upper zone from upper lobe and subcarinal or lower zone from lower lobe in addition to hilar and intrapulmonary nodes. Fortunately, none of the patients in the present study treated with sublobar resection had lymph node recurrence. However, the concept of sentinel lymph node is still controversial because of the presence of variant lymphatic vessels that sometimes cause skip mediastinal metastases, especially in cases with pleural invasion [21,22]. Intraoperative evaluation of hilar and mediastinal nodes should be considered for sublobar resection as a minimum requirement in cases with pleural involvement.

With regard to the preoperative serum CEA level, no significant impact on DFS was found in PSL patients, while the rate of serum CEA elevation was evidently higher in PSL patients than in SCDL patients. Though our previous study analysing small-sized NSCLC including GGO lesions indicated the prognostic impact of prethoracotomy CEA level on postoperative survival and a relationship with lymph node metastasis [12], the clinical significance of the CEA level is unclear in patients with pure solid small-sized lesions.

Recurrence was found mainly in the thorax, including lymph nodes, pulmonary metastases or lymphangitis, pleural dissemination and resected margin within 3 years after operation. These results indicate that solid small-sized lung cancer might be cured when sufficient intervention to achieve local control is performed. According to 'NCCN Clinical Practice Guidelines in Oncology, Non-small Cell Lung Cancer', a contrast-enhanced chest CT every 6 months for 2 years, followed by non-contrast-enhanced CT annually, is recommended in completely resected stage I–IIIA NSCLC. This guideline may also be appropriate in solid small-sized NSCLC, in which the main focus of recurrence is the loco-regional thorax. Adjuvant therapy following surgery could be discussed in NSCLC patients with a small-sized solid tumour, though a further prospective clinical study is needed.

In conclusion, the characteristics of c-stage IA lung cancer with a pure solid or solid component-dominant lesion smaller than 2 cm were addressed using survival analysis of completely resected cases. A proportion of solid-type NSCLC has malignant potential with nodal or pleural involvement. Because sublobar resection for such solid small-sized lesion might have the risk of underestimation of nodal status, the feasibility should be evaluated in a prospective clinical trial. In addition, periodic surveys for local recurrence are recommended for 3 years following surgery in patients with solid-type lung cancer.

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SUPPRESSIVE EFFECT OF ASBESTOS ON CYTOTOXICITY OF HUMAN NK CELLS

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Asbestos, a naturally occurring fibrous mineral, causes malignant mesothelioma (MM). However, it takes a very long time to develop MM, which suggests that effects other than tumorigenicity of asbestos might contribute to the development of MM, and one of the possible targets is anti-tumor immunity. Therefore, we examined the effect of asbestos exposure on human natural killer (NK) cells using the cell line of YT-A1, peripheral blood mononuclear cells (PBMCs) cultures and specimens from patients with MM. In particular, we focused on expression of NK cell-activating receptors, including NKG2D, 2B4 and NKp46. Analysis of the YT-CB5 subline of YT-A1, cultured with CB for over 5 months, showed a decrease in cytotoxicity with low expressions of NKG2D and 2B4, although there were no decreases after about one month. YT-CB5 showed decreases in phosphorylation of extracellular signal-regulated kinase (ERK) and degranulation stimulated by antibodies to NKG2D. Peripheral blood (PB-) NK cells from MM patients also showed decreased cytotoxicity compared with healthy volunteers (HV), and was accompanied with low expression of NKp46 unlike YT-CB5. PBMCs cultured with CB resulted in decreased expression of NKp46 on NK cells, although this did not occur when using glass wool, an asbestos substitute. These results indicate that asbestos has the potential to suppress cytotoxicity of NK cells. In particular, it is noteworthy that both NK cells from MM patients and those from a culture of PBMCs derived from HVs with asbestos showed the same characteristic of decreased cytotoxicity with low expression of NKp46.

Asbestos and anti-tumor immunity

Asbestos, a naturally occurring fibrous mineral, is known to cause malignant mesothelioma (MM) and lung cancer. The tumorigenic effects of asbestos, including cellular toxicity, mutagenicity and reactive oxygen species (ROS) production, have been investigated (1-3). It was shown that there was a strong correlation between levels of oxidized pyrimidines and alkylated bases and the length of occupational exposure to asbestos, and that intratracheal instillation of asbestos caused an increase in the mutation frequency of lung DNA (4, 5). However, it takes a very long time, about 40 years, to develop MM after exposure to asbestos (6-8). These findings suggest that potential effects other than tumorigenicity of asbestos might contribute to the development of MM. The immunological function, especially anti-tumor immunity, is one of the possible targets following exposure to asbestos (Fig. 1). In fact, it has been reported that rats instilled with asbestos showed delayed translocation of asbestos from lungs to draining lymph nodes, and that shipyard workers showed accumulated asbestos in draining lymph nodes and the lungs (9, 10). These observations indicate that immune-competent cells have a chance to encounter inhaled asbestos, which might cause an alteration of anti-tumor immunity. Anti-tumor immunity contributes to the removal of transformed or tumor cells, where natural killer (NK) cells play a role as first effectors, followed by clonally expanded cytotoxic T lymphocytes. In addition, it has been reported that people with low NK activity of peripheral lymphocytes show a high incidence rate of cancer (11). Therefore, we tried to examine the effect of exposure to asbestos on cytotoxicity of human NK cells using the cell line of YT-A1, peripheral blood mononuclear cells (PBMC) cultures and specimens

Key words: silica, autoimmune, Fas, regulatory T cells.

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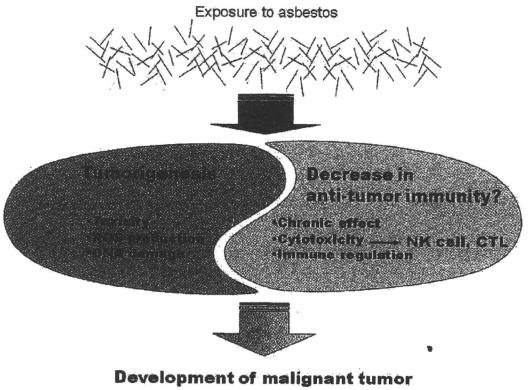

Fig. 1. *Asbestos and anti-tumor immunity. The tumorigenicity and assumed immune-suppressive effect of exposure to asbestos are illustrated. Tumorigenesis in relation to cellular toxicity, reactive oxygen species (ROS) and DNA damage is well known (left part). We propose the hypothesis that the suppression of anti-tumor immunity might be caused by chronic exposure to asbestos (right part). The effectors of NK cells and cytotoxic T lymphocytes (CTL) and regulatory T cells (Treg) play a role in injuring target cells and regulating immune status, respectively. The suppression of anti-tumor immunity might promote the development of malignant mesothelioma evoked by the tumorigenicity of asbestos.*

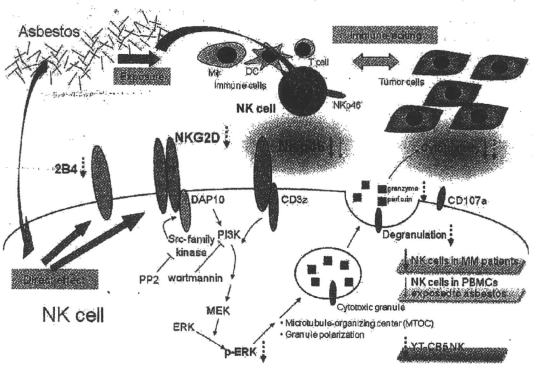

Fig. 2. *NK cell-activating receptors and the pathway from their ligation to degranulation, and summary of the study results concerning the effect of asbestos exposure on NK cells. The NK cell-activating receptors NKG2D, 2B4 and NKp46 induce phosphorylation of extracellular signal-regulated kinase (ERK), mediated by Src-family kinases and phosphoinositide 3-kinase (PI3K). ERK phosphorylation leads the movement of cytotoxic granules and the microtubule-organizing center (MTOC) to the near side of the cellular membrane, resulting in release of granzymes and perforin, i.e., degranulation. The results obtained from the studies using YT-CB5 and specimens from patients with MM and PBMC cultures upon exposure to asbestos are shown as dotted, thick and thin arrows, respectively.*

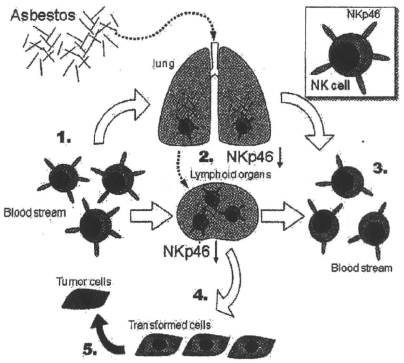

Fig. 3. *The possible mechanism of functional impairment in NK cells as caused by exposure to asbestos. 1. NK cells circulate through lymphoid and non-lymphoid organs, including the lungs. 2. Asbestos fibers accumulated in the lungs or translocated into lymph nodes cause the decrease of cell-surface NKp46 in part of the NK cells. 3. NK cells with a decrease in NKp46 re-circulate into the bloodstream. 4. These NK cells show impaired cytotoxicity against transformed cells. 5. The functional impairment of NK cells allows tumor cells to escape from surveillance by anti-tumor immunity.*

from patients with MM. In particular, we focused on expression levels of NK cell-activating receptors, utilized to recognize target cells and transduce activating signal into cytosol leading to degranulation of cytotoxic granules having granzymes and perforin.

NK cells and NK cell-activating receptors

NK cells utilize a great variety of cell-surface receptors to recognize and injure targets, transformed and tumor cells, unlike T- and B-lymphocytes, which use T-cell receptor (TCR) and surface immunoglobulin (sIg), respectively. The cytotoxicity of NK cells is induced by ligation of NK cell-activating receptors, while it is inhibited by suppressive receptors (12-14). The NKG2 family is a well-known group of NK-cell receptors that is characterized by a lectin-like domain. It includes members with activating and inhibitory functions such as NKG2D and NKG2A, respectively. NKG2D forms activating homodimers, the ligands of which are MHC class-I-chain-related protein A (MICA), MICB and the UL16-binding protein (ULBP) family in humans (15, 16). It is known that expression of MICA or MICB is upregulated by many tumor-cell lines and primary tumors of epithelial origin (17, 18). In contrast, NKG2A forms suppressive heterodimers with CD94, and its MHC class I ligand inhibits cytotoxicity of NK cells. Natural cytotoxicity receptors (NCRs), including NKp46, NKp44 and NKp30, also play a major role in NK-mediated killing of targets. Although their ligand has not been revealed as yet, previous studies have demonstrated the contribution of NKp46 to cytotoxicity against the various kinds of tumor (19-22). In addition, several kinds of signaling lymphocytic activation molecule (SLAM) family receptors can activate NK cells to exert cytotoxicity. 2B4 (CD244) is a representative SLAM family receptor expressed on NK cells, and CD48, the ligand for 2B4, can induce cytotoxicity. Following the engagement of each ligand, these NK cell-activating receptors finally provide phosphorylation of extracellular signal-regulated kinase (ERK) and Jun N-terminal kinases (JNK), by which granzymes and perforin can be released to the intercellular space between NK and target cells to induce killing activity (Fig. 2).

Impaired cytotoxicity of the human NK cell line continuously cultured with asbestos

The study concerning the effect of asbestos exposure on NK cells focused on expression level of NK cell-activating receptors and began by using the human NK cell line, YT-A1. To examine the effect of long-term exposure to asbestos, YT-A1 cells were continuously cultured with chrysotile B (CB) asbestos at 5 μg/ml, named YT-CB5, assayed for cytotoxicity and expression of NK cell-activating receptors, and compared with the other subline cultured without asbestos, YT-Org. YT-CB5 showed cytotoxicity to the same degree as YT-Org during

a month of the culture. However, after about five months YT-CB5 showed a decrease in cytotoxicity, and there was a clear difference between both sublines. Therefore, the survey concerning expression levels of NK cell-activating and suppressive receptors and others on the cell surface of YT-CB5 was conducted using flow cytometry. The expression level of CD56 and CD16, NK-cell marker and low affinity Fc receptor, respectively, altered slightly. NKG2A, which makes a suppressive receptor with CD94, also did not increase. However, YT-CB5 showed decreased expression levels of NKG2D and 2B4. In accordance with these decreases, a decrease in degranulation induced by stimulation with antibodies to NKG2D or 2B4 was observed in YT-CB5. In addition, YT-CB5 also showed decreases in intracellular granzyme A and perforin. (23). To confirm the signal transduction downstream of these receptors, phosphorylation of ERK was examined. YT-CB5 showed a decrease in phosphorylation of ERK1/2 stimulated by recognition of target cells. The antibodies to NKG2D also induced a slight ERK phosphorylation in YT-CB5, although antibodies to 2B4 did so to the same degree as YT-Org. Peripheral blood (PB-) NK cells from healthy volunteers (HV) also exhibited a gradual decrease in the phosphorylation of ERK1/2 following stimulation via NKG2D as NKG2D cell-surface expression levels decreased. Likewise, PB-NK cells exhibited decreased phosphorylation of ERK following stimulation via NKp46 as NKp46 expression became low. PB-NK cells with the lowest cytotoxicity showed low expression levels of NKG2D and NKp46 and low phosphorylation of ERK1/2 following stimulation via NKG2D or NKp46, whereas PB-NK cells with the highest cytotoxicity showed high expression levels of the aforementioned receptors and high phosphorylation levels of ERK1/2 following stimulation. These results indicate that long-term exposure to asbestos has the potential to suppress expression levels of NK cell-activating receptors on the cell surface of NK cells, resulting in an impairment of cytotoxicity due to decreased signal transduction downstream of those receptors (24).

Common decrease of NKp46 to NK cells of mesothelioma patients and asbestos-exposed PBMCs

The results obtained from the study using the NK cell line raised the question whether a functional decrease similar to YT-CB5 might be observed in the PB-NK cells of patients with MM. Therefore, cytotoxicity and cell-surface expression of NK cell-activating receptors in PB-NK cells were examined and compared between HV and MM groups. The NK cells of patients with MM exhibited lower cytotoxicity than those of HV. However, the expression levels of NKG2D and 2B4 did not differ between the HV and MM groups, unlike the results obtained from the study using the cell lines. Therefore, to explore the possibility of a decrease in the expression of an NK cell-activating receptor other than NKG2D and 2B4, an assay was performed for the surface expression of NKp46. The NK cells of patients with MM exhibited a lower expression level of NKp46 than those of HV. However, the asbestos-exposed subline YT-CB5 did not exhibit a decrease. Therefore, the effect of asbestos exposure on expression of NKp46 was examined by the other experiments in which PBMCs prepared from HV were cultured with CB at 5 μg/ml upon stimulation with IL-2, and assayed for cell-surface expression of NKp46 on $CD3^-$ $CD56^+$ NK cells. The NK cells in the culture with CB for seven days showed a lower expression level of cell-surface NKp46 compared with those in the control culture, whereas the levels of NKG2D and 2B4 on NK cells did not differ between the cultures, the characteristics of which were the same as PB-NK cells in patients with MM showed. In contrast, exposure to glass wool, which was used as a substitute for asbestos, did not cause a decrease in expression of NKp46 on NK cells in the culture. These results indicate that NK cells in patients with MM have a characteristic low cytotoxicity with decreased expression of cell-surface NKp46, a decrease which was also shown by NK cells in PBMCs exposed to asbestos.

Conclusion and discussion

The results of our studies mentioned above demonstrated that asbestos exposure has the potential to suppress cytotoxicity accompanied with alteration in expression of NK cell-activating receptors. The decreased cytotoxicity of YT-CB5 suggests that chronic exposure to asbestos inhaled into the body might gradually impair cytotoxicity of NK cells during the long period of exposure. Although the decreased expression of NKG2D was observed in YT-CB5, but not in PB-NK cells of patients with MM, it is possible that this characteristic of YT-CB5 might reflect NK cells that have been continuously and directly exposed to asbestos in the local area. NK cells of patients with MM showed low cytotoxicity accompanied with decreased expression of NKp46. It is interesting that NK cells in PBMCs cultured with asbestos, but not those cultured with non-asbestos fibers, also showed the decrease in NKp46 without decreases in NKG2D or 2B4, as in the case of MM patients. These findings suggest that asbestos exposure might cause a decrease in expression of NKp46 on the cell surface, resulting in impaired cytotoxicity of NK cells in patients with MM. The NK cells examined and compared between HV and MM groups were derived from peripheral blood. However, NK cells can circulate throughout NK cell-containing systems such as blood, spleen, lymph node, liver and lung (25).

Therefore, peripheral blood seems to include a certain level of NK cells which have experienced exposure to asbestos in the lungs and draining lymph nodes, and our findings obtained from analysis of PB-NK cells in patients with MM, namely, the decreases in cytotoxicity and NKp46, might be related to exposure to asbestos inhaled into the body (Fig. 3). The impaired cytotoxicity of NK cells in relation to a decrease in NKp46 might allow transformed cells to escape from surveillance by anti-tumor immunity, and may promote the development of MM evoked by carcinogenicity of asbestos. In PBMCs cultured with asbestos, NK cells may have received both the direct and indirect effects of exposure to asbestos, which include the effect mediated by monocytes/macrophages, dendritic cells and T cells exposed to asbestos. In addition, the body of a patient with MM includes the indirect effect of asbestos exposure mediated by non-immune and immune cells. Thus, the actual effect of exposure to asbestos inhaled into the body seems to be more complex, and might be related to the difference observed between YT-CB5 and NK cells of patients with MM and PBMCs exposed to asbestos. Although the results obtained from our studies contain an inconsistency, they may alert people to the risk of impaired anti-tumor immunity as well as carcinogenesis caused by exposure to asbestos. Furthermore, there is the possibility that the decrease in NKp46 on NK cells might be a molecular marker for either exposure to asbestos or early detection of decreased anti-tumor immunity and malignant mesothelioma. Further study is needed to clarify the suppressive effect of asbestos exposure on NK cells.

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REVIEW ARTICLE

Dysregulation of the immune system caused by silica and asbestos

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Abstract

Silica and asbestos cause pneumoconioses known as silicosis and asbestosis, respectively, that are each characterized by progressive pulmonary fibrosis. While local effects of inhaled silica particles alter the function of alveolar macrophages and sequential cellular and molecular biological events, general systemic immunological effects may also evolve. One well-known health outcome associated with silica exposure/silicosis is an increase in the incidence of autoimmune disorders. In addition, while exposure to silica—in the crystalline form—has also been seen to be associated with the development of lung cancers, it remains unclear as to whether or not silicosis is a necessary condition for the elevation of silica-associated lung cancer risks. Since asbestos is a mineral silicate, it would be expected to also possess generalized immunotoxicological effects similar to those associated with silica particles. However, asbestos-exposed patients are far better known than silicotic patients for development of malignant diseases such as lung cancer and mesothelioma, and less so for the development of autoimmune disorders. With both asbestos and crystalline silica, one important dysregulatory outcome that needs to be considered is an alteration in tumor immunity that allows for silica- or asbestos- (or asbestos-associated agent)-induced tumors to survive and thrive *in situ*. In this review, the immunotoxicological effects of both silica and asbestos are presented and contrasted in terms of their abilities to induce immune system dysregulation that then are manifest by the onset of autoimmunity or by alterations in host-tumor immunity.

Keywords: *Silica; asbestos; autoimmunity; tumor immunity*

Introduction

Silica and asbestos cause pneumoconioses known as silicosis and asbestosis, respectively, that are characterized by progressive pulmonary fibrosis (Singh and Davis, 2002; Cohen et al., 2008). Inhaled silica particles and asbestos fibers are captured by alveolar macrophages, and subsequent cellular and molecular cascaded events are known to give rise to increases in the numbers of collagenic fibroblasts and the appearance of laminated fibrous changes at areas surrounding the silica particles and silica-bearing macrophages (Nishimura et al., 2007; Hamilton et al., 2008; Thakur et al., 2008; Wells et al., 2009). These sequential cellular events have been analyzed by numerous investigators and the following mechanisms/pathways for these outcomes have been proposed:

1. Initial recognition of silica/asbestos by cell membrane receptors such as the macrophage receptor with collagenous structure, scavenger receptor (SR)-AI and SR-AII.
2. Capture of silica/asbestos by macrophages and entrapment within lysosomes.
3. Activation of nucleotide-binding domain and leucine-rich repeat containing proteins 3 inflammasome to cleave procaspase 1 to an active form.
4. Cleavage of prointerleukin-1β (proIL1β) to an active form for release to form fibrotic nodules.
5. Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the macrophages.
6. Induction of cellular and tissue damages due to the production of ROS and RNS.
7. Apoptosis of the alveolar macrophages.
8. Production of various cytokines/chemokines such as IL-1β, tumor necrosis factor-α (TNF-α), macrophage inflammatory protein-1/2, monocyte-chemoattractant

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protein-1, and IL-8 to cause chronic inflammation and proliferation of collagenic fibers.
9. Release of silica particles and asbestos fibers from alveolar macrophages and the repeating of similar cellular reactions described above by newly-recognizing nearby macrophages.
10. Transfer to silica particles and (partially cleaved) asbestos fibers to regional lymph nodes.

As these cellular and molecular reactions are continuously repeated, pulmonary fibrosis will gradually and progressively appear (Nishimura et al., 2007; Hamilton et al., 2008; Thakur et al., 2008; Wells et al., 2009).

Apart from suffering from pulmonary fibrosis, it is well known that silicosis patients (SPs) also often evince complications from autoimmune diseases such as rheumatoid arthritis (known as Caplan syndrome), systemic lupus erythematosus, systemic scleroderma, and/or anti-neutrophil cytoplasmic autoantibody-related vasculitis/nephritis (Uber and McReynolds, 1982; Steenland and Goldsmith, 1995; Shanklin and Smalley, 1998). The effect of silica on the human immune system has been considered to be a result, in part, of the potential adjuvant activity of silica. Additionally, the increasing extracellular presence of various autoantigens such as DNA, RNA, and other organelles released from apoptotic alveolar macrophages that had phagocytized silica particles accordingly increase the opportunities for reactive T-lymphocytes to encounter these autoantigens (Nishimura et al., 2007; Hamilton et al., 2008; Thakur et al., 2008; Wells et al., 2009).

As asbestos (i.e. as actinolite, amosite, anthophyllite, chrysotile, crocidolite, or tremolite) is a mineral silicate containing iron, magnesium, and calcium, it is not reasonable to question whether exposure to asbestos also causes immunological alterations like (immunomodulatory) silica (Uber and McReynolds, 1982; Steenland and Goldsmith, 1995; Shanklin and Smalley, 1998). To date, only a handful of reports have been published detailing autoimmune disorders in asbestos-exposed patients (Tellesson, 1961; Pfau et al., 2005; Noonan et al., 2006). The most typical health complications arising from asbestos exposure involve the development of malignant tumors associated with mesothelioma and lung cancers (Nicholson, 1984, 2001; Antman, 1986; Gruber, 1990; Niklinski et al., 2004). In addition, the incidence of solitary tumors of the larynx, bladder, and gastrointestinal tracts are reported to occur at a higher level among asbestos-exposed individuals than in the non-exposed population (Rolston and Oury, 2004).

With respect to these various types of cancers, it is well-established that the asbestos itself can induce malignant transformation of cells by various mechanisms. These can include an enhanced formation (non-enzymatic) of ROS and RNS that is facilitated by the iron present in asbestos, direct physical disruption of spindle bodies during cell division, and/or adhesion of various mutagens to the asbestos fibers (Lee, 1985; Rom et al., 1991, Brandt-Rauf et al., 1994, Partanen et al., 1994; Toyokuni, 1996, 2009). Despite the paucity of data pertaining to select indices of asbestos-induced immunomodulation (i.e. induction of autoimmunity), it would not be farfetched to suppose that asbestos—if acting akin to silica—might now *also* facilitate the progression (i.e. growth, expansion, metastases) of any newly-transformed cells by disrupting a host's normal abilities to recognize/remove these cells, i.e. by altering host-tumor immunity.

It remains to be determined if silica (as crystalline form) also is able to alter host-tumor immunity. Indeed, there is ample evidence that exposure to crystalline silica is associated with an increase in incidence of lung cancers among workers in several occupations (see International Agency for Research on Cancer IARC 1977; Brown, 2009; Erren et al., 2009; Lacasse et al., 2009). There is also a pool of literature that indicates that exposure to silica [by inhalation/other routes (injections)] can affect host resistance to injected/implanted syngeneic and non-syngeneic tumors (Keller, 1976; Fuji and Murakami, 1983; Sandstrom and Chow, 1987; Gresser et al., 1990); unfortunately, changes in resistance against *de novo in situ* neoplasias have not been evaluated/reported in the literature to the same degree. In many of these cited studies, it appeared that the timing of exposure to silica in relation to introduction of the tumor cells was critical, i.e. silica treatment had to occur concurrently (or near-so) with the implantation for any significant effect to manifest. This suggests that for *silica-induced tumors* to flourish in a host *via* any asbestos-like 'altered tumor immunity' route, the silica exposures would have to ongoing/continuous in nature (as opposed to the effect evolving from silica that had accumulated in the lungs in the past).

This review does not seek to educate the reader on the differing mechanisms underlying how/why various forms of silica differ in cell transforming potentials nor how/why crystalline silica differs from asbestos in these aspects. Instead, this review provides a summary of our (and others') investigations regarding the immunotoxicological effects of silica and asbestos as they pertain to autoimmunity and to disruptions of host-tumor immunity. With regard to the latter, it is hoped the information here will allow readers to glean a clearer understanding of any potential differences in mechanisms that enable the *post-transformation growth/progression* of the cancers that each agent can induce *in situ*.

Silica particles and dysregulation of immune response leading to autoimmunity

As mentioned above, SPs often manifest a disturbance of the immune system as autoimmunity. Silica is considered one of the most important environmental substances, in the fashion of vinyl chloride and other chemicals (including trichloroethene and epoxy resins), that give rise to autoimmune diseases in exposed hosts (Trice and Pinals, 1985; Goldman, 1996; D'Cruz, 2000; Hess, 2002; Cooper et al., 2009).

To ascertain how silica particles might be inducing autoimmune outcomes in exposed hosts, our research first focused its attention on the Fas/CD95 death receptor and related molecules. This is because Fas/CD95 is known as

one of the most important molecules for the induction of apoptosis of lymphocytes. Furthermore, animal models possessing a mutated *Fas* gene or *Fas ligand* gene (i.e. *mlr* or *gld* mice respectively), display various clinical manifestations of autoimmunity (Watanabe-Fukunaga et al., 1992; Watson et al., 1992; Sobel et al., 1993; Wu et al., 1993; Takahashi et al., 1994; Nagata and Suda, 1995). As such, using clinical samples derived from SPs, we examined alterations of Fas protein and related molecules in their blood and immune cells. All of the patients were Japanese brickyard workers in Bizen City (Okayama prefecture, Japan), and were monitored at either Kusaka Hospital or the Hinase-Uragami Clinic. The silica in the materials handled by these workers (e.g. dirt, sand, mud, concrete), and thus present as a potential risk for being inhaled by these individuals in their work environment, was estimated to reach levels as high as 40–60% (by mass). The subjects were diagnosed with pneumoconiosis according to the International Labor Organization (ILO) 2000 Guideline (ILO, 2002). These patients displayed neither clinical symptoms related to autoimmune diseases (e.g. sclerotic skin, Raynaud's phenomenon, facial erythema, or arthralgia) nor any cancers. The following findings were obtained from these patients (see Figure 1):

1. Detection of autoantibody to Fas and caspase-8, as well as topoisomerase I and desmoglein (Ueki A et al., 2001, 2002; Ueki H et al., 2001; Takata-Tomokuni et al., 2005).
2. Anti-Fas autoantibody detected in SPs was functionally active and caused Fas-mediated apoptosis (Takata-Tomokuni et al., 2005).
3. The level of serum soluble Fas was higher in SPs than in healthy donors (HDs), although the level of serum soluble Fas ligand did not differ between SPs and HDs (Tomokuni et al., 1997, 1999).
4. The mean fluorescent intensity (MFI) of membrane Fas was lower with lymphocytes from SPs than from HDs, although total numbers of Fas-positive lymphocytes (membrane Fas expression) did not differ between the two populations (Otsuki et al., 2006).
5. The weaker membrane Fas expressers (among lymphocytes) were identified to be weaker *Fas* message expressers (Otsuki et al., 2006).
6. The gene expression levels of extracellular inhibitor competing membrane Fas–Fas ligand binding such as soluble Fas, decoy receptor 3 (DCR3), and other alternatively-spliced variants of the *Fas* gene were higher in peripheral blood mononuclear cells (PBMC) from SPs than HDs (Otsuki et al., 2000a, b).
7. The intracellular apoptosis-inhibitory genes including *I-FLICE*, *sentrin*, *survivine*, and *ICAD* showed a lower expression in PBMC from SPs than HDs (Otsuki et al., 2000c; Guo et al., 2001).

Although significant mutations of *Fas* and *Fas ligand* genes were not detected, these results indicated that two populations of lymphocytes may exist in the peripheral blood of SPs. One population is a weaker membrane Fas expresser and these cells may have developed out of an excessive transcription of the alternatively-spliced *Fas* gene and other variant messages. Therefore, these cells may be resistant to the functional anti-Fas autoantibody, secrete higher levels of soluble Fas, DCR3, and spliced variants, and are resistant to Fas-mediated apoptosis (Otsuki et al., 2003, 2006, 2007; Murakami et al., 2009). As reported previously, patients with a weaker MFI of membrane Fas often have a higher titer of anti-nuclear antibodies, and self-recognizing clones in silicosis may be included in the fraction because these clones may survive longer and show resistance to apoptosis. The other population represents stronger membrane Fas expressers that: may be sensitive to Fas-mediated apoptosis including cell death caused by anti-Fas autoantibody; show a reduced expression of intracellular inhibitor genes of Fas-mediated apoptosis; and, undergo apoptosis. These cells may be recruited from bone marrow after reaching the final stage of cell death. This recruited fraction would not have encountered silica and would be sensitive to silica/silicate-induced apoptosis. As a result, cells in this fraction would be continuously undergoing renewal and then apoptosis (Otsuki et al., 2003, 2006, 2007; Murakami et al., 2009).

According to recent developments concerning regulation of T-lymphocyte reactions against intrinsic or foreign antigens, a modification of the population (i.e. numbers)/functions of $CD4^+CD25^+$ forkhead box P3 $(FoxP)^+$ regulatory T-lymphocytes (Treg) may be a very important factor for the manifestation of several antigen-antibody-based events, including autoimmunity, allergy, tumor immunity, resistance to infection, graft-versus-host diseases, and control of pregnancy (Fehérvari and Sakaguchi, 2004; Hori and Sakaguchi, 2004; Sakaguchi, 2005; Dejaco et al., 2006; Sakaguchi et al., 2006; Oh et al., 2010). In particular, the maintenance of hyperreactivity to autoantigens may be caused, in part, by a reduction in the functions/numbers of Treg in a host. As a result of their impact upon both autoimmunity and tumor immunity, we determined it was critical to examine Treg status (i.e. functions/numbers) in hosts suffering from silicosis.

Since $FoxP3^+$ cells cannot be readily collected for use in assays of biological function (as FoxP3 is an intranuclear molecule and the cell membrane has to be destroyed [i.e. permea-bilized] to detect it), we analyzed $CD4^+CD25^+$ cells present within PBMC derived from SPs and HDs. Overtly, it seemed that the percentage of PMBC associated with $CD4^+CD25^+$ fraction did not differ between the two groups; however, when the samples were analyzed in the context of/corrected for donor age, the values were in fact lower in the SP group than among the HD samples (Wu et al., 2006). In addition, the results of the functional assays using these same isolates (i.e. non-permeabilized) showed that the $CD4^+CD25^+$ cells from SP hosts possessed a reduced suppressive effect against alloantigen-stimulated responder T-lymphocyte ($CD4^+CD25^-$ cells) proliferation. Based on these endpoints, it was concluded that Treg function and total numbers in the peripheral $CD4^+CD25^+$ fraction was likely to have been reduced as a result of a worker's chronic exposure to silica.

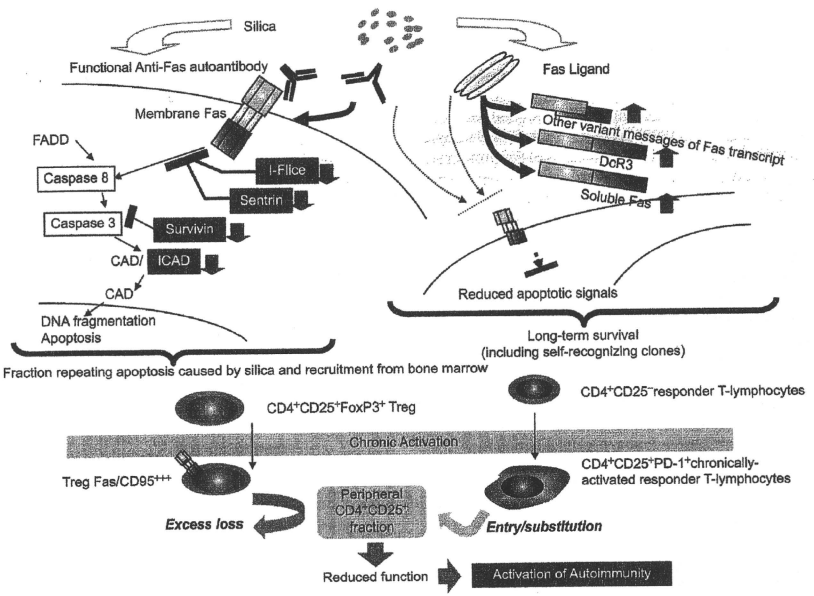

Figure 1. Schematic models of alteration in Fas and Fas-related molecules found in silicosis and speculated change of the peripheral $CD4^+CD25^+$ fraction in which the regulatory T-lymphocytes (Treg) should exist. CAD, caspase-activated deoxyribonuclease; DCR3, decoy receptor 3; ICAD, inhibitor of caspase-activated deoxyribonuclease; I-FLICE, inhibitor of FADD-like interleukin-1β-converting enzyme; FADD, Fas-associated death domain protein.

However, as we reported previously, silica can stimulate peripheral T-lymphocytes *in vitro*—as monitored by CD69, an early activation marker of T-lymphocyte expression (Hyodoh et al., 2005). If responder T-lymphocytes were activated chronically, their surface marker expression will become $CD4^+CD25^+$ and would be similar to the natural Treg. As a result, the peripheral $CD4^+CD25^+$ fraction may include true Treg and chronically-activated responder T-lymphocytes. To validate the possibility that the $CD4^+CD25^+$ fraction of SPs includes Treg and activated responder T-lymphocytes, we analyzed programmed cell death 1 (PD-1) expression, as an activated T-lymphocyte marker, in $CD4^+CD25^-$ and $CD4^+CD25^+$ fractions from SPs and HDs. Results showed that PD-1 was highly expressed in both the $CD4^+CD25^+$ and $CD4^+CD25^-$ fractions isolated from SPs, while both fractions recovered from the HDs hardly expressed this marker. Furthermore, Treg $CD4^+CD25^+FoxP3^+$ cells from SPs revealed higher membrane Fas expression than did the cells from HDs. Taken together with our previous Fas and Fas-related analyses, the following speculations arose (and which we are currently investigating):

1. Silica activates both responder T-lymphocytes and Treg (as shown in Figure 1).
2. Responder T-lymphocytes chronically-activated by silica become $CD4^+CD25^+$ ($PD\text{-}1^+$) expressers.
3. Treg activated by silica express higher Fas/CD95 and are sensitive to Fas-mediated apoptosis.
4. After an ongoing progression of these events, the composition of the peripheral $CD4^+CD25^+$ fraction in SPs changes to reflect a loss of Treg (by Fas-mediated apoptosis) and a gain of activated responder T-lymphocytes; this reduction of Treg function makes SPs more sensitive to a disruption of self-tolerance.

Disruption of tumor immunity due to silica

Based on what is known about silica and its impact on both responder T-lymphocytes and Treg cells, it would not be unreasonable to expect that silica-exposed individuals should have an increased response against tumor cells that form *in situ* (i.e. with fewer Treg present, anti-tumor responses should not be down-regulated). Yet, exposure to crystalline silica is correlated with increases in lung cancer formation. Thus, it might be that the impact of silica (in the context of altered tumor immunity) might be more 'critical' at the level of the macrophage.

The potential for silica to induce altered macrophage function and so affect neoplastic cell removal is clear. For example, Rakhmilevich et al. (Buhtoiarov et al., 2006; Lum et al., 2006) showed that while macrophages could be activated by CD40 ligation to be cytotoxic against tumor cells *in vitro and in vivo*—and that anti-CD40 monoclonal antibody (mAb) therapy could lead to inhibited tumor growth even in the absence of T-lymphocytes, natural killer (NK) cells, and neutrophils [polymorphonuclear leukocytes (PMN)]—this effect of anti-CD40 mAb was inhibited by silica (injections). A ready explanation for those findings may be that silica simply caused reductions in macrophage levels. However, as noted by Keller (1976), exposure to silica leads to increases in macrophage numbers (albeit in transitory manner). Furthermore, a hallmark of silicosis is that silica induces macrophage (and PMN) infiltration into the lungs and subsequent over-production of proinflammatory cytokines, chemokines, and ROS that eventually give rise to the associated pathologies.

If it is not an effect on macrophage numbers *per se* that may be key to any reduction in host-tumor immunity after silica exposure, then an impact on one/more critical cell function(s) is likely. In one study, mice administered silica (by intraperitoneal injection) displayed variable cell type-specific effects, i.e. accumulation of splenic macrophages and PMN, reductions in B-lymphocyte levels, and modest changes in T-lymphocyte abundance (Rao and Frey, 1998). Interestingly, while silica did not affect the spontaneous release of TNF-α or IL-1β by local (i.e. peritoneal) macrophages, IL-12 release was stimulated ≈ 5-fold; further, the ability of these cells to present antigen to T-lymphocytes (*in vitro*) or to prime antigen-specific T- and B-lymphocytes was greatly inhibited. This data suggests that silica could impact negatively on one set of macrophage functions critical to anti-tumor responses, i.e. antigen processing and presentation—even during a concurrent increase in release of an important anticancer cytokine, IL-12 (see Su et al., 2001, Weiss et al., 2007). However, given the documented shift toward induction of autoimmune responses in silica-exposed hosts, reductions in macrophage antigen processing/presentation seem incongruous. Absent a reduction in local macrophage number/capacity to act as antigen-presenting cells, it seems that modulation of other key tumor-killing functions—either directly or indirectly—would therefore underlie any change(s) in tumor immunity in silica-exposed hosts.

Because of their role in affecting lymphocyte function, the question arises as to if silica-induced increases in Fas/FasL expression might impact on lung macrophages and so reduce their role in tumor immunity. It is well known that inhaled silica causes increased FasL expression in the lungs (Borges et al., 2001, 2002; Corsini et al., 2003; Zhang et al., 2006). Consequently, an eventual increase in apoptotic death among macrophages in an exposed host's lungs is expected that, with respect to tumor immunity, should then allow any silica-transformed cells that evolve *in situ* to remain unharmed and able to proliferate. As noted above, while silica exposure leads to eventual diminution of macrophage levels, the timeframe in which exposure correlates with reductions in tumor resistance seems to be confined to a period where cells levels are *increased*.

It may be that the Fas/FasL could affect the lung macrophages so that they might not be able to activate themselves (autocrine) or other cells critical to recognizing/removing any newly-transformed cells. Unfortunately, effects of increased FasL expression on macrophage capacity to release proinflammatory appear to be variable; in some cases, the spontaneous release of TNF-α is increased by silica (and the subsequent FasL presence; see Borges et al., 2001) while in other studies, the *absence* of FasL (i.e. demonstrated using *lpr/lpr* vs. wild-type mice) results in greater spontaneous release of this and other cytokines (Brown et al., 2004) key to inflammation and anti-tumor activities. However, as the overwhelming evidence in the literature supports the former scenario (see reviews by Castranova, 2004 and Rimal et al., 2005), and an increased release in cytokines/chemokines would help move the lungs towards a silicotic state, we are left to wonder why these newly-recruited immune cells (macrophages and PMN) would be ineffective against any now-present transformed cells.

To this end, we believe there are at least two means by which the expected effects of an increased presence of Fas/FasL might be negated in a silica-exposed host's lungs (thereby mitigating any impact on macrophage numbers/activity against tumor cells). First, the presence of a DCR3 (or analogue) has been shown to abrograte the ability of FasL to induce inflammatory responses in the lungs of mice (Wortinger et al., 2003). As we noted earlier, the gene expression levels of extracellular inhibitors of FasL binding to receptors (like soluble Fas and DCR3) were higher in PBMC from SPs than in cells from HDs (Otsuki et al., 2000a, b, 2006). Second, an increased presence of transforming growth factor-β (TGF-β) could result in a shift of FasL from an inflammatory to a suppressive function. Indeed, a critical study by Chen et al. (1998) showed that an increased presence of TGF-β not only inhibited neutrophil (and likely macrophage) activation by FasL, but also led to a reduction in tumor (cell) rejection in the treated host. There is ample evidence that there is an increase in TGF-β expression in the lungs of silica-exposed hosts (Williams et al., 1993; Jagirdar et al., 1996; Ji et al., 2003a, b; Barbarin et al., 2004). If the TGF-β can mitigate effects of FasL so as to: (1) leave macrophage levels intact by diminishing induction of apoptosis; (2) lessen the release of select cytokines and/or chemokines that are essential to mounting an anti-tumor response (as well as being proinflammatory); and, (3) cause a shift in FasL-associated activities from an inflammatory to a suppressive function with other immune cell types needed to recognize and/or remove (silica-induced) transformed cells, this would provide a reasonable explanation for the 'time-sensitive' (re: exposure-to-tumor cell appearance) findings of Keller (1976) and those of other investigators noted above, as well as for how silica might disrupt tumor immunity in silicotic patients.

Disruption of tumor immunity due to asbestos fibers

The immunocompetent cells that directly kill tumor cells during a normal immune response are the NK cells and $CD8^+$ cytotoxic T-lymphocytes (CTL; Herberman, 1985; Kimber, 1985; Chambers et al., 1998; van Baarle et al., 2002; Snyder et al., 2003; Raulet and Guerra, 2009). There may be an asbestos-induced reduced function of these cells, and these modifications of tumor immunity may make patients who have been exposed to asbestos more sensitive to tumor development after a latent period of 30–40 years (Nicholson, 1984, 2001; Antman, 1986; Gruber, 1990; Niklinski et al., 2004).

To analyze the effects of chrysotile asbestos (classified in serpentine group of phyllosili-cates and the most commonly encountered form of asbestos) on human NK cells, we first utilized the human NK cell line, YT-A1 (kindly provided by Prof. Yodoi, Kyoto University, Japan). In our studies, YT-A1 cells were continuously exposed to chrysotile asbestos and the cytotoxic activity of and expressions of killing-related molecules by the cells (now designated as YT-CB5) were examined. YT-CB5 cells revealed significant reductions in surface expression levels of two NK-cell specific activating receptors, e.g. NKG2D and 2B4, and of the intracellular serine protease granzyme A that is normally secreted by the NK cells to kill tumor cells. In addition, the numbers/intensity of degranulation events normally stimulated *via* these activating receptors were also decreased. The decreased expression of these activating receptors also led to reduced phosphorylation of extracellular signal-regulated kinases in the NK cells. Although the asbestos-exposed cell line displayed decreases in NKG2D and 2B4 expression, freshly-isolated NK cells derived from mesothelioma patients and *ex vivo* expanded NK cells (from HDs) cultured with asbestos showed reduced expression of another activating receptor, NKp46. Regardless of the test system employed, our studies clearly revealed that asbestos exposure decreased the killing activity of NK cells, in part, by causing a reduced expression of cell surface activating receptors (Nishimura et al., 2009a, b, c; see Figure 2).

In addition to the above-noted endpoints, our laboratories have also been investigating the effects of asbestos exposure on CTL function, differentiation and proliferation regarding the role of various cytokines such as IL-10, TNF-α, and interferon-γ (IFN-γ). Although the results are preliminary, it seems that asbestos has an effect by reducing CTL function. The details of these investigations will be presented soon (i.e. awaiting peer-review and publication).

In addition to the above-mentioned studies and endpoints, our laboratories have also been investigating the effects of asbestos on T-lymphocyte lineages. To establish a chronic and low-dose exposure model similar to that of asbestos-exposed patients, we employed a human T-lymphocyte lymphotropic virus type 1-immortalized human polyclonal T-lymphocyte line (i.e. MT-2). This line was selected, in part, because it is

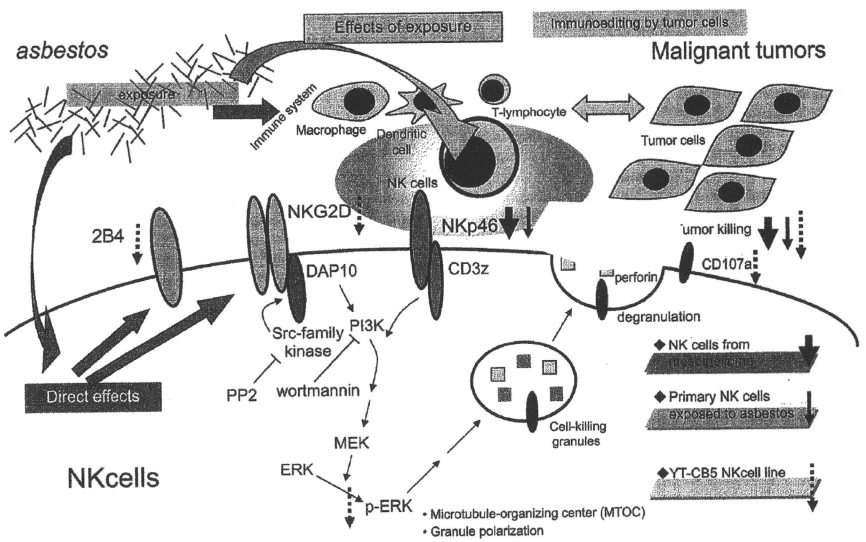

Figure 2. Effects of asbestos on human natural killer (NK) cells. The figure illustrates the down-regulation of NK cell-activating surface receptors such as 2B4, NKG2D, and NKp46, and a decrease in the levels (released levels) of the tumor-killing secreted molecule granzyme A, as found in various experiments using a cell line, as well as with freshly-isolated NK cells from healthy donors or patients with malignant mesothelioma.

not tumor-cell derived and it is reported to possess a normal karyotype. Additionally, screening for sensitivity to chrysotile asbestos revealed that the MT-2 cells and a few Epstein–Barr virus-immortalized B-lymphoblastoid cell lines were relatively more sensitive to cell death in comparison to other T- and B-lymphocyte tumor-derived lines. As it has been suggested that effects on T-lymphocytes may be more important than those on B-lymphocytes (regarding alteration of tumor immunity), this was another key reason the MT-2 cells were chosen for use in our *in vitro* experiments (Miura et al., 2006, 2008; Nishimura et al., 2006; Maeda et al., 2008; Otsuki et al., 2009).

In our experiments, short-term (i.e. 1–4 days) and high-dose exposure of the MT-2 cells to chrysotile asbestos caused progressive time- and dose-dependent apoptosis. This outcome was seen to occur in conjunction with activation of the mitochondrial apoptotic pathway, production of ROS, activation of proapoptotic signaling *via* p38 and c-Jun *N*-terminal kinase (among the mitogen-activated protein kinase pathways), as well as activation of caspases-9 and -3. These results were similar to the cellular changes that occurred in alveolar epithelial and pleural mesothelial cells exposed *in vitro* to asbestos as shown in many previous reports (BéruBé et al., 1996; Broaddus et al., 1996; Fung et al., 1997; Ollikainen et al., 2000; Aljandali et al., 2001; Buder-Hoffmann et al., 2001).

We also examined the effects from a prolonged exposed to the asbestos agent. After ≈ 1 year of continuous exposure of the MT-2 cells to chrysotile, this new continuously-exposed MT-2 subline was found to have acquired a resistance to asbestos-induced apoptosis. These cells also were found to display an: increase in activation of Src-family kinases; excess expression and secretion of IL-10; increased activation/phosphorylation of signal transducer and activator of transcription 3 by utilization of over-produced IL-10 (*via* autocrine mechanisms); and, over-expression of the anti-apoptotic molecule, Bcl-2. In addition, the continuously-exposed MT-2 subline were found to evince an excess production of TGF-β and reduced production of IFN-γ, TNF-α, and IL-6. Moreover, this subline displayed an over-expression of multiple forms of the T-lymphocyte Vβ receptors, including Vβ 5.2, 9, 13.2, 14, and 21.3, suggesting that asbestos imparted a super-antigenic activity upon human T-lymphocytes. Parts of these findings were confirmed using clinical samples derived from patients with pleural plaque, for whom there was evidence of past asbestos exposure or malignant mesothelioma. For instance, plasma levels of IL-10 and TGF-β and gene expression of *bcl-2* in peripheral $CD4^+$ T-lymphocytes were seen to be elevated in samples from SPs compared to samples from HDs, results that were in accordance with those suggested by the results seen with the chronically-exposed MT-2 cells (Miura et al., 2006, 2008; Nishimura et al., 2006; Maeda et al., 2008; Otsuki et al., 2009; see Figure 3).

Although the effects of asbestos on Treg cell function and numbers still need to be analyzed directly, most of

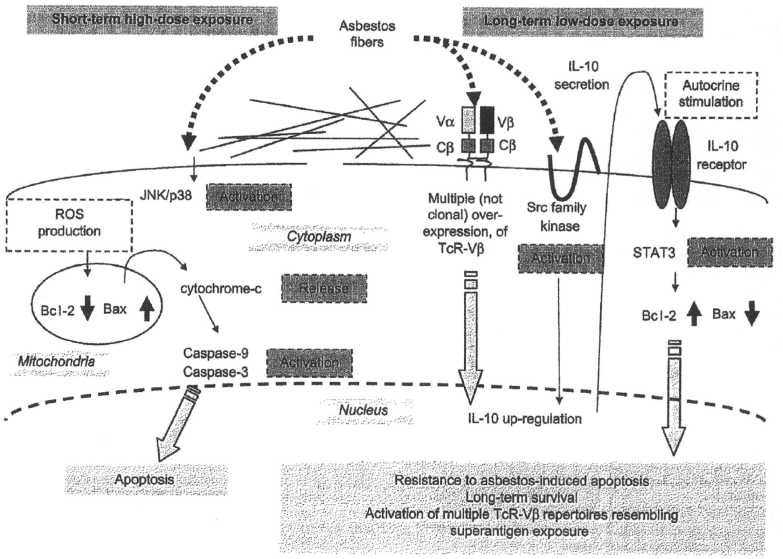

Figure 3. Experimental findings of the immunological effects of asbestos-induced by temporary high-dose exposure and continuous low-dose exposures of the human T-lymphocyte line, MT-2.

our findings indicated that chronic and low-dose exposure to asbestos by immunocompetent cells would likely cause reductions in a host's-tumor immunity. Such findings would help to explain why many patients who had been exposed to asbestos appeared to also be sensitive to the growth, expansion, and/or eventual metastatic spread of malignantly-transformed cells in their bodies.

Conclusion

While the IARC has long-recognized asbestos (i.e. as actinolite, amosite, anthophyllite, chrysotile, crocidolite, or tremolite) and silica (crystalline, *not* amorphous, form) as carcinogens, and the literature is replete with studies of the immunomodulatory effects of silica (especially autoimmune disorders), it still remains unclear whether asbestos is also a potential immunomodulant. Furthermore, if asbestos does impact upon host immune function, it remains to be determined if one key set of functions is the host immune response against neoplasia (i.e. tumor immunity). Indeed, because of its strong capacity to induce cell transformations *in situ*, one needs to consider if the asbestos also plays any role in altering tumor immunity in these now 'expected to be tumor-prone' individuals. This outcome is also important to determine with respect to crystalline silica as exposure to this form of particle is known to be associated with increases in the incidence of lung cancers. To that end, in this review, we have sought to present the reader with the most up-to-date information concerning the immunotoxicological effects of both silica and asbestos—with regard to how silica can facilitate development of an immune dysregulation that leads to autoimmunity and how both agents might target different immune cell types and cause changes in their respective functions that ultimately could lead to a reduction in immunity against tumors induced by these agents themselves.

A summary of the findings described in this review is provided in Figure 4. Recent advances in immunomolecular studies have led to detailed analyses of the immunotoxicological effects of asbestos and silica. Both agents affect immunocompetent cells and these effects may be associated with the pathophysiological development of complications in silica- and asbestos-exposed patients, such as the occurrence of autoimmune disorders and malignant tumors. Ongoing immunological analyses in our laboratories and those of other investigators may eventually lead to the discovery of new clinical tools such as cell-mediated therapies, treatments with various cytokines/chemokines, and molecule-targeting therapies that could be used to insure a better regulation of autoimmunity or a mitigation of the dysregulation that leads to altered tumor immunity that seem to be major

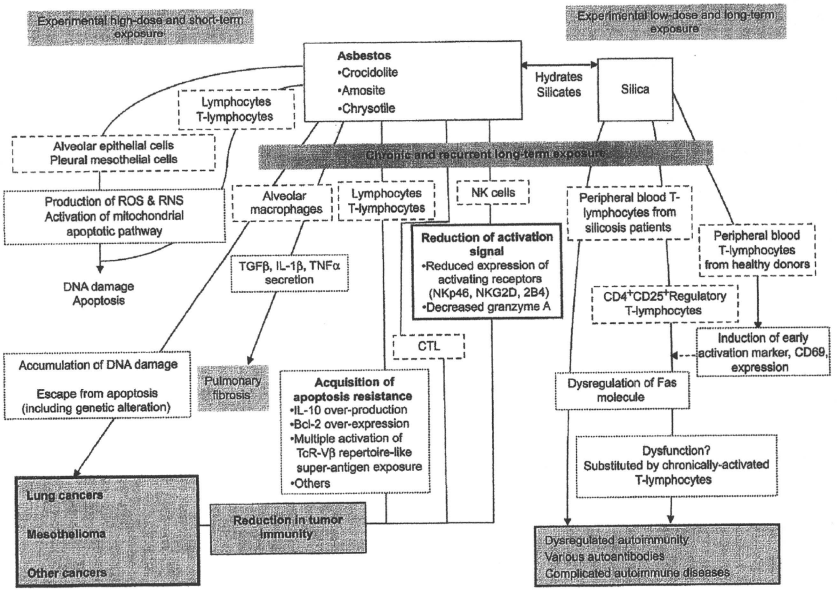

Figure 4. Summary of immunotoxicological alterations induced by silica and asbestos. CTL, cytotoxic T-lymphocytes; NK cells, natural killer cells; ROS, reactive oxygen species; RNS, reactive nitrogen species; TGF-β, transforming growth factor-β;TNF-α, tumor necrosis factor-α.

pathophysiological outcomes resulting from exposures to these two types of airborne particulate pollutants.

Declaration of interest

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診療

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鼻副鼻腔悪性黒色腫に対するホウ素熱中性子捕捉療法の治療効果

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■ はじめに

ホウ素熱中性子捕捉療法（Boron Neutron Capture Therapy：BNCT）は，ホウ素原子の熱中性子捕捉反応により生じたα線を利用する放射線治療である。エネルギーの低い熱中性子（～0.5eV）は原子核に捕獲されやすく，その確率は中性子捕獲断面積（バーン：$10^{-24}cm^2$）と呼ばれる。生体の主要な構成元素では窒素 ^{14}N が1.75バーンで最も大きく，他はこれよりも2桁以上小さい。これに対して，ホウ素の安定同位体である ^{10}B の熱中性子捕獲断面積は3,838バーンとはるかに大きい。ホウ素原子 ^{10}B と熱中性子との核分裂反応により発生したα粒子，Li反跳核の飛程はそれぞれ約9μm，5μmであり，これは細胞の直径程度に相当する。また，粒子が停止するまでに周囲に付与する単位長さあたりのエネルギー（linear energy transfer：LET）が大きい高LET放射線であり，相対的生物効果比（relative biological effect：RBE）も2.5～5.0と大きく，放射線抵抗性の細胞に対しても大きな殺細胞効果を示す。さらに，その効果はX線をはじめとする低LET放射線と異なり細胞周囲の酸素分圧の影響を受けないため，低LET放射線抵抗性の低酸素細胞に対しても有効性が高い。この反応が癌細胞内あるいはその極近傍で起こると，修復不能なDNAの2重鎖切断が生じて細胞は死滅する。したがって，ホウ素原子を癌細胞のみに取り込ませることができれば，原理的にはμmオーダで癌細胞だけを選択的に破壊することができる。

1972年，三嶋ら[1]により皮膚悪性黒色腫に対するBNCTの基礎実験が開始された。その後，1987～1994年の8年間に皮膚悪性黒色腫患者20例に対して，熱中性子およびホウ素キャリアの1つであるパラボロノフェニルアラニン（BPA）を用いたBNCTが実施され，奏功率90%，2年以上生存率78%とBNCTの優れた治療効果が示された[2]。2001年から京都大学原子炉実験所を中心に，熱中性子よりややエネルギーが高く深達度が良好な熱外中性子（0.6～10keV）を利用したBNCTの頭頸部腫瘍への適応拡大が実施され，2003年10月から川崎医科大学においても倫理委員会承認のもとBNCT医療チームを発足，再発難治性頭頸部扁平上皮癌，耳下腺悪性腫瘍および頭頸部粘膜発生悪性黒色腫に対するBNCTを行ってきた。

今回我々は日本原子力研究開発機構・東海研究所の医療用研究炉（JRR-4）において，熱外中性子およびホウ素化合物BPAを用いてBNCTを行った鼻副鼻腔悪性黒色腫の治療経験を提示し，本治療の有益性，問題点について文献的考察を加え報告する。

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〔索引用語：ホウ素熱中性子捕捉療法，悪性黒色腫，ボロノフェニルアラニン〕

❶ 症例と方法

川崎医科大学におけるBNCTの適応を以下に示す。

(1) 口腔および鼻副鼻腔粘膜に発生した悪性黒色腫で，手術または生検で病理組織学的に悪性黒色腫の確定診断がなされている
(2) CT，MRI，FDG-PETなどの画像検査で，病巣は原発巣にとどまっており，リンパ節転移および遠隔転移を認めない
(3) 放射線治療，化学療法および免疫療法がなされている場合は，それらの治療終了から1カ月以上経過している
(4) ホウ素化合物BPAは主に腎臓から排泄されるため，腎機能障害のないもの（クレアチニンクリアランスが60ml/min以上）
(5) performance status（PS）が2以下である
(6) BNCTに関するインフォームド・コンセントが本人から書面上得られている
(7) 当大学および京都大学倫理審査会での承認が得られている

2005年7月～2007年6月に当科を受診した鼻副鼻腔悪性黒色腫患者のうち，表1に示す7例が上記適応基準を満たしBNCTを行った。1例を除いた全例が手術，化学療法，放射線治療の単独あるいは併用治療をBNCT前に受けていた。BNCT時の腫瘍体積は775.5～66600.0mm^3，平均18039.0mm^{3}であった。BNCT施行時からの経過観察期間は5～50カ月，中央値17カ月であった。

BNCTの照射シミュレーションおよび線量評価はコンピュータ線量評価システムJCDS[3]で行った。BNCTにおいては前述のとおり，ホウ素原子^{10}Bの熱中性子捕捉反応により生じたα線を利用するため，熱中性子照射時の組織内ホウ素濃度が重要な鍵を握り，また線量計算には組織内に分布するホウ素濃度の値が必要不可欠である。腫瘍内および周囲正常組織内のBPA取り込みを評価するため，事前に^{18}Fで標識したBPAを放射性トレーサー（^{18}F-BPA）として用いたpositron emission tomography（PET）[4][5]を行った。BPA-PETは7例中4例に行い，得られた腫瘍／正常組織ホウ素濃度比（T/N ratio）を線量評価に用いた。BPA-PETを行わなかった3例については，福田ら[6]がまとめた悪性黒色腫患者におけるBPA投与後の血液，腫瘍および正常組織ホウ素濃度の実測値をもとに，腫瘍／血液ホウ素濃度比（T/B ratio＝3.0），正常組織／血液比（N/B ratio＝1.3）を線量評価に用いた。

BNCT当日，再発難治性頭頸部癌における投与量に準じた500mg/kg体重のBPAをフルクトース錯体溶液として2時間かけて点滴静注した。熱外中性子照射中の平均血中ホウ素濃度（ppm）は，BPA投与終了直後および熱外中性子照射終了直後に採血を行い，分光分析（ICP-AES），即発γ線を用いて測定し，これをもとに腫瘍および正常組織ホウ素濃度を算定した。中性子線およびγ線による患部以外の全身被曝を抑えるために遮蔽を行い，JRR-4照射

表1　鼻副鼻腔悪性黒色腫症例（2005年7月～2007年6月）

症例	年齢	性別	部位	既治療	腫瘍体積（mm^3）
1	55歳	男性	右鼻腔	手術，化学療法※	775.5
2	73歳	男性	右鼻腔	なし	6408.0
3	71歳	女性	右鼻腔，右上顎洞，右篩骨洞	手術，化学療法※	30778.4
4	74歳	男性	右鼻腔，右篩骨洞	手術，化学療法※	1143.1
5	74歳	女性	右鼻腔	手術，化学療法※	2881.4
6	70歳	男性	左鼻腔	手術，化学療法※，放射線治療（5 Gy×5回）	17686.6
7	69歳	男性	左鼻腔，左上顎洞	化学療法※，インターフェロン-β局注	66600.0

※化学療法　DAV：Dacarbazine＋Nimustine hydrochloride＋Vincristine