Chapter 2

Cytotoxicity Caused by Asbestos Fibers and Acquisition of Resistance by Continuous Exposure in Human T Cells

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Abstract

The cytotoxic effects of asbestos fibers on human T cells and the acquisition of resistance against asbestos-induced apoptosis have been studied. These analyses are based on the establishment of a continuous and relatively low-dose exposure model of human immune cells exposed to asbestos that resembles actual exposure in the human body. The MT-2 T cell line was selected as the candidate for the investigations. A transient and high-dose exposure to chrysotile resulted in apoptosis with production of reactive oxygen species (ROS) and activation of the mitochondrial apoptotic pathway. However, sublines continuously exposed to low dose of asbestos exhibited resistance to asbestos-induced apoptosis. The mechanism of resistance acquisition involved excess production of IL-10, activation of STAT3, and enhanced expression of Bcl-2 located downstream of STAT3. These changes were also found in a subline continuously exposed to crocidolite. Furthermore, sublines showed a marked decrease in the expression of forkhead box O1 (FoxO1) transcription factor. FoxO1 is known to regulate apoptosis and various other cellular processes. Regarding apoptosis, sublines continuously exposed to asbestos showed reduction of FoxP1-driven proapoptotic genes. This pathway is also considered one of the mechanisms that result in resistance to asbestos-induced apoptosis in sublines. These sublines also exhibited several characteristics suggesting reduction of antitumor immunity.

Keywords: cytotoxicity, asbestos, T cell, apoptosis, reactive oxygen species, FoxO1, antitumor immunity

1. Introduction

It is well known that asbestos fibers cause lung fibrosis as well as certain malignant diseases such as malignant mesothelioma, lung cancer, and other diseases (the International Agency for Research on Cancer (IARC) indicated that asbestos exposure results in a significant increased risk for ovarian and laryngeal cancers) [1–4]. A consideration of the carcinogenic mechanisms of asbestos suggests that various factors may be related. One factor involves DNA damage caused by reactive oxygen species (ROS) produced by asbestos fibers, especially iron-containing fibers such as crocidolite (CR) and amosite [5–7]. In addition to this aspect, ROS are also produced by alveolar macrophages which handle asbestos fibers as a foreign substance. However, they are not able to completely process the fibers because of the rigid and long morphological features of the fibers [8, 9]. Thus, these cells fail as a "frustrated macrophage" and begin to produce ROS [8, 9]. Another mechanism is the direct damage to DNA in cells located near the fibers since the cells possess a tendency to incorporate these foreign fibers into their interiors, but the fibers reach and damage cellular DNA directly because of the physiological features of the fibers [10, 11]. Furthermore, inhaled asbestos fibers may be found in the lung, related lymph nodes, and other pulmonary areas for a long time since they are not removed easily from the human body. Various carcinogenic substances existing in inspired air such as tobacco smoke and air pollutants are adsorbed onto the surface of the asbestos fibers. These additional substances also cause DNA damage to cells surrounding fibers [12, 13].

Cytotoxicity caused by asbestos fibers, particularly DNA damage caused by fibers, has been investigated in alveolar epithelial cells and pleural mesothelial cells since these cells are the targets of asbestos-induced cancers [14–17]. DNA damage was found when asbestos fibers were exposed to these cells using transient and relatively high doses, which cause apoptosis of cells. Subsequently, the accumulation of relatively small DNA damage that does not cause a quick cell death and/or escape from the apoptotic pathway by continuous or recurrent and relatively low-dose exposure which may exist in the bodies of asbestos-exposed populations is thought to represent the mechanism by which cancers occur in these populations.

2. Immunological effects of asbestos fibers regarding cytotoxicity

Asbestos is a mineral silicate [18]. Silica is known to affect the human immune system since silicosis patients who are chronically and recurrently exposed to silica particles by relatively low doses (inhaled as well as cells exposed to intrabody remnant silica particles) often exhibit disorders of autoimmunity [19–21]. Complications of various autoimmune diseases include rheumatoid arthritis (known as Caplan's syndrome [22]), systemic sclerosis [23, 24], and antineutrophil cytoplasmic antibody (ANCA)-related vasculitis/nephritis [25, 26]. Our investigations indicate that silica particles disturb the balance of responder T cells (Tresp), which react with antigens including foreign nonself as well as self-antigens and regulatory T cells (Treg) that control the reaction of Tresp stimulated by antigens. Silica particles can reduce Treg through Fas-mediated apoptosis by enhancing Fas expression and long-term survival of Tresp by increasing inhibitors of Fas-mediated apoptosis such as soluble Fas and decoy receptor 3 molecules [27–29].

Asbestos fibers may, therefore, affect human immune cells. To investigate the effects of asbestos fibers on human immune cells, especially T cells, a human T-cell leukemia virus 1 (HTLV-1) immortalized human polyclonal T cell line, MT-2 [30], was selected for use in the establishment of a cell line model of asbestos exposure to immune cells. To choose an MT-2 cell line, various human T or B cell-derived tumorous or virus immortalized cell lines were transiently exposed to asbestos fibers, namely, chrysotile (CH) [31]. We selected chrysotile because of its wide use around the world, and the most exposed populations are thought to have resulted mainly through inhaled chrysotile fibers, although other iron-containing fibers such as crocidolite and amosite are known to possess a much higher potential for carcinogenic activity. Among the various cell lines, MT-2 was the most sensitive (growth inhibition was the strongest). The MT-2 cell line was then used to investigate the mechanisms of cell death in MT-2 cells exposed to asbestos fibers using transient and relatively high doses (doses causing cell death in at least half of the cells) [31, 32]. Thereafter, changes of cell death in MT-2 cells by a continuous and relatively low dose (doses causing cell death in less than half of cells) were investigated to explore cellular and molecular alterations in T cells by long-term exposure to asbestos. Exposure to asbestos in a human population is thought to involve a continuous, recurrent, and low-dose exposure, even for immune cells, because the existence of asbestos fibers in the lung and related lymph nodes can cause repeated encounters between immune cells and fibers [32].

3. Transient and high-dose exposure to asbestos fibers in MT-2 cells: Cytotoxicity

The left side of **Figure 1** shows findings concerning the transient and high-dose exposure to asbestos fibers in MT-2 cells. The cells proceed to apoptosis just as alveolar epithelial cells and pleural mesothelial cells were previously reported [14–17].

Asbestos exposure caused production of ROS. **Figure 1** shows the production of superoxide anion (O_2^-) as positive for hydroethidine analyzed by flow cytometry. Proapoptotic signaling molecules in the mitogen-activated protein kinase (MAPK) pathway such as JNK and p38 were then phosphorylated. Cytochrome c in mitochondria was then released into the cytoplasm. As a result, the proapoptotic molecule BAX was upregulated in the cells. These findings indicated that the mitochondrial apoptotic pathway was activated by asbestos exposure. Caspase 9 and 3 were then truncated into active forms to cause apoptosis of cells [31, 32].

In addition, cellular phenomena such as growth inhibition, appearance of apoptosis analyzed by annexin V staining (as an early event), activation of caspase 3, positivity of Tunel staining (a late event), and ROS production were compared between MT-2 cells exposed to fibers of chrysotile and crocidolite (CH and CR, respectively in **Figure 1**). Since CR contains a massive level of iron compared with CH, ROS production was higher in MT-2 cells exposed to CR. However, other events (the degree of growth inhibition, appearance of apoptosis assayed by different methods) were stronger in MT-2 cells exposed to CH compared to CR, although these were just comparisons between these two fibers and apoptosis was certainly caused by asbestos exposure on MT-2 cells [31–33].

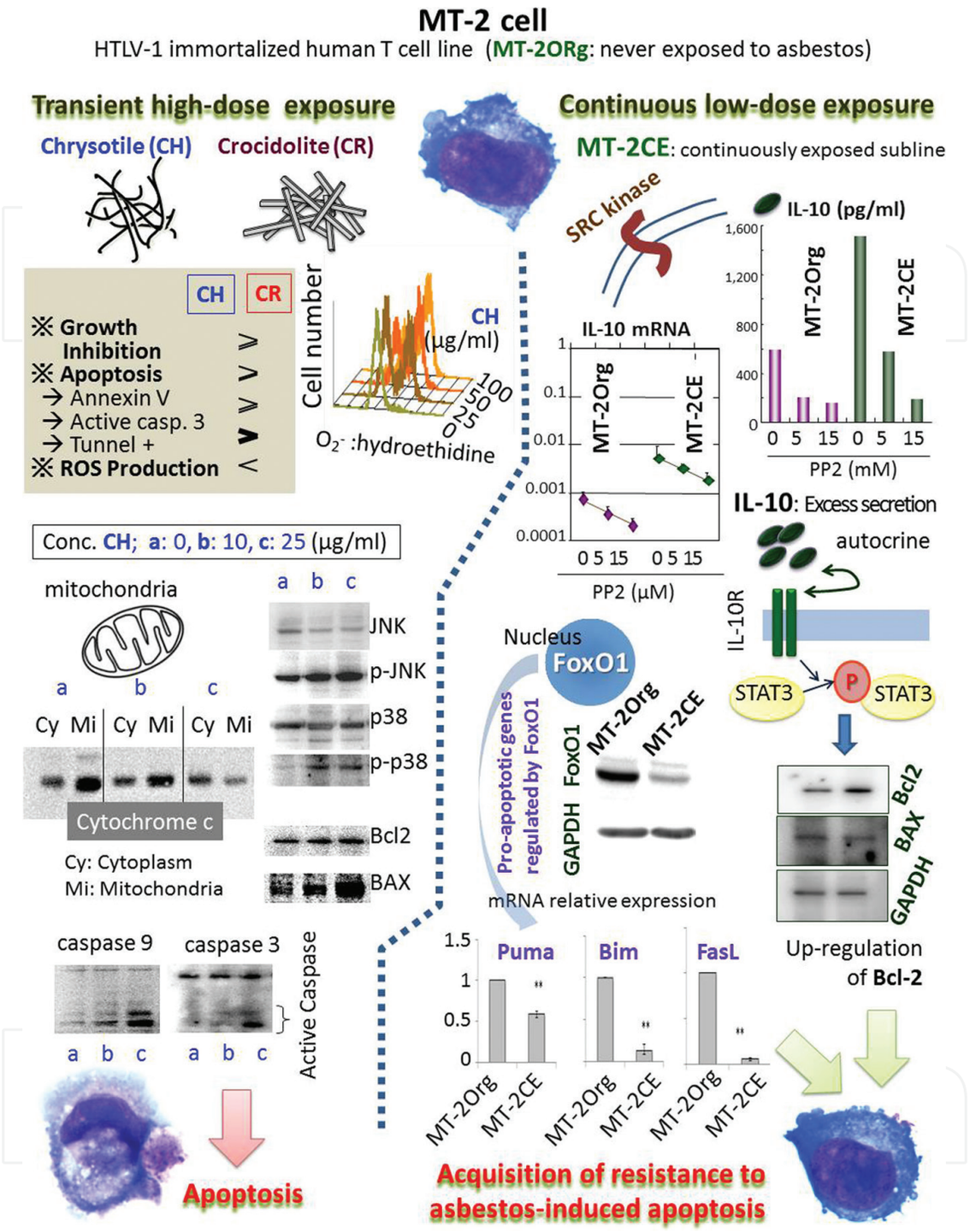

Figure 1. Schematic representation of the effects of the asbestos fibers chrysotile (CH) and crocidolite (CR) on MT-2 cells, a human T cell leukemia virus (HTLV)-1 immortalized human polyclonal T cell line [44–46]. Left side: Cellular and molecular alterations in MT-2 cells following transient and relatively high-dose exposure to CH or CR are summarized [32, 33]. Cells produced O_2^-, proapoptotic signaling molecules such as JNK and p38 were phosphorylated, cytochrome c was released from mitochondria to the cytoplasm, and caspases 9 and 3 were truncated into active forms. Cells then proceeded to apoptosis. A comparison of the effects caused by CH or CR showed that reactive oxygen species (ROS) production was greater with CR exposure, whereas growth inhibition and the level of apoptosis were greater following CH exposure. Right side: The effects of continuous and relatively low-dose exposure are summarized. MT-2 CE (a continuously exposed subline) revealed excess IL-10 production via Src kinase and phosphorylation of STAT3 resulting in upregulation of Bcl-2 [34]. In addition, the transcription factor FoxO1 was reduced in MT-2 CE, causing a reduction of proapoptotic molecules such as puma, bim, and FasL [36]. Both exposures contributed to the development of resistance to asbestos-induced apoptosis in MT-2 CE cells [44–46].

Taken together, the cytotoxicity of asbestos fibers on human T cells was caused by a mechanism similar to that demonstrated for alveolar epithelial and pleural mesothelial cells.

4. Continuous low-dose exposure to asbestos fibers in MT-2 cells: Resistance to cytotoxicity

The initial aim of asbestos exposure on the MT-2 cell line was to establish a model of continuous, recurrent, and relatively low-dose exposure on human T cells and observe which kind of alterations occur under conditions of continuous and low-dose exposure as found with human populations exposed to asbestos, such as the occurrence of cancers.

Exposed doses for continuous exposure were then determined as doses which caused apoptosis in less than half of MT-2 cells. Doses included 5 or 10 μg/ml in culture flasks, as shown on the left side of **Figure 1**. A dose of 10 μg/ml of chrysotile caused various apoptotic cellular events, although the degrees of these events were less than those resulting from exposure to 25 μg/ml of chrysotile [31, 34]. Since MT-2 cells were derived from T cells, they were grown by floating in the culture. Thus, the concentration of asbestos fibers was determined using μg/ml, although various adherent cells such as epithelial and mesothelial cells were measured using $\mu g/cm^2$ in the culture. The MT-2 cell culture was continued with a subculture twice a week and substitute asbestos fibers according to the determined concentrations. To monitor cellular alteration, the asbestos fibers were removed from continuous culture by density gradient centrifugation using LymphoPrep (gradient = 1.077) and continuously exposed cells were analyzed for the occurrence of apoptosis after transient exposure to high-dose asbestos fibers (which caused apoptosis in most of the MT-2 cells such as 25–50 μg/ml) [31, 32, 34]. After 8 months of continuous exposure, the appearance of apoptosis was reduced in the continuously exposed subline. This acquisition of resistance to asbestos-induced apoptosis was sustained in long-term cultures (until now, the sublines were cultured with asbestos fibers) [31, 34].

After 1 year of continuous exposure, the cellular features of the continuously exposed subline (MT-2 CE) were compared to those of the original MT-2 cells (MT-2Org, which were never exposed to asbestos fibers) as shown on the right side of **Figure 1**. A consideration of cytokine production showed that there was an excess production of interleukin (IL)-10 in MT-2 CE relative to MT-2Org. The regulation of IL-10 production was mediated by Src kinase, since PPT, the Src inhibitor, reduced IL-10 production in both MT-2Org and MT-2 CE cells. The excessively produced IL-10 was then utilized by the autocrine mechanism, since MT-2 possesses the IL-10 receptor (R) at its surface. As a result of IL-10 utilization, the signaling molecule, signal transducers, and the activator of transcription (STAT)-3, located downstream of IL-10R, were phosphorylated to a higher level in MT-2 CE compared to MT-2Org. Since the antiapoptotic molecule Bcl-2 is located downstream of STAT3, the expression of Bcl2 was upregulated in MT-2 CE compared to that in MT-2Org. The Bax/Bcl2 ratio was lower in MT-2 CE than in MT-2Org, as shown on the right side of **Figure 1**. In order to investigate the importance of Bcl-2 in MT-2 CE concerning acquisition of resistance to asbestos-induced apoptosis, siRNA for Bcl-2 was introduced into MT-2 CE cells and the occurrence of apoptosis and growth inhibition following transient and high-dose exposure to asbestos fibers were examined. As

suspected, Bcl-2 silenced cells exhibited much higher apoptosis and growth inhibition. Thus, the Src ➔ IL-10 ➔ STAT3 ➔ Bcl-2 axis was considered important for the acquisition of resistance to asbestos-induced apoptosis in MT-2 CE [31, 34]. Additionally, CD4-positive peripheral blood cells from asbestos-exposed patients exhibiting pleural plaque (PP) or malignant mesothelioma (MM) showed enhanced expression of Bcl-2 compared with that of healthy volunteers (HV). Thus, the MT-2 CE model may express events occurring within T cells of asbestos-exposed patients [31, 34].

Sublines continuously exposed to asbestos were established independently and comprised six sublines exposed to CH and three sublines exposed to CR. The profiles of cytokine production in these MT-2 CEs (exposed to CH or CR) were similar [31, 33, 35]. Continuous exposure caused excess production of IL-10 (as mentioned earlier) and transforming growth factor (TGF)-β, whereas interferon (IFN)-γ production was reduced in MT-2 CEs compared to that in MT-2Org. In addition, Bcl-2 upregulation was found in all MT-2 CEs and there were no differences between exposure to CH and CR [31, 33, 35]. In fact, a cDNA microarray assay using MT-2 CEs and MT-2org indicated that most of the upregulated and downregulated genes were similar in MT-2 CEs. Therefore, these MT-2 CEs could be used as a continuously asbestos-exposed immune T cell model.

The cDNA microarray assay revealed that the transcription factor forkhead box O1 (FoxO1) was expressed to a lesser degree in MT-2 CE compared to MT-2Org [36]. FoxO1 is known to regulate various genes in apoptosis, metabolism, cell growth and differentiation, and so on. In particular, FoxO1 controls various proapoptotic genes such as the p53 upregulated modulator of apoptosis (Puma), bcl-2 interacting mediator of cell death (Bim), and the Fas ligand (FasL) [36, 37]. The message expression of these proapoptotic molecules was reduced in MT-2 CE compared with that in MT-2Org (shown on the right side of **Figure 1**). In addition, following knockdown of the FoxO1 gene in MT-2Org, the level of apoptotic cells caused by transient and high-dose exposure to asbestos was reduced. Furthermore, when the expression of FoxO1 was forced in MT-2 CE, the ratio of apoptosis increased following transient and high-dose exposure to asbestos and the expression of Puma was recovered [36].

These results indicated that acquisition of resistance to asbestos-induced apoptosis by continuous and low-dose exposure to asbestos was regulated by the FoxO1 transcription factor in addition to the Src ➔ IL-10 ➔ STAT3 ➔ Bcl-2 axis [31–36].

5. Findings in MT-2CEs regarding antitumor immunity

The purpose of establishing a cell line model involving continuous and relatively low-dose exposure of human T cells to asbestos fibers was to investigate cellular and molecular alterations that may reflect the immune function in human populations exposed to asbestos as well as patients exhibiting PP or MM.

A consideration of the development of cancer in asbestos-exposed patients suggested that focus within investigations should be placed on antitumor immunity.

Our investigations showed that the CXC chemokine receptor 3 (CXCR3) exhibited reduced expression in MT-2 CEs compared to MT-2Org [38, 39]. CXCR3 is known to play an important role in antitumor immunity because it summons antitumor T cells with IFN-γ. As shown in the cell line model, investigation of freshly isolated CD4-positive T cells revealed decreased expression of CXCR3 when activated *in vitro* with CH fibers. Furthermore, peripheral blood CD4-positive cells from patients with PP or MM showed a reduction of CXCR3 and an inhibited potential for IFN-γ production when stimulated *in vitro* [31, 38, 39]. These investigations indicated that the cell line model for continuous and low-dose exposure to asbestos using the MT-2 cell line was suitable for analysis of immune alteration in asbestos-exposed human populations and patients with PP or MM [31, 38, 39].

As the MT-2 cell line was known to possess a Treg function [40, 41], the Treg function was estimated in MT-2Org and MT-2 CE. In regard to cell-cell contact, MT-2 CE enhanced its suppressive function onto Tresp cells [42]. In addition, MT-2 CE produced higher TGF-β and IL-10 in comparison to MT-2Org as described earlier. These two cytokines are the typical soluble factors for Treg in order to manifest its function. Following the knockdown of each cytokine in MT-2 CE, the suppressive function was reduced relative to that in MT-2 CE [42]. These results indicated that asbestos exposure enhanced Treg function by cell-cell contact and an increase of soluble factors [42]. In addition, FoxO1 reduced its expression in MT-2 CE as described earlier, and is known to regulate the cell cycle to suppress the accelerating genes, such as cyclins, as well as to enhance the breaking genes, such as cyclin-dependent kinase inhibitors (CDK-Is) [43]. As a consequence, cyclins were enhanced and the expression of CDK-I2 was reduced in MT-2 CE because of the reduced expression of FoxO1. Cell cycle progression was, therefore, enhanced in MT-2 CE [43]. These overall results suggest that Treg volume may also be enhanced in asbestos-exposed human populations and patients exhibiting PP or MM [42, 43]. These findings indicated that asbestos exposure causes reduction of antitumor immunity.

6. Conclusion

Investigation of cytotoxicity in human T cells caused by asbestos exposure indicated that the production of ROS and activation of the mitochondrial apoptotic pathway were the main causes for apoptosis of T cells following a transient and relatively high-dose exposure [32], similar to known mechanisms investigated previously using alveolar epithelial and pleural mesothelial cells [5–9, 44–46]. However, the continuous and relatively low-dose exposure of T cells to asbestos altered cellular and molecular events that caused acquisition of resistance against asbestos-induced cytotoxicity. Investigations revealed the importance of the Src ➔ IL-10 ➔ STAT3 ➔ Bcl-2 axis as well as the reduced expression of FoxO1 [31, 33–35]. These changes induce the reduction of antitumor immunity in an asbestos-exposed population and create an increased risk of carcinogenicity due to the transforming activity associated with asbestos fibers [44–46].

Considering the most important issue in asbestos-exposed population, the occurrence of malignancies such as mesothelioma and lung cancer after long-term latent period should be explored the mechanisms as well as be prevented [1–4]. Thus, regarding the cytotoxic effects

of asbestos fibers onto human T cell, the acquisition of reduced antitumor immunity caused by continuous exposure to fibers should be focused, since it may be possible to dissolve or recover this situation. As a result, some preventive ways for asbestos-induced cancers in exposed population will be identified.

Recovery of cellular and molecular changes in asbestos-exposed T cells using certain food constituents or physiologically active substances, including plants or other materials, may support the maintenance of antitumor activity in an asbestos-exposed population and might help to reduce the chances of carcinogenesis caused by asbestos fibers.

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References

[1] Myers R. Asbestos-related pleural disease. Current Opinion in Pulmonary Medicine. 2012;**18**:377-381. DOI: 10.1097/MCP.0b013e328354acfe

[2] Lazarus A, Massoumi A, Hostler J, Hostler DC. Asbestos-related pleuropulmonary diseases: Benign and malignant. Postgraduate Medicine. 2012;**124**:116-130. DOI: 10.3810/pgm.2012.05.2555

[3] Markowitz S. Asbestos-related lung cancer and malignant mesothelioma of the pleura: Selected current issues. Seminars in Respiratory and Critical Care Medicine. 2015;**36**:334-346. DOI: 10.1055/s-0035-1549449

[4] IARC Monographs 100c, Asbestos (Chrysotile, Amosite, Crocidolite, Tremolite, Actinolite and Anthophyllite). http://monographs.iarc.fr/ENG/Monographs/vol100C/mono100C-11.pdf

[5] Kamp DW, Graceffa P, Pryor WA, Weitzman SA. The role of free radicals in asbestos-induced diseases. Free Radical Biology & Medicine. 1992;**12**:293-315

[6] Shukla A, Gulumian M, Hei TK, Kamp D, Rahman Q, Mossman BT. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. Free Radical Biology & Medicine. 2003;**34**:1117-1129

[7] Upadhyay D, Kamp DW. Asbestos-induced pulmonary toxicity: Role of DNA damage and apoptosis. Experimental Biology and Medicine (Maywood, N.J.). 2003;**228**:650-659

[8] Simeonova PP, Luster MI. Iron and reactive oxygen species in the asbestos-induced tumor necrosis factor-alpha response from alveolar macrophages. American Journal of Respiratory Cell and Molecular Biology. 1995;**12**:676-683

[9] Murthy S, Adamcakova-Dodd A, Perry SS, Tephly LA, Keller RM, Metwali N, Meyerholz DK, Wang Y, Glogauer M, Thorne PS, Carter AB. Modulation of reactive oxygen species by Rac1 or catalase prevents asbestos-induced pulmonary fibrosis. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2009;**297**:L846-L855. DOI: 10.1152/ajplung.90590.2008

[10] Nagai H, Toyokuni S. Biopersistent fiber-induced inflammation and carcinogenesis: Lessons learned from asbestos toward safety of fibrous nanomaterials. Archives of Biochemistry and Biophysics. 2010;**502**:1-7. DOI: 10.1016/j.abb.2010.06.015

[11] Nagai H, Ishihara T, Lee WH, Ohara H, Okazaki Y, Okawa K, Toyokuni S. Asbestos surface provides a niche for oxidative modification. Cancer Science. 2011;**102**:2118-2125. DOI: 10.1111/j.1349-7006.2011.02087.x

[12] Toyokuni S. Role of iron in carcinogenesis: Cancer as a ferrotoxic disease. Cancer Science. 2009;**100**:9-16. DOI: 10.1111/j.1349-7006.2008.01001.x

[13] Chew SH, Toyokuni S. Malignant mesothelioma as an oxidative stress-induced cancer: An update. Free Radical Biology & Medicine. 2015;**86**:166-178. DOI: 10.1016/j.freeradbiomed.2015.05.002

[14] Aljandali A, Pollack H, Yeldandi A, Li Y, Weitzman SA, Kamp DW. Asbestos causes apoptosis in alveolar epithelial cells: Role of iron-induced free radicals. The Journal of Laboratory and Clinical Medicine. 2001;**137**:330-339

[15] Panduri V, Weitzman SA, Chandel NS, Kamp DW. Mitochondrial-derived free radicals mediate asbestos-induced alveolar epithelial cell apoptosis. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2004;**286**:L1220-L1227

[16] Broaddus VC, Yang L, Scavo LM, Ernst JD, Boylan AM. Asbestos induces apoptosis of human and rabbit pleural mesothelial cells via reactive oxygen species. The Journal of Clinical Investigation. 1996;**98**:2050-2059

[17] Broaddus VC, Yang L, Scavo LM, Ernst JD, Boylan AM. Crocidolite asbestos induces apoptosis of pleural mesothelial cells: Role of reactive oxygen species and poly(ADP-ribosyl) polymerase. Environmental Health Perspectives. 1997;**105**(Suppl 5):1147-1152

[18] Kohyama N, Shinohara Y, Suzuki Y. Mineral phases and some reexamined characteristics of the International Union against Cancer standard asbestos samples. American Journal of Industrial Medicine. 1996;**30**:515-528

[19] Pollard KM. Silica, silicosis, and autoimmunity. Frontiers in Immunology. 2016;**7**:97. DOI: 10.3389/fimmu.2016.00097

[20] Steenland K, Goldsmith DF. Silica exposure and autoimmune diseases. American Journal of Industrial Medicine. 1995;**28**:603-608

[21] Parks CG, Conrad K, Cooper GS. Occupational exposure to crystalline silica and autoimmune disease. Environmental Health Perspectives. 1999;**107**(Suppl 5):793-802

[22] Caplan A. Certain unusual radiological appearances in the chest of coal-miners suffering from rheumatoid arthritis. Thorax. 1953;**8**:29-37

[23] Rodnan GP, Benedek TG, Medsger TA Jr, Cammarata RJ. The association of progressive systemic sclerosis (scleroderma) with coal miners' pneumoconiosis and other forms of silicosis. Annals of Internal Medicine. 1967;**66**:323-334

[24] Sluis-Cremer GK, Hessel PA, Nizdo EH, Churchill AR, Zeiss EA. Silica, silicosis, and progressive systemic sclerosis. British Journal of Industrial Medicine. 1985;**42**:838-843

[25] Neyer U, Wöss E, Neuweiler J. Wegener's granulomatosis associated with silicosis. Nephrology, Dialysis, Transplantation. 1994;**9**:559-561

[26] Mulloy KB. Silica exposure and systemic vasculitis. Environmental Health Perspectives. 2003;**111**:1933-1938

[27] Otsuki T, Miura Y, Nishimura Y, Hyodoh F, Takata A, Kusaka M, Katsuyama H, Tomita M, Ueki A, Kishimoto T. Alterations of Fas and Fas-related molecules in patients with silicosis. Experimental Biology and Medicine (Maywood, N.J.). 2006;**231**:522-533

[28] Lee S, Hayashi H, Maeda M, Chen Y, Matsuzaki H, Takei-Kumagai N, Nishimura Y, Fujimoto W, Otsuki T. Environmental factors producing autoimmune dysregulation - chronic activation of T cells caused by silica exposure. Immunobiology. 2012;**217**:743-748. DOI: 10.1016/j.imbio.2011.12.009

[29] Lee S, Hayashi H, Mastuzaki H, Kumagai-Takei N, Otsuki T. Silicosis and autoimmunity. Current Opinion in Allergy and Clinical Immunology. 2017;**17**:78-84. DOI: 10.1097/ACI.0000000000000350

[30] Miyoshi I, Kubonishi I, Yoshimoto S, Shiraishi YA. T-Cell line derived from normal human cord leukocytes by co-culturing with human leukemic T-cells. Gan. 1981;**72**:978-981

[31] Maeda M, Yamamoto S, Hatayama T, Mastuzaki H, Lee S, Kumagai-Takei N, Yoshitome K, Nishimura Y, Kimura Y, Otsuki T. T cell alteration caused by exposure to asbestos. In: Otsuki T, Holian A, Yoshioka Y, editors. Biological Effects of Fibrous and Particulate Substances. Japan, Tokyo: Springer; 2015. pp. 195-210

[32] Hyodoh F, Takata-Tomokuni A, Miura Y, Sakaguchi H, Hatayama T, Hatada S, Katsuyama H, Matsuo Y, Otsuki T. Inhibitory effects of anti-oxidants on apoptosis of a human polyclonal T-cell line, MT-2, induced by an asbestos, chrysotile-a. Scandinavian Journal of Immunology. 2005;**61**:442-448

[33] Maeda M, Yamamoto S, Chen Y, Kumagai-Takei N, Hayashi H, Matsuzaki H, Lee S, Hatayama T, Miyahara N, Katoh M, Hiratsuka J, Nishimura Y, Otsuki T. Resistance to asbestos-induced apoptosis with continuous exposure to crocidolite on a human T cell. Science Total Environment. 2012;**429**:174-182. DOI: 10.1016/j.scitotenv.2012.04.043

[34] Miura Y, Nishimura Y, Katsuyama H, Maeda M, Hayashi H, Dong M, Hyodoh F, Tomita M, Matsuo Y, Uesaka A, Kuribayashi K, Nakano T, Kishimoto T, Otsuki T. Involvement of IL-10 and Bcl-2 in resistance against an asbestos-induced apoptosis of T cells. Apoptosis. 2006;**11**:1825-1835

[35] 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. International Journal of Oncology. 2014;**45**:2522-2532. DOI: 10.3892/ijo.2014.2682

[36] 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. Journal of Immunotoxicology. 2016;**13**:620-627. DOI: 10.3109/1547691X.2016.1143539

[37] Zhang X, Tang N, Hadden TJ, Rishi AK. Akt, FoxO and regulation of apoptosis. Biochimica et Biophysica Acta. 2011;**1813**:1978-1986. DOI: 10.1016/j.bbamcr.2011.03.010

[38] 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. American Journal of Respiratory Cell and Molecular Biology. 2011;**45**:470-479. DOI: 10.1165/rcmb.2010-0213OC

[39] 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. American Journal of Respiratory Cell and Molecular Biology. 2011;**45**:795-803. DOI: 10.1165/rcmb.2010-0435OC

[40] Chen S, Ishii N, Ine S, Ikeda S, Fujimura T, Ndhlovu LC, Soroosh P, Tada K, Harigae H, Kameoka J, Kasai N, Sasaki T, Sugamura K. Regulatory T cell-like activity of Foxp3+ adult T cell leukemia cells. International Immunology. 2006;**18**:269-277

[41] Hamano R, Wu X, Wang Y, Oppenheim JJ, Chen X. Characterization of MT-2 cells as a human regulatory T cell-like cell line. Cellular & Molecular Immunology. 2015;**12**:780-782. DOI: 10.1038/cmi.2014.123

[42] Ying C, Maeda M, Nishimura Y, Kumagai-Takei N, Hayashi H, Matsuzaki H, Lee S, Yoshitome K, Yamamoto S, Hatayama T, Otsuki T. Enhancement of regulatory T cell-like suppressive function in MT-2 by long-term and low-dose exposure to asbestos. Toxicology. 2015;**338**:86-94. DOI: 10.1016/j.tox.2015.10.005

[43] 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. International Journal of Oncology. 2017;**50**:66-74. DOI: 10.3892/ijo.2016.3776

[44] Otsuki T, Matsuzaki H, Lee S, Kumagai-Takei N, Yamamoto S, Hatayama T, Yoshitome K, Nishimura Y. Environmental factors and human health: Fibrous and particulate substance-induced immunological disorders and construction of a health-promoting living environment. Environmental Health and Preventive Medicine. 2016;**21**:71-81. DOI: 10.1007/s12199-015-0499-6

[45] Matsuzaki H, Maeda M, Lee S, Nishimura Y, Kumagai-Takei N, Hayashi H, Yamamoto S, Hatayama T, Kojima Y, Tabata R, Kishimoto T, Hiratsuka J, Otsuki T. Asbestos-induced cellular and molecular alteration of immunocompetent cells and their relationship with chronic inflammation and carcinogenesis. Journal of Biomedicine & Biotechnology. 2012;**2012**:492608. DOI: 10.1155/2012/492608

[46] Kumagai-Takei N, Maeda M, Chen Y, Matsuzaki H, Lee S, Nishimura Y, Hiratsuka J, Otsuki T. Asbestos induces reduction of tumor immunity. Clinical & Developmental Immunology. 2011;**2011**:481439. DOI: 10.1155/2011/481439