

# Exploration of Biomarkers for Asbestos Exposure and Occurrence of Malignant Mesothelioma Based on the Immunological Effects of Asbestos

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## Abstract

Investigation was carried out on the immunological effects of asbestos on individuals and found that asbestos exposure not only reduces the effectiveness of tumor surveillance but also makes asbestos exposed individuals sensitive to tumor development. The continuous experimental exposure of T cells to asbestos has enhanced production of IL-10 and TGF- $\beta$ . It has also provoked over expression of Bcl-2 with reduced expression of cell-surface CXCR3 and suppressed IFN- $\gamma$  production with decreased expression of activating receptors such as Nkp46 in NK cells. These alterations were also detected in asbestos-exposed patients such as those with pleural plaque or mesothelioma. Such changes in combination with certain markers produced by mesothelioma cells may therefore be considered as an initial screening system for asbestos exposure and developing mesothelioma using peripheral blood.

**Keywords:** Asbestos; Mesothelioma; T cell; NK cells

## Introduction

Asbestos causes not only non-malignant changes to the pulmonary system such as lung fibrosis (asbestosis), pleural thickening and benign pleural effusion, but also malignant diseases such as lung cancer and malignant mesothelioma (MM) [1,2]. In addition, people exposed to asbestos are at a higher risk of developing cancers of the larynx, gastrointestinal (GI) tract and bladder [3-5]. In particular, MM is considered very difficult to handle clinically, especially because it is thought to appear approximately 40 years after the initial exposure to asbestos. In addition, the degree of exposure associated with the development of MM is relatively lower than that which causes lung fibrosis and lung cancer [6,7]. Thus, high incidence of asbestosis is observed in workers associated with asbestos-processing industries. However, MM might also occur in family members of those workers if they reside near the asbestos-handling plants, and also in people who doesn't have any past history of exposure to asbestos.

Clinical issues concerning MM include difficulty of diagnosis, treatment, and poor prognosis [6-10]. The latency period following the initial exposure to asbestos is around 40 years, and MM usually progresses rapidly once it has developed at the diagnostic level. The half-survival time is still around one year, even after the medication with new chemotherapeutic drugs such as pemetrexedare [6-10].

An early diagnosis with subsequent surgical resection of the tumor represents the strongest strategy to cure MM patients [11,12]. Diagnosis is done using radio-diagnostic screening for workers in asbestos-handling industries. If the workers show evidence of pleural plaque (PP) they are examined once every 6 months, otherwise they are examined using a standard chest X-P approximately once every three years. However, there are some limitations to this approach. For example, although PP is evidence of asbestos exposure, it does not represent a pre-malignant state; therefore MM may develop in asbestos-exposed people without PP. Furthermore, even if patients with PP manifest signs of MM, examination once every 6 months might be too infrequent and may allow MM cells to proliferate to an inoperable

volume. Precisely, there is almost no way to detect the occurrence of MM among people who lived near asbestos-handling industries.

Various biomarkers for the early detection of MM have been explored and reported during this decade [13-15]. These include soluble mesothelin-related peptide (SMRP) [16-19], mesothelin [20,21], osteopontin [22,23], platelet-derived growth factor (PDGF) [24], and vascular endothelial growth factor (VEGF) [25]. Recent trials have published more candidate molecules like: fibulin-3 [26], megakaryocyte potentiating factor (MPF) [27,28], chemokine (C-C motif) ligand 2 (CCL2) [29], the secretory leukocyte peptidase inhibitor (SLPI) [29], and interleukin (IL)-4 receptor (R)  $\alpha$  [30]. Moreover, some micro RNAs (miR) such as miR-625-3p [31], miR-126 [32], miR-34b/c [33] and miR-103 [34] may be potential candidates. More recently, microarray assays using serum from asbestos-exposed people with or without MM indicated the potential usefulness of various marker combinations for the screening of MM.

In addition to the search for biomarkers of MM, a screening system to detect asbestos exposure and the occurrence of MM has been presented in this manuscript. Asbestos is a mineral silicate that includes the core elements of Si and O [1,2]. Exposure to silica (SiO<sub>2</sub>) causes lung fibrosis and dys-regulation of autoimmunity [35-37]. The condition of silicosis patients is often complicated with rheumatoid arthritis (known as Caplan's Syndrome [38,39]) and systemic sclerosis,

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and some recent reports have shown complications involving antineutrophil cytoplasmic auto antibody (ANCA)-related vasculitis/nephritis [35-37]. Since silica's direct affect on human T cells through perturbation of autoimmune regulation has been shown [40,41], asbestos may also result in these immunological effects. Based on the complications found in asbestos-exposed individuals, the malignant diseases are the most crucial complications, and if the immunological effects promote the development of tumor, those effects might involve reduction of tumor surveillance and a decrease of anti-tumor immunity as shown in figure 1.

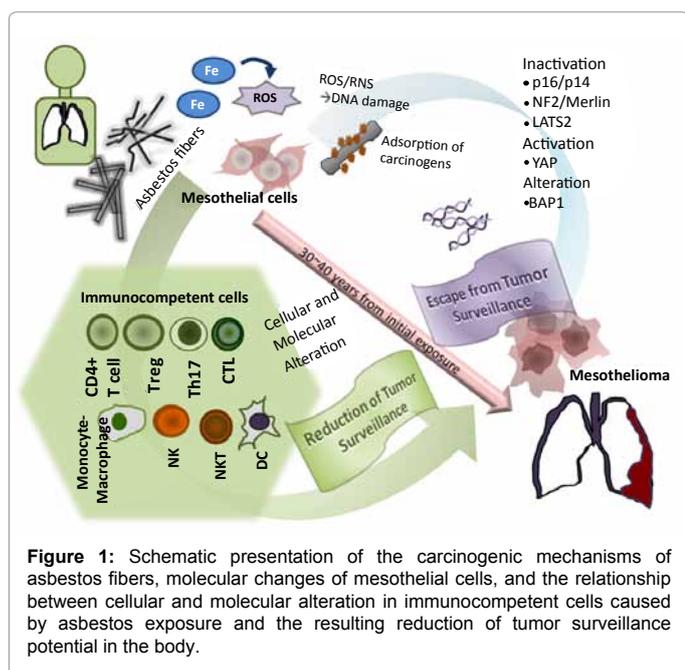
In addition to these immunological effects, asbestos alters mesothelial cells via the production of reactive oxygen/nitrogen species (ROS/RNS) caused by iron included in the asbestos fibers and adsorption of other carcinogens in the lung [42-45]. Mesothelial cells then acquire molecular changes that include inactivation of some tumor suppressors such as p16<sup>ink4</sup> [46,47], merlin/Neurofibromatosis 2 (NF2) [48] and large tumor suppressor 2 (LATS2) [49], activation of Yes-associated protein (YAP1) [50], and alteration of BRCA1-associated protein-1 (BAP1) [51,52] as shown in figure 1.

To explore these aspects, the effects of asbestos on T cells, natural killer (NK) cells, and other types of immune competent cells were investigated. In this review, we detail the altered molecules in T cells and NK cells. Additionally, we show that our experiments have yielded new insights concerning the biological effects of asbestos, and that these findings support the use of immunological biomarkers for the screening of asbestos exposure and MM.

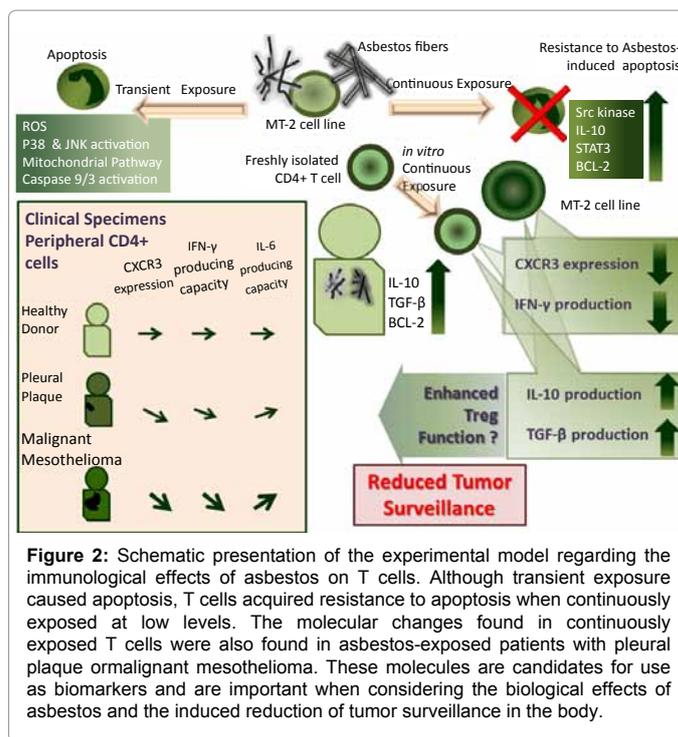
### Continuous Asbestos Exposure Model for T cells

As shown in figure 2, we started by investigating the immunological effects of asbestos on T cells [53] because we had already investigated alteration of Fas/CD95 and its related molecules in T cells following silica exposure [40,41], and it is known that T cells and NK cells play an important role for tumor immunity.

Initially, a human T cell leukemia/lymphoma virus-1 (HTLV-1) immortalized polyclonal T cell line, MT-2 [54], was used to observe



**Figure 1:** Schematic presentation of the carcinogenic mechanisms of asbestos fibers, molecular changes of mesothelial cells, and the relationship between cellular and molecular alteration in immunocompetent cells caused by asbestos exposure and the resulting reduction of tumor surveillance potential in the body.



**Figure 2:** Schematic presentation of the experimental model regarding the immunological effects of asbestos on T cells. Although transient exposure caused apoptosis, T cells acquired resistance to apoptosis when continuously exposed at low levels. The molecular changes found in continuously exposed T cells were also found in asbestos-exposed patients with pleural plaque or malignant mesothelioma. These molecules are candidates for use as biomarkers and are important when considering the biological effects of asbestos and the induced reduction of tumor surveillance in the body.

the effects of transient and high-dose (causing apoptosis) exposure to chrysotile, which is known as the white asbestos. Chrysotile was chosen on the basis of its global use as an economic form of asbestos. We hoped to observe the immunological effects caused by the fibers of this most frequently used form of asbestos, even though it is carcinogenic potential, is considered much less than that of brown (amosite) and blue (crocidolite) asbestos, both of which includes iron [55].

The transient exposure to chrysotile caused the production of ROS, activation of pro-apoptotic signaling molecules such as p38 and c-Jun N-terminal kinase (JNK), activation of the mitochondrial apoptotic pathway, and finally induced apoptosis. We then tried to establish a continuous exposure model involving the MT-2 cell line [53]. Starting from the chrysotile dose that caused apoptosis in less than half of cells subjected to transient exposure, the continuous exposure experiment was maintained for more than eight months. This subline showed transient chrysotile-induced apoptosis [56]. The molecular status of this subline in comparison with the original MT-2 cells showed involvement of Src family kinases, upregulation of IL-10, phosphorylation of Signal Transducers and Activators of Transcription (STAT) 3, and upregulation of Bcl-2 [56]. An additional five chrysotile-continuous exposure subline models and three crocidolite continuous exposure models were established independently [57]. A comparison of the cellular and molecular features of these sublines indicated that all sublines exhibited a similar mRNA expression pattern as analyzed by cDNA microarray, and showed the same particular characteristics concerning acquisition of resistance to apoptosis [57].

These sublines revealed the reduced cell surface expression of chemokine (C-X-C motif) receptor 3 (CXCR3) in CD4+ T cells, as well as suppression of interferon (IFN)-γ production [58,59]. Furthermore, the secretion of transforming growth factor (TGF)-β from the sublines was also enhanced when compared with that of the original MT-2 line. These findings were also confirmed when freshly isolated peripheral CD4+ T cells derived from several healthy donors were activated and cultured *in vitro* with chrysotile fibers.

Reduction of CXCR3 with suppressed IFN- $\gamma$  production results in the reduction of anti-tumor immunity. Moreover, overproduction of IL-10 and TGF- $\beta$  (both cytokines are known as the typical soluble factor for CD4+25+ and fork head box P3 (FoxP3) positive regulatory T cells (Treg)) in the MT-2 line, which is assumed to be an *in vitro* model of Treg, indicated that asbestos exposure may enhance Treg function and reduce anti-tumor immunity. We also compared the inhibitory function of MT-2 original MT-2 cells (never exposed to asbestos) and sublines (continuously exposed to chrysotile or crocidolite) in regard to Treg, namely, suppression of the proliferation activity of responder T cells against foreign antigens, and found that asbestos exposure enhanced Treg function by cell-cell contact via upregulation of some cell surface molecules (unpublished data). The results indicated that asbestos exposure on Treg enhances its function via excess production of soluble factors and increased cell contact.

Although these findings summarized in figure 2 are interesting when considering the biological effects of asbestos in the various contexts of asbestos-induced carcinogenesis, these cellular and molecular changes may be utilized as biomarkers for asbestos exposure and the presence of MM. As expected, we found that plasma levels of IL-10 and TGF- $\beta$  were higher in MM patients, and Bcl-2 expression in peripheral CD4+ T cells derived from MM was higher than that associated with healthy donors.

Regarding CXCR3 and IFN- $\gamma$ , peripheral CD4+ T cells from PP and MM showed reduced expression of CXCR3 when compared with that of healthy donors, a finding that resembles the results we found for *in vitro* assays using the MT-2 cell line or freshly isolated peripheral CD4+ T cells [58,59]. In addition, peripheral CD4+ cells from PP or MM showed a reduced potential to produce IFN- $\gamma$ , although the capacity to produce IL-6 was enhanced in these CD4+ T cells from asbestos-exposed patients.

### Change of NKp46 expression for NK cells in Asbestos-Exposed Patients

As described above, asbestos exposure altered T cell functions and the expression of certain molecules related to anti-tumor immunity. These findings may indicate that asbestos causes a gradual reduction of tumor surveillance potential and makes people sensitive to transforming activity, which results in the development of tumors such as lung cancer and MM, as well as producing a higher risk of developing other tumors of the larynx, GI tract and bladder [3-5].

The direct and main player of tumor surveillance is the NK cell. Therefore, we examined the cellular and molecular alteration of NK cells caused by asbestos exposure. As performed with T cells, the NK cell line YT-1 (kindly provided by Prof. Yodoi of Kyoto University) and freshly isolated NK cells from healthy donors were exposed continuously *in vitro*. Results showed that the expression of some activation receptor molecules such as NKG2D and 2B4 was reduced after long-term exposure to chrysotile. There was also a reduction of killing activity with a decrease of phosphorylation of Extracellular Signal-regulated Kinase (ERK) signaling molecule and inhibition of degranulation of killing molecules such as granzyme and perforin. These results indicated that asbestos exposure causes suppression of NK cell activity and reduced expression of activating receptors [60,61].

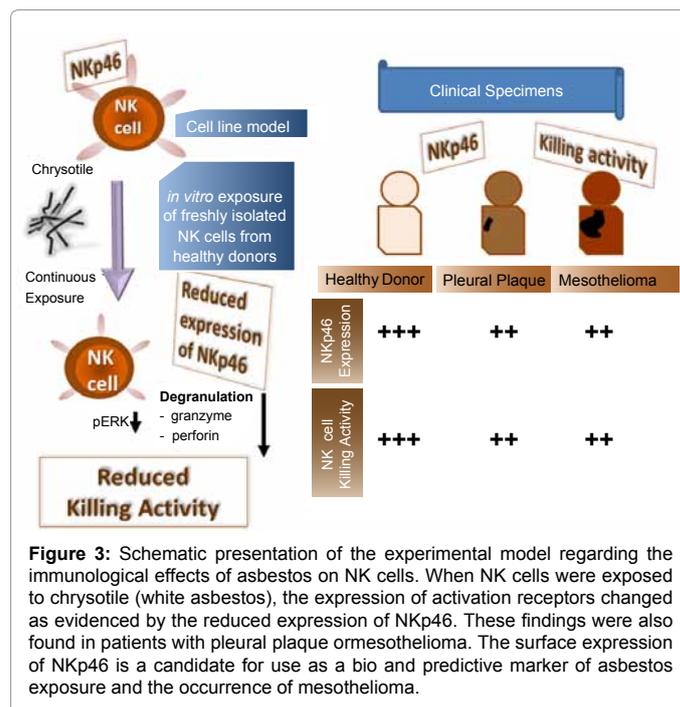
The killing activity and expression of activating receptors in NK cells freshly isolated from asbestos-exposed patients such as PP and MM were then compared with those of healthy donors. As shown in figure 3, the expression of one NK cell activating receptor, NKp46, was decreased and positively related with killing activity [62,63].

These findings are of interest when considering the biological effects of asbestos in regard to reduced tumor surveillance. Furthermore, the expression of NKp46 in NK cells may be utilized as a marker of asbestos exposure and the development of MM in asbestos-exposed patients.

### Other Trials

A recent report involving a comprehensive analysis using proteomics in serum [64] was performed in asbestos-exposed people with or without MM and indicated several molecules as markers for the detection of MM, and their potential usefulness as candidates for the screening of asbestos-exposed people using blood. The taking of blood samples has more advantages compared with radiological examinations. It is therefore possible to select 10 to 20 candidate molecules that change in asbestos-exposed individuals and/or patients with MM, and use these molecules for a screening system concerning the early detection of asbestos exposure and MM. We may also decide, for example, that a radiological examination is necessary if more than half of these molecules have changed in an individual.

To further investigate this type of approach, we recently performed comprehensive analyses of immunological molecules in plasma such as interleukins, chemokines, and other molecules related to immune responses because asbestos influences immunocompetent cells as shown above, and changes of immune cells caused by asbestos exposure can be detected using peripheral blood. Our investigations have not only focused on detecting the differences among healthy donors, PP and MM patients, but we have also recently examined alterations of these molecules in plasma between asbestos- and silica-exposed patients. As mentioned above, it may be interesting to compare findings obtained from silicosis patients with those derived from certain autoimmune diseases such as rheumatoid arthritis and systemic sclerosis to further elucidate the effects of silica exposure. The results of such an investigation will be presented in the near future.



**Figure 3:** Schematic presentation of the experimental model regarding the immunological effects of asbestos on NK cells. When NK cells were exposed to chrysotile (white asbestos), the expression of activation receptors changed as evidenced by the reduced expression of NKp46. These findings were also found in patients with pleural plaque or mesothelioma. The surface expression of NKp46 is a candidate for use as a bio and predictive marker of asbestos exposure and the occurrence of mesothelioma.

## Conclusion

Discovery of the alteration of immune cells such as T cells and NK cells following asbestos exposure is interesting for a consideration of the biological effects of asbestos, as well as the mechanisms surrounding asbestos-induced carcinogenesis [65,66]. Additionally, the changes of immune cells are easily detected using peripheral blood. Taking a peripheral blood sample from a patient is an easier procedure to perform relative to other procedures; it can potentially be performed frequently, and involves less risk of irradiation and a lower cost compared with radiological examinations. Moreover, the monitoring of 10 to 20 altered molecules using the blood sample represents the best approach for an initial screening system concerning the detection of asbestos exposure and developing MM. Although tumor biomarkers have a certain limitation in that serum levels of molecules produced by tumors are dependent on tumor volumes, the combined use of immunological molecules and molecules produced by tumors may allow us to determine whether an examined individual should proceed towards detailed examinations such as radiological analyses using X-P, CT and MRI.

In this review, we briefly introduced our findings examining the immunological effects of asbestos and the method by which these findings could be used clinically for an early screening system for asbestos exposure and developing MM.

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