〈Review〉

Skewing T helper cells exposed to asbestos fibers toward reduction of tumor immunity or activation of autoimmunity

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ABSTRACT Asbestos fibers cause malignant tumors such as lung cancer and malignant mesothelioma. Furthermore, with carcinogenic activity, they alter the human immune system. Our previous findings indicated that immune cells exposed to asbestos revealed cellular and molecular alterations that resulted in a reduction in anti-tumor immunity. Focusing on regulatory T cells (Tregs), we found enhanced function and proliferating activity resulting from asbestos exposure using an *in vitro* cell line model. Additionally, surface C-X-C motif chemokine receptor 3 (CXCR3) levels in T helper (Th) cells as well as the capacity to produce interferon γ were reduced, which lead to decreased anti-tumor immunity. However, our recent findings showed that IL-17 production in the intracellular CXCR3-rich fraction in Th cells was enhanced by asbestos exposure. Regarding alterations of autoimmunity, some investigators have reported the detection of autoantibodies against endothelial cells and mesothelial cells in an asbestos-exposed mouse model and suggested that these autoantibodies contribute to the formation of pulmonary fibrosis. However, the precise mechanisms by which asbestos affects the human immune system and causes cancers and the production of certain autoantibodies to create pathophysiological conditions found in asbestos-exposed patients remains to be elucidated.

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INTRODUCTION

Asbestos fibers are mineral silicates that include Si and O[1,2)]. In addition to these elements, iron (Fe), magnesium (Mg) and sodium (Na) also comprise various asbestos fibers such as chrysotile, possessing the serpentine group $Mg_3(Si_2O_5)(OH)_4$, and amosite

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and crocidolite which possess the amphibole groups $Fe_7Si_8O_{22}(OH)_2$ and $Na_2Fe^{2+}{}_3Fe^{3+}{}_2Si_8O_{22}(OH)_2$, respectively[1, 2].

Silicosis patients exposed to silica particles often reveal complications of autoimmune diseases[3-6] such as rheumatoid arthritis, known as Caplan's syndrome[7], systemic sclerosis[8], as well as anti-neutrophil cytoplasmic autoantibody (ANCA)-related vasculitis[9, 10]. Although the actions of silica particles to induce disruption of autoimmunity have been considered as adjuvant effects which generate small auto-antigens recognized by the immune system[11, 12], our previous investigations showed that silica particles induce chronic activation of T helper (Th) responder (Tresp) cells as well as regulatory T (Treg) cells. As a result, Tresps begin to display certain activation markers such as soluble interleukin-2 receptor (sIL-2R)[13-15], program death-1 (PD-1)[16] and CD69[17]. Additionally, Tresps displayed certain surviving molecules such as soluble Fas/CD95 (sFas)[18, 19] and decoy receptor 3 (DcR3)[20] to prevent an attack of cell death ligands such as fas ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Thus, the imbalance between Tresps and Tregs manifests as higher Tresps and lower Tregs[14, 15]. This tendency forms the basis of the disruption of autoimmunity and people who possess this Tresp/Treg tendency may be prone to the onset of autoimmune diseases[14, 15].

As asbestos fibers are mineral silicates, they may affect human immune cells. From our previous investigations, natural killer (NK) cells possess reduced cytotoxic activity following asbestos exposure due to a reduction in surface expression of one of the activation receptors, NKp46[21, 22]. Additionally, clonal expansion of CD8+ cytotoxic T lymphocytes (CTLs) was inhibited by the addition of chrysotile asbestos when assayed by the mixed lymphocyte reaction (MLR)[23-25].

In this review, findings regarding anti-tumor immunity in Th cells and Tregs exposed to asbestos fibers are introduced. Additionally, our recent findings regarding IL-17 production in the intracellular C-X-C motif chemokine receptor 3 (iCXCR3)-rich fraction following asbestos exposure have also been reported[26]. Thereafter, the tendency toward anti-tumor immunity and disruption of autoimmunity caused by asbestos exposure is discussed.

EFFECTS OF ASBESTOS ON TH (CD4+) CELLS WITH A FOCUS ON CXCR3

To investigate the effects of asbestos on human T cells, the human T cell leukemia virus (HTLV)-1 immortalized human polyclonal T cell line MT-2 was employed to establish continuous (more than eight months) and relatively low-dose (causes less than half of the cells to proceed to apoptosis when transiently exposed) exposure models[27-32]. After continuous and low-dose exposure, MT-2 sublines showed cellular and molecular changes related to the acquisition of resistance to asbestos-induced apoptosis.

Among the various changes between original MT-2 cells not exposed to asbestos and MT-2 sublines independently established by continuous and low-dose exposure to asbestos fibers such as chrysotile and crocidolite, surface expression of CXCR3 (sCXCR3) was examined since CXCR3 plays an important role in anti-tumor immunity by marshaling interferon (IFN)-γ producing T cells to the area surrounding tumor cells. Using an MT-2 cell line model in addition to *ex vivo* CD4+ T cells freshly isolated from healthy volunteers never exposed to asbestos, it was found that exposure to asbestos fibers resulted in reduced expression of surface CXCR3. Additionally, the cell line model and *ex vivo* cultivation model revealed a decrease in intracellular IFN-γ expression. These findings were confirmed using freshly isolated CD4+ T cells derived from patients exposed to asbestos and

diagnosed with pleural plaque (PP) or malignant mesothelioma (MM)[28, 29].

MT-2 cells possess Treg-like function as previously reported[33, 34], and given the initial observations regarding changes in CXCR3 expression using MT-2 cells, the result might be considered to reflect a decrease in surface CXCR3 in Treg following asbestos exposure. However, CXCR3 is widely expressed in human immune cells such as CD4+ Th, CD8+ T and NK cells. Thus, as mentioned above, an *ex vivo* exposure model was employed using freshly isolated peripheral CD4+ T cells from healthy volunteers. An *ex vivo* continuous exposure model (with exposure lasting four weeks with chronic stimulation with IL-2 in the absence or presence of asbestos fibers) was utilized as the next step from cell line model to confirm that freshly isolated cells might be useful for further analyses using these T cells from exposed patients. Since Tregs represented a small percentage of peripheral CD4+ cells, most of this population comprised Th cells. Following confirmation of the decrease in CXCR3 expression in CD4+ Th cells by the *ex vivo* model cultured with asbestos fibers, the assay was then utilized to examine CXCR3 expression in asbestos-exposed patients with as PP and MM. Surface expression of CXCR3 in peripheral CD4+ cells were compared among healthy volunteers, and patients with PP and MM. A reduction of CXCR3 in peripheral CD4+ Th cells in these patients was demonstrated. Although these experiments did not exactly differentiate any changes in CXCR3 expression in Th or Treg cells, most of the analyzed cell population comprised Th cells. It was then considered that the reduction in CXCR3 expression leads to a decrease in anti-tumor immunity.

Thereafter, we investigated changes in intracellular (iCXCR3) expression in freshly isolated CD4+ T cells from healthy volunteers using an *ex vivo* model. After dividing CD4+ T cells by sCXCR3, no marked differences were found in the expression of intracellular IFN-γ, IL-17 or CXCR3 when sCXCR3+ and sCXCR3- subpopulations were compared. In this model, peripheral CD4+ cells were stimulated with IL-2 following initial activation using anti-CD3 and anti-CD28 antibodies in the absence or presence of various amounts of chrysotile asbestos fibers. Additionally, after removing the asbestos fibers, cells were restimulated with mitogens (phorbol 12-myristate 13-acetate (PMA) and ionomycin). During this second stimulation, IL-17 production in the culture medium showed a dose-dependent increase depending on the initial supplemented chrysotile concentration (even though there was no statistical significance due to larger error bar values), although IFN-γ production showed no marked changes following the addition of chrysotile fibers[28]. Thereafter, we examined mRNA expression of T-bet (T-box transcription factor for differentiation to Th1), IFN-γ, retinoic acid-related orphan receptor γt (RORγt; transcription factor for skewing to Th17) and IL-17 in CD4+sCXCR3+ T cells and CD4+sCXCR3- T cells cultured in the absence or presence of chrysotile fibers following stimulation with mitogens. As a result, no significant changes were found in mRNA levels of T-bet, IFN-γ or RORγt, while mRNA expression of IL-17 increased significantly in the CD4+sCXCR3+ fraction in a dose-dependent manner of the supplemented chrysotile fibers[28]. Unfortunately, expression of CCR6, the other special marker for Th17 cells, was not examined in this study. Moreover, the discrepancy between increased IL-17 production as well as mRNA expression and unchanged RORγt expression was hitherto unresolved.

EFFECTS OF ASBESTOS ON TREG FUNCTION AND PROLIFERATION

Since MT-2 cells possess Treg-like function[33, 34], Treg cellular function and proliferation were examined and compared between MT-2 original

cells and sublines continuously exposed to asbestos. As previously reported, we found that function was enhanced by cell-cell contact as well as over-production of soluble factors such as IL-10 and transforming growth factor (TGF)-β[35]. Additionally, sublines showed acceleration of cell cycle progression by increased expression of accelerating molecules such as cyclin D1 and decreased expression of braking molecules such as cyclin-dependent kinase inhibitors (CDK-Is) including $p21^{Cip1}$, $p27^{Kip1}$ and $p16^{CDKN2A}$ [36]. These changes were regulated by forkhead box protein O1 (FoxO1) transcription factor[37]. Expression of FoxO1 in MT-2 sublines exposed to asbestos was markedly lower compared with that of MT-2 original cells.

Taken together, asbestos causes an increase and enhancement of Tregs. This is consistent with our hypothesis in which asbestos causes a reduction in anti-tumor immunity.

AUTOANTIBODY PRODUCTION BY ASBESTOS EXPOSURE

As we previously reported[38–42], silicosis patients showed the appearance of various autoantibodies (AAb) such as anti-Scl-70 (topoisomerase I) -AAb[38], anti-centromere (CENP-B) -AAb[39], anti- desmoglein-AAb[40], anti-Fas-AAb[41] and anti-caspase-8-AAb[42]. Other investigations have demonstrated AAb production with asbestos exposure such as anti-endothelial cell AAb, anti-fibroblast antibody, and anti-mesothelial antibody[43–45]. Although some AAbs were found in a mouse model, some AAbs were found in humans comprising a "Libby Amphibole (LA)"-exposed population. LA comprises a mixture of amphibole fibers that contain contaminated vermiculite, and is mined at Libby, Montana, U.S.A[43–45]. The researchers considered that these antibodies may play a role in the formation of lung and pleural fibrosis caused by asbestos exposure.

INTERACTION BETWEEN TH17 AND TREG CELLS

As shown above, we found an enhancement of function and proliferation of Tregs by asbestos exposure. Unfortunately, it was impossible to measure peripheral and/or tumor surrounding Tregs in asbestos-exposed population. However, our findings support the notion that a reduction in anti-tumor immunity is induced by these Treg changes caused by asbestos exposure, in addition to a reduction in sCXCR3 and IFN-γ in CD4+ Th cells, decreased differentiation of CTLs[23–25], as well as impaired function of NK cells[21, 22].

On the other hand, the iCXCR3 population in CD4+ Th cells showed an increased capacity to produce IL-17. Since IL-17 is mainly produced by Th17 cells, asbestos exposure can skew some of the Th cells toward Th17 cells. Essentially, Th17 cells are considered to possess an important role in the disruption of autoimmunity, and that the increase in Th17 may facilitate the onset of autoimmune diseases.

Additionally, the skewing toward Th17 or Treg cells is thought to be induced by cytokine conditions surrounding Th naïve cells. Although only TGF-β can facilitate the differentiation of naïve T cells into Treg cells, TGF-β with IL-6 can induce differentiation of Th naïve cells into Th17 cells[46–49]. As we previously reported, T cells from patients with PP or MM showed increased capacity to produce IL-6. Additionally, in patients with MM, serum IL-6 levels were higher compared with healthy volunteers and patients with PP[50].

CONCLUSION

Fig. 1 schematically shows findings described in this review.

These complex findings suggest that (1) asbestos exposure induces enhancement of Tregs, (2) asbestos exposure causes differentiation of a small population of Th cells (sCXCR3+ cells) toward Th17 cells

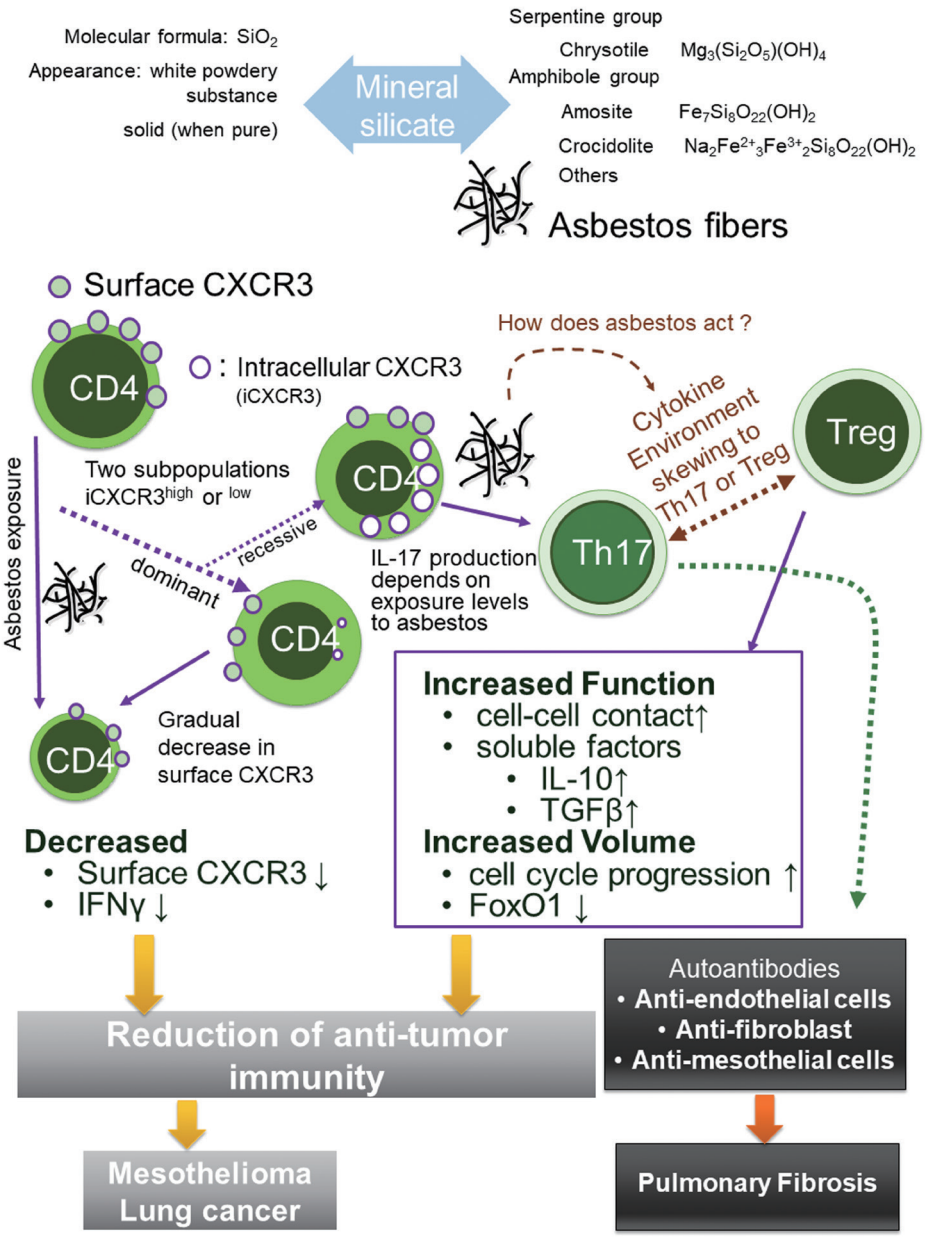

Fig. 1. The effects of asbestos fibers on Th, Treg and Th17 cells are schematically summarized in this figure from the viewpoint of decreased anti-tumor immunity and disruption of autoimmunity. Asbestos is a mineral silicate containing iron, magnesium and so on. Although the physical properties of fibrous substances differ from that of silica particles, asbestos may affect immune cells, as silica caused a disruption in autoimmunity. Regarding CXCR3 in CD4+ helper T cells, asbestos exposure resulted in a decline in surface (s) CXCR3 expression. Examination of CD4 T cells exposed to asbestos revealed a gradual decrease in sCXCR3 as well as intracellular (i) CXCR3. Additionally, the capacity to produce IFN γ was also reduced. However, recessive populations of CD4+ cells exposed to asbestos still maintained sCXCR3 expression, gradually began to produce IL-17, and were skewed toward Th17 cells. Th17 cells in the presence of asbestos possess the capacity to produce auto-antibodies against endothelial, fibroblast and mesothelial cells. These autoantibodies are reported to contribute towards the formation of pulmonary fibrosis. On the other hand, asbestos exposure may alter the cytokine environment surrounding T cells. Although further studies are required, the altered environment may modify the skewing conditions and outcomes, especially between Th17 and Treg cells. Asbestos exposure enhanced Treg function and led to increased volume. All of these effects of asbestos on human immune cells lead to a reduction in anti-tumor immunity and facilitate the onset of pulmonary fibrosis.

when increased IL-6 is present, although sCXCR3+ T cell levels decreased with asbestos exposure, and (3) a small fraction of IL-17-producing Th cells may possess the capacity to produce AAbs found in asbestos-exposed populations and may contribute toward the onset of pulmonary fibrosis. However, since these possibilities have yet to be completely investigated, additional investigations will be required.

Further studies are required to identify the kinds of alterations that occur to the immune system resulting from continuous exposure to asbestos, and which contribute to asbestos-induced pathophysiological conditions such as the occurrence of malignant tumors, formation of pulmonary fibrosis, as well as the appearance of autoimmune disorders. Additionally, with regard to the disruption of autoimmunity, the effects of silica and asbestos exposure on the immune system should be examined and any similarities and differences be noted. This would help to clarify immune modifications, and together with improved knowledge of certain substances in foods, plant extracts, and others sources, may assist in the development of strategies to facilitate recovery of the immune system, and help prevent future occurrences of silica- and asbestos-induced diseases.

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CONFLICT OF INTEREST

All authors declare there are no conflicts of interest regarding the contents of this review.

REFERENCES

1）Roggli VL, Coin P: Mineralogy of Asbestos. *In* Pathology of Asbestos–Associated Diseases (Roggli VL, Oury TD, Sporn TA, eds). New York, USA, Springer-Verlag New York. 2004, pp1-16

2）Craighead JE, Gibbs AR, Pooley F: Mineralogy of Asbestos. *In* Asbestos and Its Diseases (Craighead JE, Gibbs AR, eds). New York, USA, Oxford University Press. 2008, pp23-38

3）Uber CL, McReynolds RA: Immunotoxicology of silica. Crit Rev Toxicol 10: 303-319,1982

4）Pollard KM: Silica, Silicosis, and Autoimmunity. Front Immunol 7: 97, 2016

5）Steenland K, Goldsmith DF: Silica exposure and autoimmune diseases. Am J Ind Med 28: 603-608, 1995

6）CAPLAN A: Certain unusual radiological appearances in the chest of coal-miners suffering from rheumatoid arthritis. Thorax 8: 29-37, 1953

7）Haustein UF, Anderegg U: Silica induced scleroderma--clinical and experimental aspects. J Rheumatol 25: 1917-1926, 1998

8）Tervaert JW, Stegeman CA, Kallenberg CG: Silicon exposure and vasculitis. Curr Opin Rheumatol 10: 12-17, 1998

9）Saeki T, Fujita N, Kourakata H, Yamazaki H, Miyamura S: Two cases of hypertrophic pachymeningitis associated with myeloperoxidase antineutrophil cytoplasmic autoantibody (MPO-ANCA)-positive pulmonary silicosis in tunnel workers. Clin Rheumatol 23: 76-80, 2004

10）Bartůnková J, Pelclová D, Fenclová Z, Sedivá A, Lebedová J, Tesar V, Hladíková M, Klusácková P: Exposure to silica and risk of ANCA-associated vasculitis. Am J Ind Med 49: 569-576, 2006

11）Picha GJ, Goldstein JA: Investigation of silicone oil and fumed silica in an adjuvant animal model. Plast Reconstr Surg 100: 643-652, 1997

12）Parks CG, Conrad K, Cooper GS: Occupational exposure to crystalline silica and autoimmune disease. Environ Health Perspect 107: 793-802, 1999

13）Hayashi H, Maeda M, Murakami S, *et al*.: Soluble interleukin-2 receptor as an indicator of immunological disturbance found in silicosis patients. Int J Immunopathol Pharmacol 22: 53-62, 2009

14）Lee S, Matsuzaki H, Kumagai-Takei N, *et al*.: Silica exposure and altered regulation of autoimmunity.

Environ Health Prev Med 19: 322-329, 2014

15) Lee S, Hayashi H, Mastuzaki H, Kumagai-Takei N, Otsuki T: Silicosis and autoimmunity. Curr Opin Allergy Clin Immunol 17: 78-84, 2017

16) Hayashi H, Miura Y, Maeda M, Murakami S, Kumagai N, Nishimura Y, Kusaka M, Urakami K, Fujimoto W, Otsuki T: Reductive alteration of the regulatory function of the CD4(+)CD25(+) T cell fraction in silicosis patients. Int J Immunopathol Pharmacol 23: 1099-1109, 2010

17) Wu P, Hyodoh F, Hatayama T, Sakaguchi H, Hatada S, Miura Y, Takata-Tomokuni A, Katsuyama H, Otsuki T: Induction of CD69 antigen expression in peripheral blood mononuclear cells on exposure to silica, but not by asbestos/chrysotile-A. Immunol Lett 98: 145-152, 2005

18) Tomokuni A, Aikoh T, Matsuki T, Isozaki Y, Otsuki T, Kita S, Ueki H, Kusaka M, Kishimoto T, Ueki A: Elevated soluble Fas/APO-1 (CD95) levels in silicosis patients without clinical symptoms of autoimmune diseases or malignant tumours. Clin Exp Immunol 110: 303-309, 1997

19) Otsuki T, Sakaguchi H, Tomokuni A, Aikoh T, Matsuki T, Kawakami Y, Kusaka M, Ueki H, Kita S, Ueki A: Soluble Fas mRNA is dominantly expressed in cases with silicosis. Immunology 94: 258-262, 1998

20) Otsuki T, Tomokuni A, Sakaguchi H, *et al*.: Over-expression of the decoy receptor 3 (DcR3) gene in peripheral blood mononuclear cells (PBMC) derived from silicosis patients. Clin Exp Immunol 119: 323-327, 2000

21) Nishimura Y, Maeda M, Kumagai N, Hayashi H, Miura Y, Otsuki T: Decrease in phosphorylation of ERK following decreased expression of NK cell-activating receptors in human NK cell line exposed to asbestos. Int J Immunopathol Pharmacol 22: 879-888, 2009

22) Nishimura Y, Miura Y, Maeda M, Kumagai N, Murakami S, Hayashi H, Fukuoka K, Nakano T, Otsuki T: Impairment in cytotoxicity and expression of NK cell- activating receptors on human NK cells following exposure to asbestos fibers. Int J Immunopathol Pharmacol 22: 579-590, 2009

23) Kumagai-Takei N, Nishimura Y, Maeda M, Hayashi H, Matsuzaki H, Lee S, Hiratsuka J, Otsuki T: Effect of asbestos exposure on differentiation of cytotoxic T lymphocytes in mixed lymphocyte reaction of human peripheral blood mononuclear cells. Am J Respir Cell Mol Biol 49: 28-36, 2013

24) Kumagai-Takei N, Nishimura Y, Maeda M, Hayashi H, Matsuzaki H, Lee S, Kishimoto T, Fukuoka K, Nakano T, Otsuki T: Functional properties of CD8(+) lymphocytes in patients with pleural plaque and malignant mesothelioma. J Immunol Res 2014: 670140, 2014

25) Kumagai-Takei N, Nishimura Y, Matsuzaki H, Lee S, Yoshitome K, Hayashi H, Otsuki T: The Suppressed Induction of Human Mature Cytotoxic T Lymphocytes Caused by Asbestos Is Not due to Interleukin-2 Insufficiency. J Immunol Res 2016: 7484872, 2016

26) Maeda M, Chen Y, Lee S, *et al*.: Induction of IL-17 production from human peripheral blood CD4+ cells by asbestos exposure. Int J Oncol 50: 2024-2032, 2017

27) Miura Y, Nishimura Y, Katsuyama H, *et al*.: Involvement of IL-10 and Bcl-2 in resistance against an asbestos-induced apoptosis of T cells. Apoptosis 11: 1825-1835, 2006

28) Maeda M, Nishimura Y, Hayashi H, Kumagai N, Chen Y, Murakami S, Miura Y, Hiratsuka J, Kishimoto T, Otsuki T: Reduction of CXC chemokine receptor 3 in an in vitro model of continuous exposure to asbestos in a human T-cell line, MT-2. Am J Respir Cell Mol Biol 45: 470-479, 2011

29) Maeda M, Nishimura Y, Hayashi H, Kumagai N, Chen Y, Murakami S, Miura Y, Hiratsuka J, Kishimoto T, Otsuki T: Decreased CXCR3 expression in CD4+ T cells exposed to asbestos or derived from asbestos-exposed patients. Am J Respir Cell Mol Biol 45: 795-803, 2011

30) Maeda M, Yamamoto S, Chen Y, *et al*.: Resistance to asbestos-induced apoptosis with continuous exposure to crocidolite on a human T cell. Sci Total Environ 429: 174-182, 2012

31) Maeda M, Chen Y, Kumagai-Takei N, Hayashi H, Matsuzaki H, Lee S, Hiratsuka J, Nishimura Y, Kimura Y, Otsuki T: Alteration of cytoskeletal molecules in a human T cell line caused by continuous exposure to chrysotile asbestos. Immunobiology 218: 1184-1191, 2013

32) Maeda M, Chen Y, Hayashi H, Kumagai-Takei N, Matsuzaki H, Lee S, Nishimura Y, Otsuki T: Chronic exposure to asbestos enhances TGF-β1 production in the human adult T cell leukemia virus-immortalized T cell line MT-2. Int J Oncol 45: 2522-2532, 2014

33) Hamano R, Wu X, Wang Y, Oppenheim JJ, Chen X: Characterization of MT-2 cells as a human regulatory T

cell-like cell line. Cell Mol Immunol 12: 780-782, 2015

34) Chen S, Ishii N, Ine S, *et al*.: Regulatory T cell-like activity of Foxp3+ adult T cell leukemia cells. Int Immunol 18: 269-277, 2006

35) Ying C, Maeda M, Nishimura Y, *et al*.: Enhancement of regulatory T cell-like suppressive function in MT-2 by long-term and low-dose exposure to asbestos. Toxicology 338: 86-94, 2015

36) Lee S, Matsuzaki H, Maeda M, Yamamoto S, Kumagai-Takei N, Hatayama T, Ikeda M, Yoshitome K, Nishimura Y, Otsuki T: Accelerated cell cycle progression of human regulatory T cell-like cell line caused by continuous exposure to asbestos fibers. Int J Oncol 50: 66-74, 2017

37) Matsuzaki H, Lee S, Maeda M, Kumagai-Takei N, Nishimura Y, Otsuki T: FoxO1 regulates apoptosis induced by asbestos in the MT-2 human T-cell line. J Immunotoxicol 13: 620-627, 2016

38) Ueki A, Isozaki Y, Tomokuni A, Ueki H, Kusaka M, Tanaka S, Otsuki T, Sakaguchi H, Hyodoh F: Different distribution of HLA class II alleles in anti-topoisomerase I autoantibody responders between silicosis and systemic sclerosis patients, with a common distinct amino acid sequence in the HLA-DQB1 domain. Immunobiology 204: 458-465, 2001

39) Lee S, Hayashi H, Kumagai-Takei N, *et al*.: Clinical evaluation of CENP-B and Scl-70 autoantibodies in silicosis patients. Exp Ther Med 13: 2616-2622, 2017

40) Ueki H, Kohda M, Nobutoh T, Yamaguchi M, Omori K, Miyashita Y, Hashimoto T, Komai A, Tomokuni A, Ueki A: Antidesmoglein autoantibodies in silicosis patients with no bullous diseases. Dermatology 202: 16-21, 2001

41) Takata-Tomokuni A, Ueki A, Shiwa M, *et al*.: Detection, epitope-mapping and function of anti-Fas autoantibody in patients with silicosis. Immunology 116: 21-29, 2005

42) Ueki A, Isozaki Y, Tomokuni A, Hatayama T, Ueki H, Kusaka M, Shiwa M, Arikuni H, Takeshita T, Morimoto K: Intramolecular epitope spreading among anti-caspase-8 autoantibodies in patients with silicosis, systemic sclerosis and systemic lupus erythematosus, as well as in healthy individuals. Clin Exp Immunol 129: 556-561, 2002

43) Pfau JC, Li S, Holland S, Sentissi JJ: Alteration of fibroblast phenotype by asbestos-induced autoantibodies. J Immunotoxicol 8: 159-169, 2011

44) Noonan CW, Pfau JC, Larson TC, Spence MR: Nested case-control study of autoimmune disease in an asbestos-exposed population. Environ Health Perspect 114: 1243-1247, 2006

45) Pfau JC, Serve KM, Noonan CW: Autoimmunity and asbestos exposure. Autoimmune Dis 2014: 782045, 2014

46) Afzali B, Lombardi G, Lechler RI, Lord GM: The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol 148: 32-46, 2007

47) Kitani A, Xu L: Regulatory T cells and the induction of IL-17. Mucosal Immunol 1: S43-S46, 2008

48) Lee YK, Mukasa R, Hatton RD, Weaver CT: Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol 21: 274-280, 2009

49) Kimura A, Kishimoto T: IL-6: regulator of Treg/Th17 balance. Eur J Immunol 40: 1830-1835, 2010

50) Murakami S, Nishimura Y, Maeda M, Kumagai N, Hayashi H, Chen Y, Kusaka M, Kishimoto T, Otsuki T: Cytokine alteration and speculated immunological pathophysiology in silicosis and asbestos-related diseases. Environ Health Prev Med 14: 216-222, 2009