Chapter 8

Autoantibodies in Silicosis Patients: Silica-Induced Dysregulation of Autoimmunity

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http://dx.doi.org/10.5772/intechopen.72999

Abstract

Silica particles cause silicosis (SIL) and represent one of the most typical environmental and occupational substances that induce autoimmune disorders among the exposed population. Anti-nuclear antibody (ANA), anti-Sjögren's-syndrome-related antigen A (SS-A), anti-centromere protein B (CENP)-B, and anti-scleroderma (Scl)-70 autoantibodies were examined in SIL and compared with those in healthy volunteers (HV) and patients with systemic sclerosis (SSc). Individuals with SIL were prone to autoimmune diseases and some autoantibodies seemed to be important as an estimation of this condition. Anti-Fas autoantibody found in SIL was functionally capable of inducing apoptosis in Fas-expressing cells, and this may cause a decrease of regulatory T cells (Tregs) expressing Fas in SIL. Moreover, responder T cells (Tresps) in SIL seemed to be activated chronically and protected from Fas-mediated apoptosis. Thus, an imbalance of Tresps (dominant) and Tregs (less) occurred in SIL. All of these causes of SIL are ready to further develop autoimmune diseases.

Keywords: silicosis, anti-CENP-B autoantibody, anti-Fas autoantibody, apoptosis, regulatory T cell, responder T cell

1. Introduction

Many environmental and occupational substances such as vinyl chloride, epoxy resins, solvents, pesticides, paraffin/silicone and silica particles cause dysregulation of autoimmunity [1, 2]. Silica-exposed patients suffer from silicosis (SIL), a condition that is well known to complicate with various autoimmune diseases [3, 4]. Of course, silica exposure produces typical

pneumoconiosis [5, 6], which is defined as lung inflammation and fibrosis with scarring in the form of nodules in the middle to upper lungs. Although various clinical types such as acute, progressive and chronic SIL are distinguished depending on the exposed dosage of silica particles and duration, patients clinically exhibit dyspnea, fatigue, cough, chest pain and other pulmonary symptoms. There are several typical pulmonary complications such as pulmonary tuberculosis, tuberculous pleurisy, pneumothorax, bronchiectasis and lung cancer [7, 8].

In addition to these lung complications, it is well known that the condition of SIL patients is often complicated with autoimmune diseases. The classical disease is known as Caplan's syndrome, complicated with rheumatoid arthritis (RA) [9]. The initial description reported by Caplan involved 51 cases among coal miners. Thereafter, many epidemiological reports revealed high odds ratios for the occurrence of RA in SIL [10, 11]. Furthermore, other autoimmune diseases such as systemic sclerosis (SSc) [12, 13], systemic lupus erythematosus (SLE) [14, 15] and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis/nephritis [16, 17] have been reported in case reports and epidemiological investigations.

We have been studying the direct effects of silica particles on human lymphocytes, especially responder T (Tresp) and regulatory T (Treg) cells [18–20], as well as investigating autoantibodies found in SIL [21–28]. In this chapter, clinical evaluation, epitope search and functional assays of several autoantibodies found in SIL are described and mechanistic analyses of T cells exposed to silica particles are conducted.

2. Anti-CENP-B and Scl-70 autoantibodies

The clinical evaluation of anti-centromere protein B (CENP-B) and scleroderma (Scl)-70 autoantibodies in SIL patients was performed and reported [29].

Figure 1 shows the titers of anti-nuclear antibody (ANA), anti-Sjögren's-syndrome-related antigen A (SS-A) antibody (Ab), anti-CENP-B and anti-Scl-70 (also known as anti-topoisomerase I) Abs in healthy volunteers (HV), SIL and SSc. All subjects were Japanese. 19 HV [median age = 46.0 years old (y.o.); mean ± standard deviation (SD) = 44.8 ± 8.6 y.o.; male:female (M:F) = 8:11], 20 SIL [median age = 73.5 y.o.; mean ± SD = 74.9 ± 5.4; male:female (M:F) = 19:1] and 25 SSc [median age = 65.0 y.o.; mean ± SD = 62.3 ± 12.1; male:female (M:F) = 3:22] were included in the study. All SIL were brickyard workers in Bizen City, Okayama prefecture, Japan, and were diagnosed according to the ILO 2000 guideline for pneumoconiosis. They were clinically followed in Kusaka Hospital or Hinase Uragami Iin according to Japanese law regarding the medical care of pneumoconiosis patients. The amount of free silica inhaled by these patients was estimated as high as 40 to 60% as determined from the work environment. These individuals did not show any symptoms of autoimmune diseases such as sclerotic skin, Raynaud's phenomenon, facial erythema or arthralgia. The SSc patients were diagnosed and monitored by the Department of Dermatology, Kawasaki Medical School Hospital, Kurashiki, Japan [29].

As shown in **Figure 1A**, investigation of the titers of ANA in HV, SIL and SSc revealed that a few SIL cases showed a higher titer of ANA, but there was no statistical significance. Not surprisingly, most of the SSc cases showed significantly higher ANA (compared to HV and SIL). Interestingly,

titers of anti-Sjögren's-syndrome-related antigen A (SS-A) in SIL and SSc were higher than those of HV (**Figure 1B**). SS-A may be detected not only in Sjögren's syndrome, but also in other autoimmune diseases such as SSc and SLE. However, it may be interesting to note that SIL without any symptoms related to autoimmune diseases exhibited a higher titer for anti-SS-A Ab. Although clinical evaluation of anti-SS-A Ab in SIL has not been investigated, it is worth mentioning that SIL showed a pre-clinical status for autoimmune diseases as indicated by various epidemiological studies [9–17].

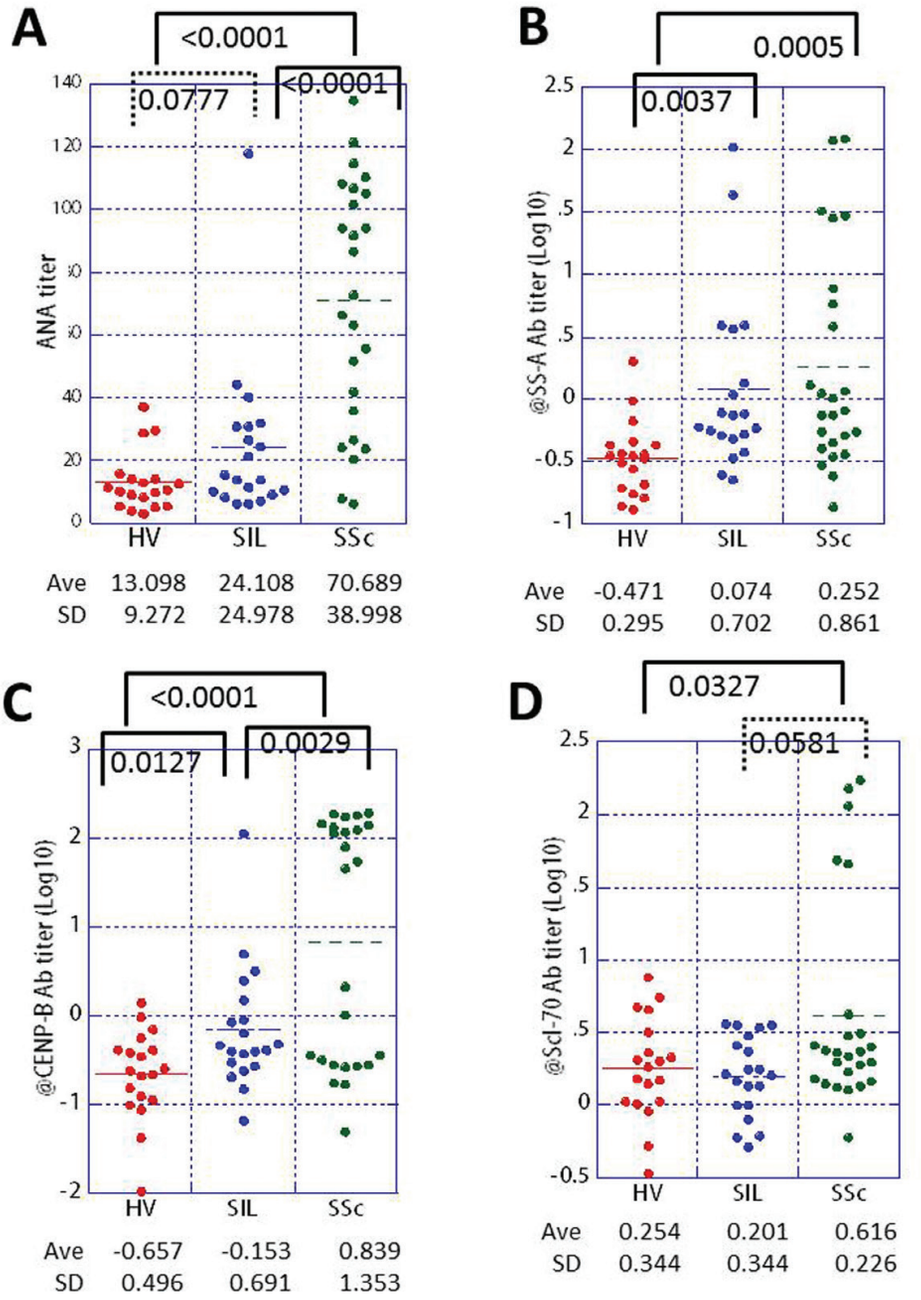

Figure 1. Comparison of titers for anti-nuclear antibody (ANA), anti-SS-A antibody (Ab), anti-CENP-B Ab and anti-Scl-70 Ab among healthy volunteers (HV), silicosis cases (SIL) and patients with systemic sclerosis (SSc). Except for ANA, titers are shown as logarithmic values. Statistical significance was examined using the student T-test and $p < 0.05$ was defined as significant. All titers were measured using a multiplex ELISA kit for ANA.

The evaluation of SSc showed that anti-CENP-B and anti-Scl-70 Abs were typical autoantibodies. Anti-CENP-B Ab is usually thought to be found in SSc cases with a type of localized skin lesion. On the other hand, SSc cases positive for anti-Scl-70 Ab are regarded as a generalized type with diffuse and extensive skin lesions [30–32]. Our results shown in **Figure 1C** (anti-CENP-B Ab) and **1D** (anti-Scl-70 Ab), for both Abs, demonstrated that there were clear breaks between positive and negative (close to levels of HV) cases in SSc. Regarding SIL, anti-CENP-B Ab was significantly higher than that in HV with the highest case whose titer was just as high as the positive case in SSc [29]. However, there was no case that showed higher anti-Scl-70 Ab in this series of SIL cases [29].

Thus, the clinical evaluation of anti-CENPN Ab in SIL was performed [29]. There was no correlation with other immunological or respiratory parameters in SIL such as titer of ANA, immunoglobulin (Ig) G, Ig A, Ig M, age, radiological classification of SIL (PR: profusion ratio), exposure years, percentage vital capacity (VC), forced expiratory volume 1.0 (SEC) (FEV1.0 (%)) or forced expiratory flow at 25% of vital capacity divided by body height (V25/H) except positive for anti-Scl-70 Ab titers, although anti-Scl-70 titers were similar to the those of HV. Factor analysis was performed using these immunological and respiratory clinical parameters [29]. As a result, anti-CENP-B Ab was found to contribute to the second and fourth factors. Factor 2 (17.7% contribution ratio) comprised the titer indices of anti-CENP-B and Scl-70 Abs, Ig G and age, all with positive values. This factor is understood as an immunological factor with aged patients showing a tendency for higher antibodies and Ig G. The fourth factor with a 13.2% contribution ratio was formed by the titer index of anti-CENP-B Ab with a negative value, the anti-Scl-70 autoantibody with a positive value, in addition to the Ig A level with a positive value. As found in the analyses of individual correlations, the titer index of anti-Scl-70 Ab and Ig A showed a positive correlation. This fourth factor indicated that even though the titer index of anti-Scl-70 autoantibody was located in the range of HV, among these titers, there is a correlation with Ig A and this tendency was the opposite of that observed for the titer index of anti-CENP-B auto-Ab. Thus, even with lower levels of titers, higher SIL cases with anti-CENP-B or anti-Scl-70 Ab differed as both Abs were divided in subtypes of SSc. Taken together, both Abs, especially anti-CENP-B (as well as anti-SS-A Ab), may indicate a pre-clinical status for forthcoming manifestations of autoimmune disease in SIL [29].

3. Autoantibodies against apoptosis-related molecules

Our previous reports indicated that autoantibodies against molecules related to apoptosis, Fas and caspase-8, were found in SIL [26–28]. These molecules may be expressed when cells in the body progressed to apoptosis in physiological as well as pathological situations.

Regarding anti-caspase-8 auto-Ab, although HV and cases comprised a different series from the aforementioned volunteers and cases, anti-caspase-8 auto-Ab was detected in 70% of HV, 62% of SIL, 90% of SSc and 60% of SLE cases, using four fragments of caspase-8 protein [26]. As a result, the positivity was not unique to autoimmune diseases and SIL. It was easily detected even in sera of HV. The report that revealed these positivities for anti-caspase-8 auto-Ab examined the epitope mapping. The epitopes were widely spread from the death effector domain to caspase regions and there was no specific epitope expressed in specific disease types such as SSc, SLE, SIL or HV [26].

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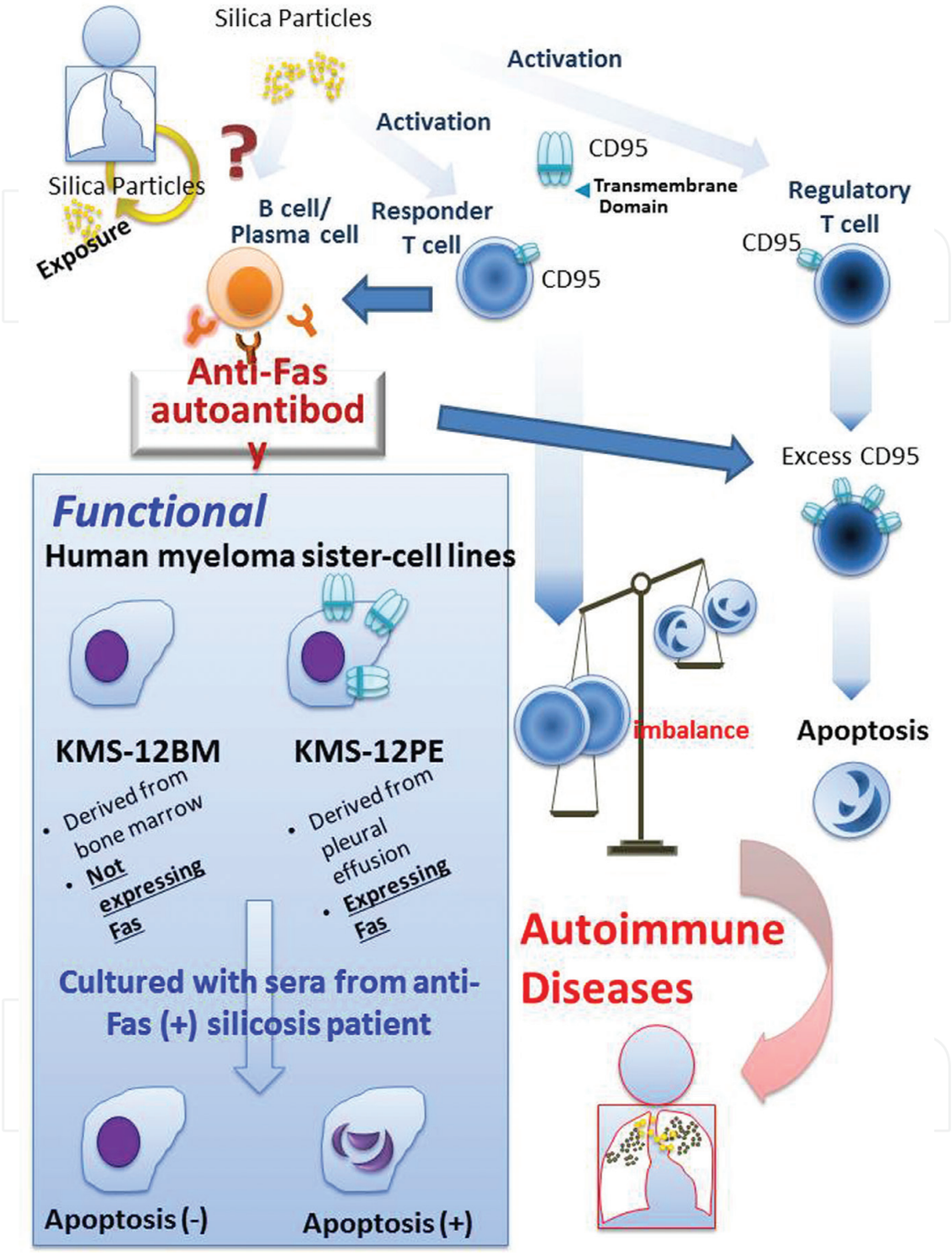

Figure 2. Anti-Fas auto-Ab was found in ca. 25% of SIL. The function of anti-Fas auto-Ab was examined using sister human myeloma cell lines, KMS-12PE and KMS-12BM. Only Fas-expressing KMS-12PE proceeded onto apoptosis when cultured with sera from SIL which revealed the highest titer for anti-Fas auto-Ab. Since anti-Fas auto-Ab seems to be functional, regulatory T cells (Tregs) in SIL with this auto-Ab may fall into apoptosis, given the higher expression of Fas in Tregs from SIL compared to responder T cells (Tresps) from SIL or Tregs from HV. As a result, an imbalance of Tresps (dominant) and Tregs (less) will occur.

The anti-Fas auto-Ab was also found in SIL cases [28]. This was detected as 23.1% in SIL, 53.3% in SLE and 46.7% in SSc, but not detected in HV. For the anti-Fas auto-Ab, epitope mapping was also performed and there was no special site, with epitopes being widely spread from

the cysteine-rich domain (CRD) in extracellular sites to the death domain in intracellular sites. However, in contrast to anti-caspase-8 auto-Ab (caspase-8 is an intracellular molecule), anti-Fas-auto-Ab can bind to the Fas molecule which is present on the cell surface and, if this auto-Ab is functional, cells presenting Fas/death receptor may be induced toward apoptosis. Thus, we examined whether anti-Fas auto-Ab is functional, whereby it can cause cell death and growth inhibition in Fas-expressing cells [28]. For this purpose, two myeloma cell lines established in our laboratory, called KMS-12PE and KMS-12BM, were employed which were sister cell lines derived from the same Japanese myeloma patients [33]. KMS-12PE was derived from an earlier stage of patients and from pleural effusion, while KMS-12BM was derived from the terminal stage and from bone marrow. Interestingly, Fas expression was higher in KMS-12PE, but very scant in KMS-12BM [28, 33]. Thus, we incubated both cell lines with sera from SIL which showed the highest titer for anti-Fas auto-Ab. As a result, KMS-12PE progressed to apoptosis, but 12BM did not [28]. From these analyses, anti-Fas auto-Ab functions to induce apoptosis against Fas-expressing cells. From our previous study [34], it was found that Tregs in SIL expressed higher levels of Fas molecules compared to Tregs derived from HV. Taken together, if SIL patients possessed anti-Fas auto-Ab in their serum, Tregs may easily proceed to apoptosis and be reduced [34]. The imbalance of Tregs and Tresps (dominant Tresps and less Tregs) is a typical situation that induces the occurrence of autoimmune disorders. Thus, functional anti-Fas auto-Ab is a key molecule involved in dysregulation of autoimmunity (**Figure 2**).

4. Other autoantibodies and silicosis associated with autoimmune diseases

Some reports have identified anti-desmoglein auto-Ab in SIL [21, 22]. Thus, SIL showed various auto-Abs against ANA, Scl-70, CENP-B, SS-A, Fas and caspase-8. How are these various auto-Abs manifested in SIL without any autoimmune symptoms? As mentioned above, Tregs may be reduced in SIL, especially SIL with anti-Fas auto-Ab. Therefore, what about Tresps? If the imbalance defined by dominant Tresps and less Tregs is important for the onset of autoimmune diseases, what kinds of alterations were found in Tresps derived from SIL?

We found that there were many T cell activation markers in SIL, such as higher soluble interleukin (IL)-2 receptor [34], higher program death (PD)-1 expression in Tresps (T helper (Th) 4 cells) as well as Tregs [35], and an *in vitro* assay showed that Tresps expressed CD69 as the earliest activation marker of T cells when peripheral blood mononuclear cells (PBMCs) were cultured with silica particles [36]. In addition to this evidence of chronic activation of Tresps, there were many inhibitors of Fas-mediated apoptosis present in SIL serum, for example, soluble Fas (sFas) [37] and Fas-alternatively spliced variants (lacking the transmembrane domain, but maintaining the Fas-ligand binding domain) [38]. Additionally, PBMCs from SIL showed higher decoy receptor 3 (DcR3; which acts similar to sFas binding with trail-apoptosis induced at the extracellular area) expression compared to PBMCs from HV [39] (**Figure 3**). Taken together, Tresps in SIL are stimulated and survive longer by inhibition of Fas-mediated apoptosis. These Tresps can encounter various self-antigens and force B cells to produce auto-Abs [18–20].

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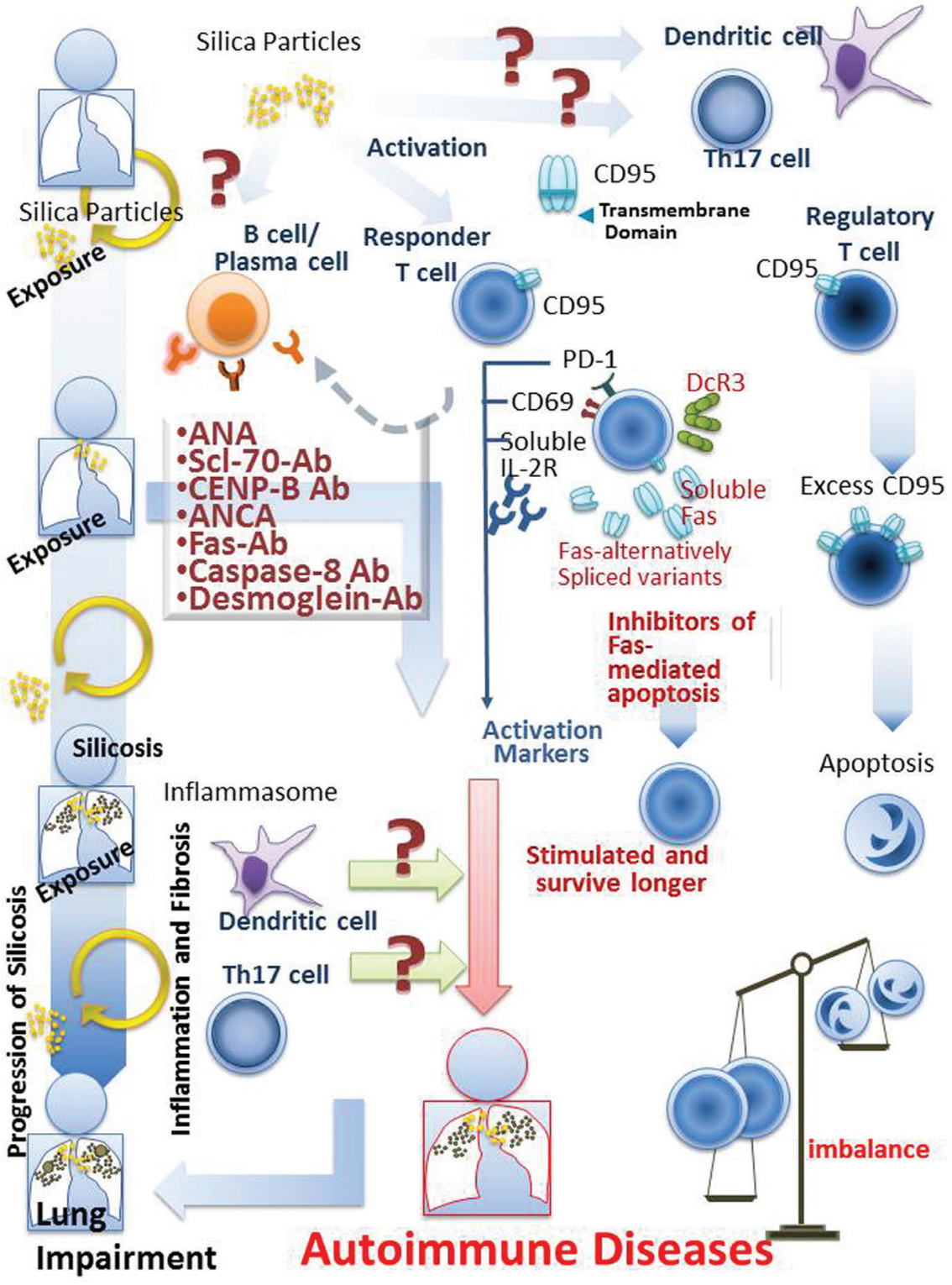

Figure 3. In addition to various auto-Abs found in SIL, and earlier apoptosis of Tregs in SIL, Tresps in SIL revealed a chronically activated status with CD69 and PD-1 expression as well as higher serum soluble IL-2 receptor. Additionally, Tresps in SIL inhibited Fas-mediated apoptosis by excess soluble Fas and similar molecules such as DcR3. Thus, Tresps in SIL survive longer and encounter various autoantigens. Moreover, the imbalance between Tresps and Tregs may be enhanced.

5. Conclusion

SIL is prone to autoimmune diseases. SIL patients were positive for various auto-Abs such as ANA, SS-A, CENP-B and Fas. Some auto-Abs possess certain clinical values that reflect pre-autoimmune status. These auto-Abs are produced from B cells/plasma cells that receive some commands to generate these Abs from T cells. In T cells in SIL, an imbalance exists between Tresps and Tregs. Both are chronically activated by long-term silica exposure. Thereafter, Tresps survive longer and Tregs proceed to apoptosis. However, the cytokine status in SIL needs to be examined and compared with HV as well as some autoimmune diseases, SSC, SLE or ANCA-related vasculitis. Additionally, the role and alteration of Th17 cells require investigation from the viewpoint of autoimmune diseases, since these are considered to be important in modifying autoimmune status and dendritic cells which initially recognize silica particles.

Acknowledgements

The authors appreciate the assistance of former colleagues from the Department of Hygiene, Kawasaki Medical School, Kurashiki, Japan, Professor Ayako Ueki, Drs. Yasuhiko Kawakami, Fuminori Hyodoh, Akiko Takata-Tomokuni, Yoshie Miura, Shuko Murakami, Ping Wu, Ying Chen and Megumi Maeda. The authors are also grateful for technical support from Ms. Yumika Isozaki, Haruko Sakaguchi, Tamayo Hatayama, Shoko Yamamoto and Miho Ikeda.

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