ILO pneumoconiosis radiological classification, 2011 revised guidelines, 1 to 4), subjective dyspnea (numbered 1 according to the ILO pneumoconiosis radiological classification, 1 to 4), respiratory parameters, and exposure parameters including age, exposure years, profusion rate (PR; according to the ILO pneumoconiosis radiological classification, 2011 revised guidelines, 1 to 4), subjective dyspnea (numbered 1 according to the ILO pneumoconiosis radiological classification, 1 to 4), peak flow rate (PFR) at 25% forced vital capacity (FVC)/Height (v25/H), and percent volume capacity (%VC) were all performed. As a result, it was found that the level of sIL-2R was not related to respiratory parameters, and was positively correlated with ANA, anti-Scl-70 and anti-CENP-B titers. The stepwise regression test showed that sIL-2R was significantly regulated by IgG and tended to be regulated by anti-CENP-B. Moreover, from the factor analysis, sIL-2R was extracted with various immunological parameters including IgG, sFas, CD4+CD25+ cell percentage, and anti-CENP-B. Taken together, sIL-2R levels in SIL indicated immunological disturbance without any manifestations of autoimmune symptoms, and suggested the presence of chronic activated T cells in SIL [47].

Although foreign- and/or self-antigen-stimulated T cells proceed to apoptosis in a process referred to as activation-induced cell death, this is usually mediated by Fas/CD95. sFas is secreted from cells and binds with Fas-ligand at the extracellular area which interferes with the binding between Fas ligand and Fas located on the cell surface [48]. In fact, serum levels of sFas were higher in SIL patients compared with HV, and a similar case was found with SLE [48]. Additionally, mRNA analysis showed that expression of the sFas transcript was higher in PBMCs derived from SIL patients compared with HV [49]. In addition to the sFas transcript, higher levels of other Fas alternatively spliced variants, which have lost the transmembrane domain but maintain the Fas ligand binding domain similar to sFas and were assumed to act in a similar way as sFas in preventing Fas-mediated apoptosis in T cells, were detected in PBMCs derived from SIL patients compared with HV [50].

Moreover, decoy receptor 3 (DcR3) is known to possess functionality that resembles that of sFas with respect to Trail-induced apoptosis. As reported, DcR3 mRNA expression in PBMCs derived from SIL patients was higher compared with HV [51]. Taken together, rTh cells in SIL are supposed to be activated chronically and survive for longer periods by the inhibition of Fas-mediated apoptosis as shown in Figure (2). These cell populations would include self-antigen recognizing clones.

REGULATORY T (TREG) CELLS

Treg cells are well-known as the regulator subpopulation of T helper cells, and enable rTh cells to cease stimulation-induced proliferation physiologically. The decrease and/or reduced function of Treg cells may induce autoimmune diseases or allergic conditions since rTh cells stimulated by self or foreign antibodies are not quite activated. In contrast, increased and/or enhanced Treg cell activity may result in cancer development and proliferation since T cells attacking cancer cells may be over-suppressed by Treg cells [52-54].

From this viewpoint, the Treg, CD4+CD25+ cell fraction, in
the peripheral blood of SIL patients was examined with respect to Treg function and compared with HV [55]. As a result, this fraction from SIL patients showed reduced Treg function compared with HV [55]. Consequently, it had to be considered that rTh cells obtained from SIL patients are chronically activated by exposure to silica particles as described above and that activated rTh cells possess the CD25 molecule, unlike the case with resting T cells. Taken together, the CD4+CD25 fraction in cases of SIL included true Treg cells and chronically activated rTh cells, and as a result Treg function was reduced in this fraction [46]. In addition to these findings, when Treg cells are chronically activated, Fas is over-expressed on the surface, which facilitates earlier apoptosis [46]. In fact, expression of cell surface Fas was higher in peripheral blood Treg cells derived from SIL patients compared with HV. Furthermore, when PBMCs from HV were cultured with silica particles, Treg cell expression of CD4, CD25 and intranuclear FoxP3 gradually decreased, although the CD25-expressing cell percentage remained unchanged. These results indicated that the presence of silica particles lead to Treg cell apoptosis by Fas-mediated apoptosis, and also result in the activation of rTh cells [46].

These findings regarding rTh and Treg cells suggested that the balance between rTh and Treg cells in SIL is disturbed, where levels of the former increase as shown in Figure (3), and the latter decrease. This imbalance may provide the framework for the onset of autoimmune disorders.

**DENDRITIC CELLS (DCS) IN THE PULMONARY REGION**

DCs, alveolar macrophages (AMs), are the first responders following the inhalation of foreign substances including silica particles. Once DCs recognize foreign bodies, NACHT, LRR and PYD domains-containing protein (NALP)-3 inflammasome is activated to produce IL-1β due to lysosomal damage and cathepsin B [56,57]. Although the roles of DCs with respect to forthcoming disorders of autoimmunity in SIL are not well understood, the alterations in DCs related to the development of lung inflammation have been documented in detail. One of the key molecules involved in the development of silica-induced pulmonary inflammation is the macrophage receptor with collagenous structure (MARCO), which is the predominant scavenger receptor for the recognition and binding of silica particles [58-61]. The role of MARCO seemed to prevent silica-induced inflammation [58-61]. Mice null for MARCO showed

**Figure 2** The effects of silica particles on human responder T cells. Responder T cells exposed chronically and recurrently to silica particles are chronically activated and expressed PD-1 and CD69, and produce soluble IL-2R as activation markers. In addition, various molecules such as soluble Fas, DcR3, and other Fas-alternatively spliced variants are produced from responder T cells to inhibit Fas-mediated apoptosis. Therefore, these responder T cells including self-recognizing clones survive longer with chronically activated status, subsequently cause autoimmune diseases.

**Figure 3** In addition with effects of silica particles on responder T cells shown in Figure 2, these particles modify and chronically activate the regulatory T cells. As the results, regulatory T cells express excess Fas molecule resulting early apoptotic loss. Therefore, the imbalance of responder and regulatory T cells occur to trigger to be prone to autoimmune diseases.
a greater degree of lung inflammation, increased activation of inflammasome as revealed by the release of IL-1β, increased release of cathepsin B, and excessive activation of caspase-1. Additionally, silica is capable of inducing the production of reactive oxygen species (ROS) on the particle surface as well as cells responding to silica such as AMs. These cellular alterations are causative for pulmonary inflammation. Moreover, activated AMs proceed to apoptosis, and thereafter phagocytized proteins and nucleotides may be released [58-61]. These proteins and nucleotides may be recognized as auto-antigens by rTh cells. Of course, rTh cells are also stimulated by activated AMs as well as silica particles as described above. Taken together, AM activation may also function as one of the triggers involved in the dysregulation of autoimmunity.

TH17 CELLS

The Th17 subpopulation of helper T cells is known to be important in the development of autoimmune disorders. Both the decrease in Treg cells resulting from the suppression of its inhibitory function and enhancement of the effectiveness of Th17 cells and/or increase in Th17 cell numbers are considered to make the human body prone to autoimmune disorders. Additionally, the polarization of Treg or Th17 cells is regulated by immunological circumstances surrounding T helper cells. For example, Treg cells mainly differentiate via transforming growth factor (TGF)-β, however, Th17 cells can be promoted by TGF-β and IL-6 with IL-23 as the expansion and survival factor [63-66]. Thus, if the cytokine-based circumstances change, the balance of Treg and Th17 cells is also modified and the human body may become prone to autoimmune disorders. With this in mind, the direct effects of silica particles on Th17 cell polarization and Treg cell differentiation should be investigated for a better understanding of silica-induced dysregulation of autoimmunity.

Several reports have detailed the role of Th17 cells in silica-induced lung fibrosis. These reports have indicated that IL-1β and IL-17 enhance fibrosis [67,68]. Additionally, 4-1BB/CD137 (tumor necrosis factor receptor superfamily member 9; TNFRSF9), which is induced by lymphocyte activation and plays a role as an immune checkpoint molecule in a co-stimulatory manner, also stimulated the development of silica-induced lung fibrosis [69]. In contrast, myeloid differentiation primary response gene 88 (MyD88), which is an adaptor protein and plays an important role in the signals of toll-like receptor (TLR), nucleotide-binding oligomerization domain receptor (NLR) and IL-1R superfamily to the downstream activation of nuclear factor-kB (NFkB), blocked fibrosis formation [70]. The Wnt signaling pathway and β-catenin also inhibited the progression of fibrosis. These findings were derived from animal models in addition to the blocking of certain molecules by antibodies, and the use of specific inhibitors or comparisons between knock-out and wild-type mice [71]. Thus, future investigations will need to focus on the relations of these key molecules in terms of their involvement in human SIL. The roles of DCs and Th17 in forming lung fibrosis are show in Figure (4).

CONCLUSION

Figure (1) shows the summarized findings described in this review. All of the alterations that lead to the development of lung fibrosis as well as autoimmune diseases in SIL are also shown. Experimental results obtained by examining the effects of exposing various immune cells to silica particles have indicated that silica-exposed patients possess altered immune functions and are prone to lung fibrosis and autoimmune disorders. These findings may be utilized as clinical markers for follow-up SIL in terms of immunological modifications. It seems that not all SIL patients develop pulmonary pathologies and immunological abnormalities. Approximately one-fifth to one-fourth of SIL patients might reveal a better immunological status with the development of pulmonary fibrosis [72]. The opposite pattern, comprising very slow progression of lung fibrosis with accelerated immunological deterioration, was also reported for a similar subpopulation [72].

Taken together, in addition to considering the management of SIL patients, these findings may assist in our understanding of the pathophysiological changes involved in various general autoimmune diseases.

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