Chapter 1
Suppressive Effects of Asbestos Exposure on the Human Immune Surveillance System

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Abstract Asbestos exposure causes malignancies such as mesothelioma and lung cancer. Asbestos induces carcinogenic activity, and its fibers may cause immune-modifying effects that impair the immune surveillance system in regard to cancer cell monitoring. Impairment of natural killer (NK) cells, cytotoxic T lymphocytes (CTLs), T helper 1 (Th1) cells, and regulatory T (Treg) cells was investigated using cell lines and freshly isolated peripheral blood immune cells derived from health donors, as well as peripheral immune cells from asbestos-exposed patients with pleural plaque and malignant mesothelioma (MM). All findings showed that asbestos exposure caused reduction of antitumor immunity. Therefore, the carcinogenic and immune-modifying effects indicate that the immune surveillance system in relation to cancerous cells may be impaired by asbestos exposure.

Keywords Asbestos • NK cell • CTL • Th1 cell • Treg • Immune surveillance

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1.1 Asbestos, Malignant Mesothelioma (MM), and Immune Function

Asbestos fibers have been used in many industrial fields worldwide because they are a natural mineral exhibiting high flexibility; resistance against heat, fire, friction, acids, and alkalis; as well as high electrical conductivity with a relatively low price for supply [1–4]. However, the majority of advanced nations have banned the use of asbestos due to its carcinogenicity (International Agency for Research on Cancer; IARC categorized asbestos as a definitive carcinogen in group 1), especially its association with lung cancer and MM, although many developing countries continue to use asbestos and several countries are currently exporting this mineral [5–8].

MM is a malignant tumor occurring in mesothelial cells located in the pleura, peritoneum, testicular serosa, and pericardium [9–11]. The major cause of MM is considered exposure to asbestos, with scant cases involving exposure to uranium and erionite (the frequent occurrence of MM in Cappadocia, Turkey, one of world's heritage sites, is known to be caused by erionite) [12–15]. It should be noted that MM in individuals exposed to asbestos results from relatively low to middle doses of exposure, compared with asbestosis/asbestos-induced lung fibrosis, a type of pneumoconiosis, which patients acquire after having been exposed to relatively high doses (according to the asbestos fibers found in the lung, i.e., more than two million fibers in 1 g dry lung tissue). In addition, the latent period is estimated as 30–50 years from the initial exposure to asbestos [9–11].

The carcinogenic actions of asbestos fibers are thought to be due to (1) oxygen stress, (2) chromosome tangling, and (3) absorption of other carcinogens in the lung. Considering these processes, crocidolite is thought to possess the strongest carcinogenic activity because it possesses the highest content of iron [16–20]. In addition, among the various asbestos fibers, the amphibole group, which includes crocidolite and amosite, is considered to have a stronger carcinogenic activity than the serpentine group, which only includes chrysotile, because of its physiological peculiarities and rigid form, and these considerations provide a basis for the above-mentioned carcinogenic hypotheses [16–20].

However, if we consider the long latent period, there may be biological effects of asbestos that cause malignancies other than the direct actions on alveolar and mesothelial cells. An insight may be gained by considering the immunological effect because asbestos fibers are found in various lymph nodes and mainly in pulmonary regions, not only in asbestos-handling workers, but also in individuals exposed from the environment. In particular, investigation of individuals experiencing non-work-related exposure revealed higher asbestos contents in lymph nodes rather than the lung [21, 22]. The overall findings suggest a frequent association between asbestos fibers and immune cells, and recurrent and continuous exposure to asbestos may alter the cellular, molecular, and functional features of immune cells. A consideration of malignant tumors in asbestos-exposed people indicates that the immune effects of asbestos may comprise a reduced tumor immunity that makes individuals more prone to cancers after a long latent period.

1.2 Alteration of Various Immune Cells Caused by Asbestos Exposure

1.2.1 Natural Killer (NK) Cell

1.2.1.1 Human NK Cell Line, YT-A1

A human NK cell line, YT-A1, was cocultured continuously with 5 μg/ml of chrysotile B (CB) for more than 5 months (YT-CB5). The aforementioned concentration of CB was chosen for these experiments because it did not induce apoptosis or growth inhibition. Meanwhile, the cytotoxicity against K562, a human erythroleukemia cell line, and cell surface expression of various receptors were compared with those of the YT-A1 original cell line, which was never exposed to asbestos. After 5 months of cultivation, YT-CB5 showed decreased cytotoxicity with reduced expression of NK cell-activating receptors such as NKG2D and 2B4, whereas other surface markers such as CD16, NKG2A, and CD94 were not changed. Although killing of K562 cells is not dependent on the 2B4 receptor, YT-CB5 showed impairment of 2B4-dependent cytotoxicity as analyzed by a reverse antibody-dependent cellular cytotoxicity (ADCC) assay using the anti-2B4 antibody. In addition, YT-CB5 showed decreased phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 after cultivation with K562 and stimulation with anti-NKG2D antibody. These results indicate that exposure to asbestos causes a reduction of NK cell cytotoxicity and decreased expression in activating receptors [23–28].

1.2.1.2 NK Cell Cytotoxicity in MM Patients

We investigated the state of NK cell cytotoxicity in patients with MM who might have been exposed to asbestos through the presence or absence of an occupational history with the mineral. After adjusting peripheral blood mononuclear cells (PBMC) from MM patients, cytotoxicity against K562 cells caused by 5,000 NK cells in PBMC and expression of NK cell-activating receptors were compared with those from healthy donors (HDs). NK cells derived from MM patients showed reduced cytotoxicity when compared with those from HD. Interestingly, although surface expression of NKG2D and 2B4 on NK cells from MM did not alter, another activating receptor, NKp46, exhibited reduced expression [23]. In response to this finding, we analyzed the expression of activating receptors on NK cells derived from HD and stimulated by interleukin (IL)-2 in vitro with or without 5 μg/ml of CB. Similar to NK cells from MM patients, there was a remarkable decrease in NKp46 expression, whereas expression of NKG2D and 2B4 did not decrease. In addition, the control substance used for CB, glass wool, did not induce these changes of expression of activating receptors. Thus, asbestos exposure specifically reduced the expression of NKp46 and resulted in reduced function of NK cells [23–28].

1.2.1.3 Relationship Between Killing Activity and Receptor Expressions

The relationship between expression levels of NK cell-activating receptors with the degree of cytotoxicity in HD and strength of downstream signaling receptors was examined. The killing activity against K562, expression levels of activating receptors, and phosphorylation levels of ERK 1/2 in NK cells derived from HD were measured. HDs whose NK cells revealed stronger killing activity showed higher expression of NKG2D and NKp46, as well as strong phosphorylation levels of ERK 1/2 molecules, when NK cells were simulated by anti-NKG2D or anti-NKp46 antibodies. However, killing activity was not correlated with expression levels of 2B4 or phosphorylation levels of ERK 1/2 in NK cells after stimulation by anti-2B4 antibody. The overall results showed that surface expression levels of NKG2D and NKp46 were important for cytotoxic activity in NK cells derived from asbestos-exposed patients. The overall results showed that the impairment of NK cells in these patients may be caused by decreased expression of these molecules induced by the continuous and recurrent exposure to asbestos [23–28].

1.2.2 Cytotoxic T Lymphocyte (CTL)

1.2.2.1 CTL Differentiation and Proliferation in Mixed Lymphocyte Reaction (MLR)

The MLR assay was used to evaluate CTL function derived from naïve CD8+ T cells cultured with allo-PBMC or splenic cells in vitro. Thus, to examine the effects of CB on CTL function, PBMC derived from HD were utilized for MLR using allo-PBMC with or without 5 μg/ml of CB. The increase of CD8+ cells in MLR was suppressed by addition of CB and cytotoxicity targeting the allo-PBMC using sorted CD8+ cells after cultivation was also reduced. Consistent with these findings, intracellular positivity of effector molecules such as granzyme B and interferon (IFN)-γ in CD8+ cells cultured with CB during the MLR was reduced. Regarding the differentiation of CD8+ cells, CD45RA as the marker for naïve CD8+ cells remained relatively high, while CD25 and CD45RO as markers of effector/memory CD8+ cells were not elevated when cultured with CB, compared with no CB MLR. In addition, the proliferation of CD8+ cells examined by the carboxyfluorescein succinimidyl ester (CFSE) labeling method was also inhibited when cultured with CB, although apoptotic cells of CD8+ cells were not changed regardless of whether the MLR was cocultured with or without CB. Furthermore, production of tumor necrosis factor (TNF)-α and IFN-γ in culture supernatants decreased when MLR was performed with CB, whereas IL-2 did not change irrespective of the presence of CB. These findings show that asbestos exposure causes inhibitory effects on CTL induction from CD8+ naïve T cells [29–31].

1.2.2.2 Characteristics of CTL in Asbestos-Exposed Patients

CTL differentiation and proliferation were impaired by asbestos exposure in asbestos-exposed patients with MM and pleural plaque (PP), who showed plaque in pleura as the marker of asbestos exposure and no other health effects in their body. The status of CTL characteristics in PP and MM patients was not identical. Analysis of CD8+ cells from PP patients revealed an increase of the perforin-positive and CD45RA-negative population in peripheral blood, as well as an increase of the perforin- and IFN-γ-positive fraction after in vitro stimulation, whereas MM patients showed a decrease of perforin-positive CD8+ cells in peripheral blood. Furthermore, this reduction was not due to the excess degranulation.

Details regarding the features of CTL in PP and MM were published in our previous reports. In particular, the reduction of killing activity in CD8+ cells caused by asbestos exposure suggests that the pathophysiological status such as the presence or absence of cancers may alter the function of CTL. This issue needs to be resolved in future studies [29–31].

1.2.3 Alteration of T Cell Caused by Asbestos Exposure

1.2.3.1 T Helper 1 (Th1) Cell

Asbestos Exposure on Human T Cell Line, MT-2

A human T cell line, MT-2 (human T cell leukemia/lymphoma virus 1 (HTLV-1) immortalized polyclonal T cell line), was utilized to establish a continuous and recurrent asbestos exposure model (more than 1 year exposure to low-dose asbestos fibers and details were reported previously [32]). Six independent continuously exposed sublines of MT-2 cocultured with CB were established, and cDNA microarray analysis was performed to compare the sublines with the original MT-2 cells, which were never exposed to asbestos. All six sublines showed a similar expression pattern of cDNA, and 84 up- and 55 downregulated genes were specified. Network analysis using the MetaCore™ system indicated that the IFN-γ pathway was involved in the sublines. All sublines showed a decrease in IFN regulatory factor 9 (IRF9) and IFN-stimulating gene factor 3 (ISGF3), as well as a decrease of CXC chemokine receptor 3 (CXCR3), which was regulated by IRF9. Since it is known that the Th1 cell shows higher IFN-γ and CXCR3 expression, mRNA and protein expression in the MT-2 sublines were reanalyzed. Results indicated that all sublines showed a decrease of CXCR3 and IFN-γ productive capacity, whereas the other Th1-type chemokine receptor, C-C chemokine receptor type 5 (CCR5) which had been chosen as the comparison, did not change [33–35].

CXCR3 and IFN-γ in Peripheral Th1 Cells Cultured with CB

Following analysis of the cell line model, in which it was supposed that asbestos exposure may inhibit Th1 function, and to confirm these findings in freshly isolated CD4+ Th cells from HD, these cells were stimulated and activated using anti-CD3 and anti-CD28 antibodies with IL-2 and cocultured with or without 5 or 10 μg/ml of CB. The CB-exposed Th cells showed decreased surface and mRNA expression of CXCR3 and intracellular IFN-γ-positive cells after 4 weeks of cultivation. These results indicated that ex vivo exposure to asbestos causes a distinct reduction of Th1 function as revealed by expression of CXCR3 and IFN-γ [33–35].

CXCR3 Expression of Peripheral CD4+ Cells in MM and PP Patients

In response to the experimental results of the cell culture, the expressions of CXCR3 and IFN-γ were analyzed in peripheral blood CD4+ cells derived from PP and MM patients, and results were compared with those from HD. Results showed that the CXCR3 expression level in CD4+ cells from PP and MM patients decreased and that of MM was lower than that of PP, whereas expression of CD4+CCR5+ cells in peripheral blood did not differ between the PP, MM, and HD groups. Moreover, CD4+ Th cells derived from MM showed decreased mRNA expression of IFN-γ when cells were ex vivo stimulated. A consideration of the overall results and experimental findings indicates that clinical exposure to asbestos induces dysfunction of Th1 cells as specifically revealed by decreased expression of CXCR3 and IFN-γ. Since both CXCR3 and IFN-γ are known to be important for antitumor immunity, these findings support the hypothesis that asbestos exposure induces a gradual decrease of antitumor immunity in the body of an exposed individual, which eventually causes lung cancer and MM in these individuals after a long latent period following the initial exposure to asbestos [33–35].

1.2.3.2 Regulatory T Cell (Treg)

In Vitro Assessment of Treg Function Exposed to Asbestos Using MT-2

The MT-2 cell line is known to possess Treg functionality, since HTLV-1 has a high affinity for Treg cells and adult T cell leukemia/lymphoma is considered a malignancy of Treg [36–38]. Treg is important for various pathophysiological states; for example, if the quality or quantity of Treg is decreased, allergy and autoimmune disorders may occur because the reaction of responder T cells against self- or non-self-antigens is not suppressed by Treg. In addition, if the function or volume of Treg is upregulated, antitumor immunity may decrease because responder T cells that recognize the tumor antigen and CTL suppress their function [39–41].

The abovementioned cell line model continuously exposed to asbestos was established using MT-2, and analyses of Treg function using the MT-2 sublines and the original MT-2 cell line, which was never exposed to asbestos fibers, may clarify the alteration of Treg following asbestos exposure [42].

Treg expresses suppressive activity by cell-cell contact and soluble factors, typically known as IL-10 and transforming growth factor (TGF)β. Interestingly, all sublines showed overproduction of IL-10 and TGFβ. The overproduced IL-10 was regulated by Src family kinases in MT-2 because of the reduction of IL-10 production and expression by the Src family inhibitor 4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2). This IL-10 was utilized by MT-2 cells through autocrine mechanisms and caused phosphorylated activation of signal transducer and activator of transcription (STAT)3, as well as Bcl-2 located downstream of STAT3. These conditions made the sublines resistant to asbestos-induced apoptosis. In addition, the overproduced TGFβ resulted in TGFβ-induced growth inhibition in MT-2 original cells, with over-phosphorylation of the downstream signaling molecule p38, as well as the TGF pathway signaling molecule SMAD3, but resulted in a decrease of SMAD2 [32, 43].

The contact inhibitory function of the MT-2 sublines exposed continuously to asbestos was then examined. Freshly isolated CD4+ cells were stimulated with anti-CD3 and auto-peripheral blood monocyte-derived dendritic cells to induce proliferation of cells. Instead of autologous Treg, irradiated cells from the original MT-2 cell line or an asbestos-exposed subline were added to this culture. Results revealed that the subline showed a greater enhanced suppressive activity than the original MT-2 cells, indicating that asbestos exposure caused an increase of Treg function [42].

Furthermore, the overproduced soluble factors IL-10 and TGFβ were evaluated by individually knocking down both cytokines using the siRNA method. The abovementioned assay for Treg suppressive function was then performed using a transwell culture plate with the MT-2 subline, MT-2 original cells, and IL-10 or TGFβ knocked-down sublines and permitting only the soluble factor to affect CD4+ cell proliferation activity. Results indicated that the IL-10 and TGFβ knocked-down sublines had reduced suppressive activity. These findings show that asbestos-exposed Treg exhibits an enhanced inhibitory function by a cell-cell contact mechanism, as well as excess production of functional soluble factors [42].

Analysis of the number and/or function of Treg may be better for an evaluation of tumor-surrounding Treg, although we have not had the opportunity to analyze Treg surrounding MM or asbestos-induced lung cancer cells. Our current findings are therefore limited due to the experimental assay. However, asbestos may cause the reduction of antitumor immunity by altering Treg function. The examination of the level of proliferating activity and inhibitory function of Treg should be performed in future investigations [42].

1.3 Summary of Asbestos-Induced Reduction of Antitumor Immunity

Table 1.1 summarizes investigation of the experimental immunological effects of asbestos exposure on various immune cells and alteration of immune function in asbestos-exposed patients with PP and MM. Additionally, Fig. 1.1 summarizes the typical findings described in this chapter.

As mentioned at the beginning of this chapter, the carcinogenic actions of asbestos fibers are attributed to (1) oxygen stress, (2) chromosome tangling, and (3) absorption of other carcinogens in the lung [16–20]. Due to these or other mechanisms, mesothelial cells may tend to change their cellular and molecular characteristics toward an abnormal and transformed cell type. For example, p16 cyclin-dependent kinase inhibitor, NF2, neurofibromatosis type 2, BAP1, and breast cancer susceptibility gene 1 (BRCA1)-associated protein-1 (ubiquitin carboxy-terminal hydrolase) are the typical altered tumor suppressor genes in MM [44–48]. However, many of these transforming cells are usually monitored by immune surveillance and then removed from the body. However, asbestos-exposed individuals may possess an impaired immune surveillance system as described in this chapter,

Table 1.1 Investigations of the experimental immunological effects of asbestos exposure on immune cells and alteration of immune function in asbestos-exposed patients

Immune cell type	Kinds of asbestos fiber for exposure or analyzed patients exposed to asbestos	Findings	References
NK cell			[23–28]
Human NK cell line, YT-A1	Cultivation with chrysotile	Reduction of cytotoxicity	
		Reduction of surface expression of NKG2D and 2B4	
		Decreased phosphorylation of ERK signaling molecule	
Peripheral CD56+ NK cells	Malignant mesothelioma	Low cytotoxicity	
		Low surface expression of NKp46-activating receptor	
Freshly isolated NK cells derived from healthy donors	Cultivation with chrysotile during in vitro activation	Reduction of surface expression of NKp46	
Cytotoxic T cell			[29–31]
Human CD8+ cells in mixed lymphocyte reaction (MLR)	Cultivation with chrysotile during MLR	Reduction of allogenic cell killing	
		Decrease of intracellular IFN-γ and granzyme B	
Peripheral CD8+ T cell	Pleural plaque	Relatively high perforin+ cell	

(continued)

Table 1.1 (continued)

Immune cell type	Kinds of asbestos fiber for exposure or analyzed patients exposed to asbestos	Findings	References
T helper cell			[32–34, 42, 43]
Human T cell line, MT-2	Continuous cultivation with chrysotile	Acquisition of asbestos-induced apoptosis	
		Excess expression and production of IL-10	
		Overexpression of Bcl-2	
		Reduced production of IFN-γ, TNF-α, and CXCL10	
		Reduction of CXCR3 surface and mRNA expressions	
		Hyperphosphorylation of β-actin	
		Excess binding capacity to chrysotile in vimentin, myosin 9, and tubulinβ2	
		Excess production of TGFβ with phosphorylation of p38 and SMAD3	
		Resistance to TGFβ-induced growth inhibition	
	Continuous cultivation with crocidolite	Acquisition of asbestos-induced apoptosis	
		Excess expression and production of IL-10	
		Enhancement of Bcl-2/Bax expression ratio	
		Reduced production of IFN-γ and TNF-α	
Freshly isolated CD4+ cells derived from healthy donors	Cultivation with chrysotile during in vitro activation	Reduced expression of surface CXCR3	
		Reduction of intracellular IFN-γ	
Peripheral CD4+ T cell	Pleural plaque	Low expression of surface CXCR3	
	Malignant mesothelioma	Remarkably lower expression of surface CXCR3	
		Low IFN-γ mRNA expression	
		High Bcl-2 mRNA expression	
Regulatory T cell			[41]
Human T cell line, MT-2	Continuous cultivation with chrysotile	Enhanced suppressive activity in cell-cell contact assay	
		Enhanced production of functional soluble factors such as IL-10 and TGFβ	

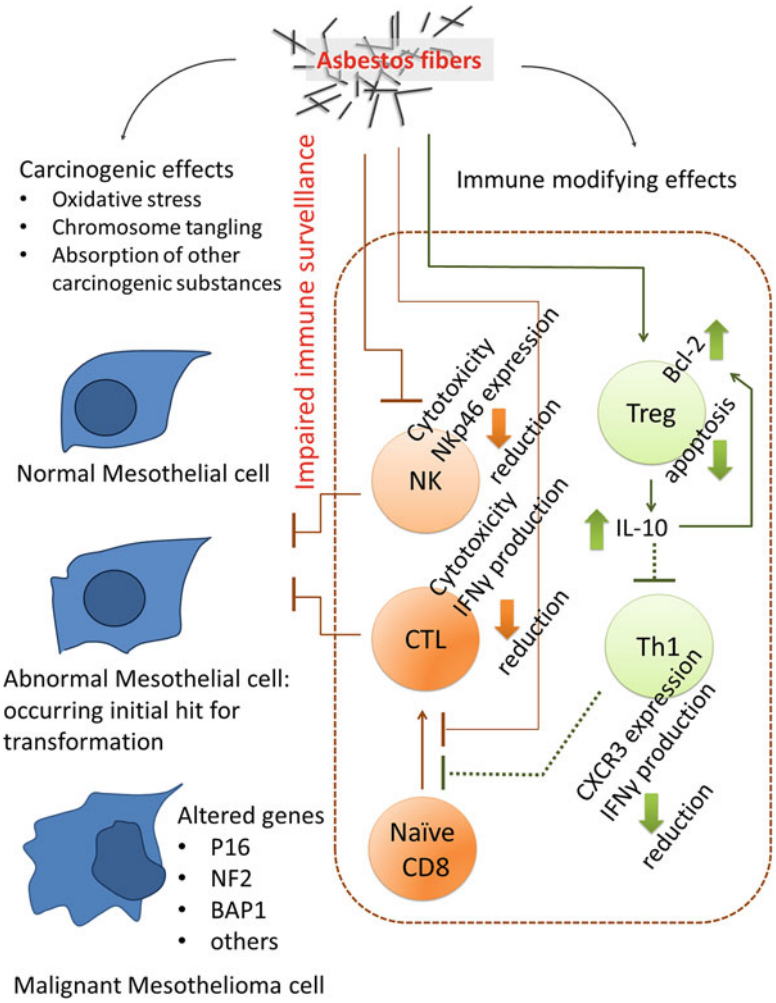

Fig. 1.1 Summarized schematic effects of asbestos fibers on various immune cells such as natural killer (NK), cytotoxic T lymphocyte (CTL), naïve CD8+, T helper 1 (Th1), and regulatory T (Treg) cells (*right side* of figure). The carcinogenic effects of asbestos fibers are shown on the *left side*, and normal mesothelial cells are gradually transformed toward malignant mesothelioma cells with alteration of tumor suppressor genes such as p16, NF2, and BAP1. Between these two effects, the usual immune surveillance system regarding cancerous cells may be impaired by asbestos exposure

and this impairment may result in MM and other cancers in these individuals after a long latent period [49–53].

Future investigations aimed at neutralizing the immune surveillance system in the asbestos-exposed population through physiologically active substances in foods, plants, and other materials are necessary in order to prevent the occurrence of cancerous diseases in asbestos-exposed individuals.

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