

Review of Reduced Tumor Immunity Caused by Asbestos Exposure to Immunocompetent Cells Such as T and Nk Cells

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Abstract

Although the immunological effect of silica, SiO₂, has been well investigated and involves the dysregulation of autoimmunity because of complications of autoimmune diseases found in silicosis, the immunological effects of asbestos, a mineral silicate possessing Si and O as core atoms, have not been well investigated. Asbestos can cause alteration of immunocompetent cells to result in a decline of tumor immunity. The experimental continuous exposure of chrysotile asbestos onto a human T cell line or NK cell line resulted in reduction of tumor immunity in both cell types. In the T cells, important molecules for tumor immunity such as CXCR3 chemokine receptor and IFN- γ exhibited reduced expression and production, respectively, not only for the cell line mode, but also for freshly isolated CD4⁺ T cells from healthy donors exposed *ex vivo*, as well as T cells derived from asbestos-exposed patients such as those with pleural plaque (PP) or malignant mesothelioma (MM). The activating receptors in NK cells such as NKG2D, 2B4 or NKp46 exhibited reduced expression in the asbestos-exposed NK cell line and freshly isolated NK cells exposed to asbestos *ex vivo*, from healthy donors as well as patients with PP or MM. Additionally, activation of the ERK signaling molecule is reduced in asbestos-exposed NK cells. These results strongly indicate asbestos exposure causes reduction of tumor immunity. Furthermore, improving these functions through certain bioactive substances in foods, plants or microorganisms may be a good tool for chemoprevention in asbestos-exposed people concerning future development of cancers.

Keywords: Asbestos; T cell; NK cell; Tumor immunity

Introduction

Asbestos exposure causes a variety of tumors such as malignant mesothelioma and lung cancers [1,2], in addition to laryngeal, gastrointestinal and bladder cancers [3-5].

Issues involving asbestos usage are also varied. Although the majority of developed countries have already banned the use of asbestos, a few nations are still exporting asbestos to developing countries and people in these asbestos-importing nations encounter many chances of being exposed to asbestos. Moreover, asbestos is still present in the buildings, water-pipes and other structures of nations that have even banned the material, and many workers such as the wrecking crews of buildings are under threat of attack from asbestos exposure.

In Japan, the asbestos issue erupted in the summer of 2005 [6-8]. Residents were suddenly informed that asbestos, which was used in large amounts from the early 1950s up to the early 1990s in Japan with a maximum usage of approximately 352,000 tons in 1974, caused malignant mesothelioma (MM). Residents that lived near the asbestos handling manufacturer Kubota Corporation, in Amasasaki City, Hyogo Prefecture, developed MM. They had never worked in the asbestos-handling manufacture industry. In addition, medical information regarding MM induced anxiety in Japanese people, since the prognosis is very poor and there is no certain way to detect the cancer in the very early stage of the disease. Furthermore, people could not remember being exposed to asbestos 30 to 40 years ago. These matters increased the anxiety of residents. Furthermore, there is concern for people who are involved in processing earthquake rubble following Japan's 2011 earthquake, since a news report revealed a case of mesothelioma for an

individual who handled earthquake rubble after the Japanese Hanshin-Awaji earthquake in 1995 [9].

Reports of other issues regarding asbestos-induced malignant tumors such as malignant mesothelioma have not provided sufficient clinical, cellular or molecular details for an elucidation of these diseases in regard to better prevention, early diagnosis, and improvement of prognosis [10,11]. Of course, it is certain that recent advances in medical science regarding mesothelioma are progressing towards an understanding of the cellular and molecular aspects of mesothelioma cells that will contribute to newer diagnostic and therapeutic developments [12-14].

For example, the alteration of oncogenes and tumor suppressor genes in mesothelioma was recognized as a loss of p16-ink4 family cyclin dependent kinase-inhibitor (CDK-I) in the majority of cases of mesothelioma cells, and neurofibromin 2 (NF2)/merlin tumor suppressor was silenced by genomic deletion of epigenetic alteration in approximately half of the cases [15,16]. However, a large tumor suppressor homolog 2 (LATS2) gene located on chromosome 13q12

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was recently reported as a newer tumor suppressor in mesothelioma. LATS2 encodes a serine/threonine kinase, a component of the Hippo tumor-suppressive signaling pathway localized down-stream of MF2 [17,18]. Transduction of LATS2 in mesothelioma cells with its mutation inactivated oncoprotein YAP, a transcriptional coactivator, via phosphorylation and inhibited cell growth. Moreover, it was reported that the BRCA1 (breast cancer susceptibility gene 1) associated protein-1 (ubiquitin carboxy-terminal hydrolase)/BAP1 gene is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma [19, 20].

On the other hand, the cellular and molecular effects of asbestos regarding carcinogenesis are considered to be caused by DNA damage due to production of reactive oxygen/nitrogen species (ROS/RNS) by iron-including asbestos [21,22], chromosome tangling due to direct physiological attack by asbestos fibers on cellular chromosomes, and adsorption of various carcinogens from cigarette smoking and air pollutants surrounding asbestos in the lung as shown in figure 1 [23-25].

We have been investigating the immunological effects of environmental substances such as silica and asbestos, which is a mineral silicate including SiO₂ with magnesium, calcium and iron [26-28]. Silicosis patients suffer not only from pulmonary fibrosis, but often experience complications from autoimmune diseases [29,30] such as rheumatoid arthritis (known as Caplan's syndrome) [31,32], Anti-Neutrophil Cytoplasmic Antibody (ANCA)-related vasculitis/nephritis [33,34], and systemic sclerosis [35,36]. Although the cause of this dysregulation of autoimmunity was thought to be due to the adjuvant effects of silica, we have demonstrated that alteration of CD95/Fas, the death receptor inducing cellular apoptosis, in silicosis patients [37-39] and direct activation of responder T cells and CD4+25+ forkhead box P3 (FOXP3) positive regulatory T (Treg) cells by silica exposure and resulting loss of Treg and imbalance of responder T cells and Treg causes reduction of the inhibitory effects of Treg against activation of responder T cells including auto-reacting T cells [40-43].

From these investigations, we thought that asbestos may affect the human immune system and that these effects would tend to reduce tumor immunity because of malignant complications in asbestos-exposed patients. In this review, we introduce our experimental studies regarding asbestos exposure and alteration of tumor immunity using cell lines, freshly isolated human immunocompetent cells from healthy donors (HD), patients with pleural plaque (PP) who have a history of past exposure to asbestos, but not having malignancies, and patients with mesothelioma (MM) as shown in figure 1.

Continuous and Low-Dose Exposure of Asbestos onto T Cells

Temporary and high-dose exposure of chrysotile asbestos on a human HTLV-1 (Human Adult T Cell Leukemia Virus1) immortalized polyclonal T cell line, Mt-2

First of all, we started to investigate the immunological effects of asbestos using a human T cell line and chrysotile. We used a cell line in order to further investigate cellular and molecular alteration of T cells, and chrysotile was selected because it was the most frequently used asbestos and resembled silica chemically.

As we reported previously, a temporary and relatively high-dose (causing massive apoptosis for three days of *in vitro* exposure) exposure of chrysotile onto MT-2 cells caused apoptosis in a dose- and time-

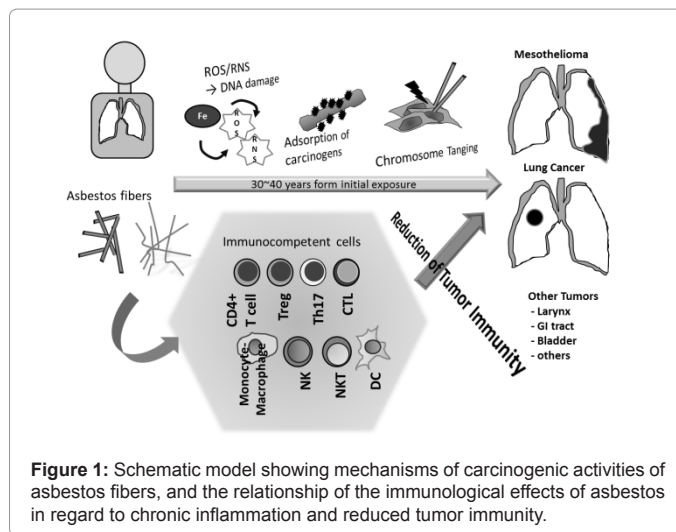


Figure 1: Schematic model showing mechanisms of carcinogenic activities of asbestos fibers, and the relationship of the immunological effects of asbestos in regard to chronic inflammation and reduced tumor immunity.

dependent manner, activation of the mitochondrial apoptotic pathway involving phosphorylation of c-Jun N-terminal kinase (JNK) and p38 in the Mitogen-Activated Protein Kinase (MAPK) pathway, increase of BAX expression, release of cytochrome-c from mitochondria to the cytoplasm, and production of superoxide. These results indicated that asbestos can cause cellular and molecular alteration in lymphocytes similar to phenomena reported in pulmonary epithelial and mesothelial cells temporarily exposed to asbestos [44].

Continuous and low-dose exposure of chrysotile onto the Mt-2 cell line

After characterization of cellular and molecular alteration of MT-2 cells temporarily exposed to chrysotile, we attempted a continuous and relatively low-dose (doses causing less than half of cells to proceed to apoptosis when exposed temporarily) exposure experiment that was maintained for more than eight months. Thereafter, when a continuously exposed subline was released from chrysotile exposure and re-exposed to a high dose temporarily, it exhibited resistance to chrysotile-induced apoptosis. The cellular and molecular characterization of the subline revealed activation of Src-family kinases, upregulation of expression and secretion of interleukin (IL)-10, phosphorylation of Signal Transducer and Activator of Transcription 3 (STAT3), and upregulation of Bcl-2 localized down-stream of STAT3. These cascaded mechanisms were identified as cellular and molecular changes in MT-2 cells continuously exposed to chrysotile. In addition, other cellular changes involved upregulation of transforming growth factor (TGF)- β and reduced production of various cytokines. Furthermore, we established another five sublines that were exposed to chrysotile in a similar manner and independently. All of the six sublines exhibited similar gene expression profiles according to cDNA microarray analysis, and the effects on these cell lines are mostly confirmed as the effects of chrysotile on human T cells and not due to alterations obtained by chance. As further confirmation of these findings, serum TGF- β and IL-10 levels and the gene expression of Bcl-2 derived from peripheral CD4+ T cells from PP and MM patients exhibited increases of both cytokines and enhanced expression of Bcl-2 compared to HD [44,45].

Reduction of Cxcr3 chemokine receptor and production of IFN- γ

To investigate alteration of cellular and molecular markers in MT-2 sublines continuously exposed to chrysotile, the CXC chemokine

receptor 3 (CXCR3) and interferon- γ were examined since cDNA array, pathway and signaling canonical analysis, as well as network analysis, indicated that these molecules were involved and altered in all sublines when compared with the original (never exposed) MT-2 line [46,47].

Thus, the expression of CXCR3 in MT-2 sublines and in long-term exposure to chrysotile using freshly isolated peripheral CD4⁺ T cells were analyzed. All of the MT-2 sublines showed reduction of CXCR3 expression in mRNA and protein (examined via Western blotting, flow cytometry, and fluorescent immunohistological staining). In addition, four weeks of exposure to chrysotile caused reduction of CXCR3 expression when freshly isolated CD4⁺ T cells derived from six HDs were cultured with IL-2 and chrysotile. Similar to CXCR3, other Th1 type molecules such as IFN- γ , C-X-C motif chemokine 10 (CXCL10)/IFN- γ -induced protein 10 (IP-10) as the ligand for CXCR3, and chemokine (C-C motif) ligand 4 (CCL4)/Macrophage inflammatory protein-1 (MIP-1)- β exhibited reduced production or mRNA expression in all of the MT-2 sublines. In addition, the peripheral CD4⁺ T cells from patients with PP or MM exhibited increased IL-6 production compared with cells from HDs when these cells were stimulated *in vitro* with anti-CD3/CD28 antibodies for five days. Finally, peripheral CD4⁺ T cells derived from PP or MM patients showed CXCR3 surface expression [46,47].

Since CXCR3 is a G-protein-coupled seven-transmembrane receptor expressed on various lymphocytes including T, B and natural killer (NK) cells, it binds to IFN- γ -inducible chemokines such as CXCL9/Monokine induced by IFN- γ (MIG), CXCL10/IP10 and CXCL11/IFN-inducible T-cell alpha chemoattractant (I-TAC) that recruit leukocytes to inflammatory sites such as tumors. In the case of CD4⁺ T cells, the CXCR3 is preferentially expressed and IFN- γ -producing Th1/effector T cells exhibit a high-level production of inflammatory cytokines. As described above, there was a high production of anti-inflammatory cytokine IL-10 in MT-2 sublines and also in plasma from PP or MM patients. However, CD4⁺ T cells from these patients showed a high production potential of IL-6. Taken together, continuous exposure to asbestos caused reduction of tumor immunity induced by CXCR3 and IFN- γ cytokine networks, whereas the lesions where asbestos fibers are localized may be modified immunologically and there may be chronic inflammation suggested by potential IL-6 production and molecular changes leading to carcinogenesis. IL-10 may have an effect later when cells start to transform at the locus. As shown in figure 2, the overall results indicate that the effects of asbestos on CD4⁺ T cells modify the inflammatory and transforming status in the lesion, where asbestos and mesothelial cells are co-localized such that carcinogenesis develops slowly and progressively over 30 to 40 years, which is regarded as the latency period for the occurrence of mesothelioma [46-49].

Effects of asbestos on T Cells such as Treg

The function and numbers of Treg are very important for a consideration of tumor immunity. As mentioned above, silica exposure can activate Treg to express the CD95/Fas molecule and induce early loss of Treg to create an imbalance between Treg and responder T cells which include self-antigen recognizing T cells. These effects may partially explain the occurrence of autoimmune diseases such as the complications of silicosis.

We have been analyzing Treg functions in regard to asbestos using a continuous and low-dose exposure cell line or *ex vivo* stimulating models. However, we have so far not obtained results that have been confirmed, and will report the *in vitro* modification of Treg function using human cells in the near future.

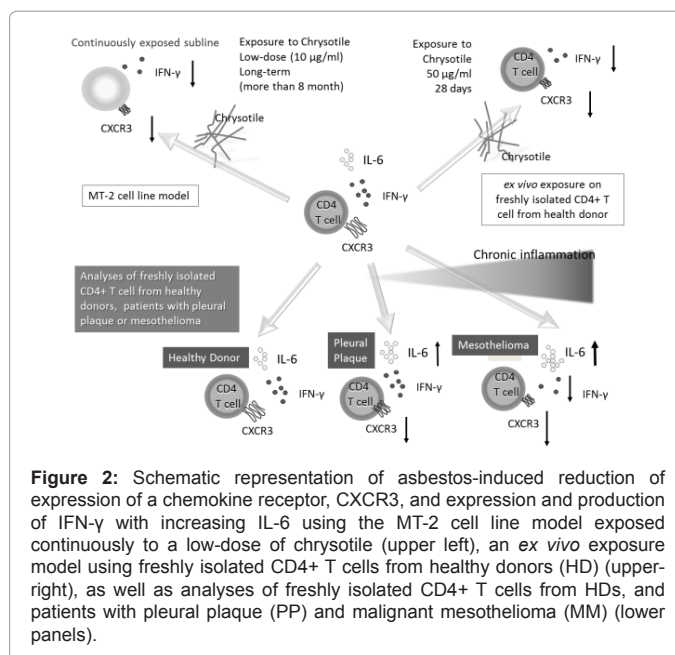


Figure 2: Schematic representation of asbestos-induced reduction of expression of a chemokine receptor, CXCR3, and expression and production of IFN- γ with increasing IL-6 using the MT-2 cell line model exposed continuously to a low-dose of chrysotile (upper left), an *ex vivo* exposure model using freshly isolated CD4⁺ T cells from healthy donors (HD) (upper-right), as well as analyses of freshly isolated CD4⁺ T cells from HDs, and patients with pleural plaque (PP) and malignant mesothelioma (MM) (lower panels).

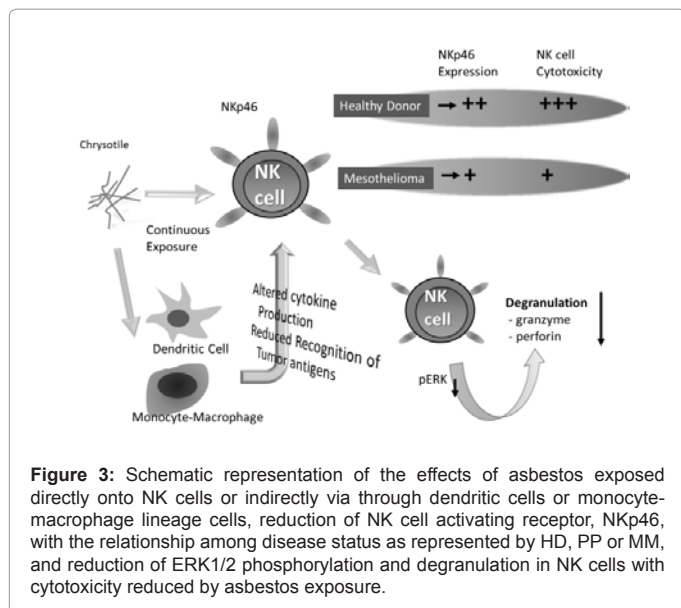
Alteration of NK cells Cytotoxicity and Expression of Activating Receptor

Impairment of cytotoxicity of NK cells exposed to asbestos

Similar to the above-mentioned analyses targeting human T cells, the effects of asbestos on the function of NK cells, direct killers of tumor cells, were analyzed using a human NK cell line (YT-A1, kindly provided from Dr. Y. Yodoi, Kyoto University) and freshly isolated NK cells from HD exposed to chrysotile *ex vivo*. NK cell cytotoxicity was reduced when YT-A1 cells were continuously exposed to a relatively low-dose concentration (the same concentration used for the MT2 culture). Regarding cytotoxic granules, granzyme B and perforin exhibited reduced intracellular expression as analyzed by flow-cytometry. Moreover, among the various NK cell-activating receptors, the expressions of NKG2d and 2B4, which belong to the NKG2 family characterized by a lectin-like domain, decreased during continuous exposure. In addition, freshly isolated NK cells from HDs exposed *ex vivo* showed the same reduction of cytotoxicity, whereas the key molecule influenced by chrysotile exposure was Nkp46-activating receptor belonging to the NCR (natural cytotoxicity receptor) group with Nkp44 and Nkp30. Furthermore, freshly isolated NK cells from patients with MM exhibited a reduction of Nkp46, but not NKG2D or 2B4. Taken together and considering that the results obtained from the cell line may not be natural, the target of asbestos that reduces NK cell cytotoxic activity is the Nkp46 molecule. It may be possible to use the expression level of Nkp46 in peripheral NK cells as a marker for asbestos-exposure or tumor-bearing in people who have handled asbestos through the work environment or those who lived near asbestos-handling industries as shown in figure 3 [50].

Signaling disturbance in NK cells exposed to chrysotile

As mentioned above, NK cell activity was reduced by asbestos exposure. Alteration of the signaling pathway was analyzed according to the reduction of NKG2D and 2B4 expression levels in the NK cell line we used. Although NK cell-activating receptors possess different adaptor molecules to induce degranulation, the most important



among these molecules is extracellular signal-regulated kinase (ERK). Thus, the phosphorylation status of ERK1/2 was examined using the YT-A1 subline continuously exposed to chrysotile. In spite of the enhancement of ERK1/2 phosphorylation when YT-A1 original cells were cultured with target tumor cells, the K562 subline did not show any increase of phosphorylation of ERK1/2. In addition, the reduction of phosphorylation of ERK1/2 in the YT-A1 cell line was also obtained when cells were treated with wortmannin and PP2, inhibitors of phosphoinositide 3-kinase (PI3K) and Src-family kinase, respectively, and when NK cells were cultured with target K562 cells and anti-2B4 or NKp46 antibodies were added to the culture [51].

These results strongly suggest that NK cells exposed to asbestos reduced their cytotoxic activity via inhibition of signaling pathways involving the ERK1/2 molecule and decrease of degranulation in perforin and granzyme B as shown in figure 3. Further investigation will be conducted regarding modification of NK cell activity influenced by asbestos-exposed dendritic cells or monocyte/macrophage lineage cells, since their production of cytokines may affect K cell activity and production may be altered by asbestos exposure onto these types of cells [50-52].

Conclusion

As described above, the effects of asbestos on T cells and NK cells reduce their activity against tumor cells. However, other immunocompetent cells such as Treg, Th17, CD8+ cytotoxic T cells (CTL), and natural killer T (NKT) cells, as well as antigen presenting cells such as D, may influence their function following continuous exposure to asbestos. Alteration of the functions and differentiations of these various cells affected by asbestos exposure should be investigated to better understand the reduction of tumor immunity caused by chronic asbestos exposure. In particular, the effects on Treg, CTL and Th17 cells should be analyzed immediately and it should be determined how asbestos influences total tumor immunity and how strongly individual cell type is involved in reduced tumor immunity. Moreover, the effects of other fibers such as crocidolite and amosite should be investigated since these iron-including fibers are thought to possess stronger carcinogenicity than chrysotile.

The processes involved in tumor immunity in asbestos-exposed people should be elucidated for the purpose of chemoprevention of asbestos-induced malignancies. Some bioactive substances including foods, plants or microorganisms may recover the reduced function of immunocompetent cells caused by asbestos-exposure. These types of trials may help to relieve the anxiety experienced by most people who worked in the asbestos-handling industries or lived near these manufacturers.

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