The immunological effects of asbestos exposure on various lymphocytes such as the regulatory T cell (Treg), responder CD4 +T helper cell (Tresp), CD8 +cytotoxic T lymphocytes (CTL) and natural killer (NK) cells were investigated. Results show that asbestos exposure impairs anti-tumour immunity through enhancement of regulatory T cell function and volume, reduction of CXCR3 chemokine receptor in responder CD4 +T helper cells, and impairment of the killing activities of CD8 +cytotoxic T lymphocytes (CTL) and NK cells. These findings were used to explore biological markers associated with asbestos exposure and asbestos-induced cancers, and suggested the usefulness of serum/plasma IL-10 and TGF-β, surface CXCR3 expression in Tresp, the secreting potential of IFN-γ in Tresp, intracellular perforin level in CTL, and surface expression NKp46 in NK cells. Although other unexplored cytokines in serum/plasma and molecules in these immunological cells, including Th17, should be investigated by experimental procedures in addition to a comprehensive analysis of screening methods, biomarkers based on immunological alterations may be helpful in clinical situations to screen the high-risk population exposed to asbestos and susceptible to asbestos-related cancers such as mesothelioma

557 CONTACT DERMATITIS AMONG WORKERS OCCUPATIONALLY EXPOSED TO FERRONICKEL ALLOYS

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Introduction Many studies have shown that nickel and its alloys can be potential irritants and sensitizers among workers engaged in ferronickel alloy production, and provoke occupational contact dermatitis.
Objective To assess the prevalence of contact dermatitis focusing on allergic contact dermatitis in workers exposed to nickel while producing ferronickel alloys.
Methods A cross-sectional study included 103 male workers (mean age=49.1±10.1) employed as ferronickel smelters (duration of exposure 18.2±11.9) with direct contact to nickel. Their findings were compared with a control group of 37 male office workers (mean age=46.7±10.6), employed in the same facility, without direct nickel contact, matched for age, smoking habits and socioeconomic status. Evaluation of examined subjects included Nordic Questionnaire on Occupational Skin Diseases (NOSQ-2002/long), physical examination of the skin changes on hands, wrists and forearms, and patch test with $NiS0_4$ (5%).
Results and discussion Skin rush during six months was registered in 21 (20,4%) exposed worker, and in 4 (10,8%) controls. The prevalence of skin changes, chronic rhinitis, conjunctivitis, and asthma was higher in exposed workers, but without statistical significance. Hand skin efflorescence due to non-occupational substances were present in 10 (9,8%) of exposed workers, and among 2 (5,5%) of controls. There was no significant difference concerning urticaria between two groups, and non-occupational nickel sensitisation (metal buttons, jewellery, etc.). Positive patch test by 5% $NiSO_4$ was registered in 20 (19.5%) exposed workers and in 2 (5.4%) controls ($p<0.05$). Significant difference was found between the two groups concerning improvement of skin lesions after temporary elimination of workplace exposure. Positive elimination test was registered among 5 (4.9%) exposed workers with hand contact dermatitis.
Conclusion Our data confirmed that workplace nickel exposure can cause occupational allergic contact dermatitis among workers producing ferronickel alloys, and determined the need of preventive activities in order to decrease the pathogenic dermal effect of nickel.

638 EFFECTS OF IL-15 ADDITION ON THE SUPPRESSED INDUCTION OF CTL UPON EXPOSURE TO ASBESTOS

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Introduction Asbestos exposure can cause malignant mesothelioma and lung cancer. However, in contrast, its effect on anti-tumour immunity remains unclear. Our previous study reported that asbestos exposure suppressed the induction of CTL during mixed lymphocyte reactions (MLR), accompanied by the decrease in proliferation of $CD8^+$T cells. Recently, we reported that IL-2 showed a tendency to increase% granzyme B^+ cells in the CFSE-positive $CD8^+$ lymphocytes without proliferation upon exposure to asbestos. Therefore, we investigated whether IL-15 addition might improve the suppressed induction of CTL upon exposure to asbestos.
Methods For MLR, human PBMCs were cultured with irradiated allogenic PBMCs upon exposure to chrysotile B asbestos at 5 μg/ml for 7 days. After 2 days of culture, IL-15 was added at 1 ng/ml. After 7 days of MLR, PBMCs were collected and analysed for phenotypic and functional markers of $CD8^+$ T cells with fluorescence-labelled anti-CD3, anti-CD8, anti-CD45RA, anti-CD45RO, and anti-granzyme B Abs using flow cytometry.
Result IL-15 didn't recover the asbestos-caused decreases in% $CD25^+$ and% $CD45RO^+$ cells and increase in% $CD45RA^+$ cells, but recovered the decrease in cell numbers of $CD3^+CD8^+$ cells and% granzyme B^+ cells, in contrast to IL-2.
Discussion These results indicate that IL-15 is more effective on recovery from asbestos-caused suppressed induction of CTL than IL-2, although the interfered expressions of cell surface markers were not recovered even by addition of IL-15. Further study about the characteristics of $CD3^+CD8^+$granzyme B^+ cells induced by addition of IL-15 will contribute to clarification for the mechanism of asbestos-caused suppression in CTL induction and to finding out a clue to restore it.

640 EFFECT OF LONG-TERM EXPOSURE TO ASBESTOS ON FUNCTIONAL PROPERTIES OF HUMAN CD8+T CELL LINE

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Introduction The tumorigenicity of asbestos, which is thought to cause mesothelioma, has been clarified, whereas its effect on anti-tumour immunity remains unclear. In ICOH Congress 2015, we have reported the enhanced decrease in% $perforin^+$ cells of stimulated $CD8^+$ cells of the patients with malignant

mesothelioma (MM). This result suggest the decrease in stimulation-induced cytotoxicity in MM patients. Therefore, we hypothesised that chronic exposure to asbestos might affect anti-tumour immunity of CTL, and examined this possibility by comparing long-term cultures of human $CD8^+$T cell line EBT-8 between with and without exposure to chrysotile (CH) asbestos as an *in vitro* model to analyse the effects of exposure on CTL.

Methods EBT-8 cells were continuously cultured with CH asbestos at 0, 5, or 30 μg/ml, and were designated EBT-8-Org, EBT-8-CH5, and EBT-8-CH30, respectively. The cells were regularly separated from asbestos using a Ficoll-Hypaque density gradient, before being assayed for MFI of granzyme B and percentage of $perforin^+$ cells by flow cytometry. The cells were also stimulated with PMA/ionomycin for 4 hour or beads coated with anti CD3 Ab for 48 hour and assayed for IFN-λ production by flow cytometry and ELISA, respectively.

Result The long-term exposure to CH asbestos at 5 μg/ml or 30 μg/ml did not suppress the MFI of granzyme B in EBT-8 cells. In contrast, both the doses of CH exposure suppressed the percentage of $perforin^+$ cells. Although the exposure to CH did not suppress intracellular level of IFN-λ induced by PMA/ionomycin, the secreted production of IFN-λ stimulated via CD3 decreased by CH exposure.

Discussion These results indicate that long-term exposure to asbestos has the potential to suppress the perforin level and the production of IFN-λ of human $CD8^+$T cells. Further study is needed to clarify the mechanism of asbestos-caused suppressed function of CTL.

665 EFFECT OF ASBESTOS ON FOXP3 EXPRESSION IN HUMAN T CELL LINE MT-2

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Introduction Asbestos fibres cause mesothelioma and lung cancer. We propose that asbestos suppress anti-tumour immune system in addition to transformation of mesothelial and lung epithelial cells. It is considered that regulatory T cells, Treg produce inhibitory cytokines to suppress immune reaction against tumour cells. We employed human T cell line MT-2 cell as a model of Treg and cultured them with low concentration of asbestos for 8 months. MT-2 cells exposed with low-concentration asbestos for long term showed higher viability after treatment with high lethal dose of asbestos than original MT-2 cells, and they were designated as MT-2Rst. However, it is still unclear how asbestos induces apoptosis in MT-2 cells and molecular basis of resistance of MT-2Rst cells to high concentration of asbestos. Recently, we found that forkhead transcription factor FoxP3 plays an important role in regulation of apoptosis induced by asbestos.

Methods We analysed the regulation of FoxP3 transcription in MT-2Org and MT-2Rst cells using luciferase reporter plasmids containing promoter sequence of FoxP3 gene.

Result There was no significant difference in FoxP3 reporter activity between MT-2Org and MT-2Rst cells.

Discussion Our result indicates that long-term exposure with asbestos suppressed FoxP3 transcription through the epigenetic modification, such as DNA methylation.

667 EFFECT OF SHORT-TERM EXPOSURE OF ASBESTOS ON HUMAN T CELL LINE MT-2

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Introduction Asbestos fibres cause mesothelioma and lung cancer. We propose that asbestos suppress anti-tumour immune system in addition to transformation of mesothelial and lung epithelial cells. It is reported that asbestos induces apoptosis of epitherial cells through various processes such as, mitochondrial dysfunction, DNA damage, ER stress. On the other hand, we proposed that asbestos fibres affects on immune cells to attenuate immune response to tumour cells. We employed MT-2 cells as a model of Treg and maintained them with low concentration of asbestos for longer than 8 months. MT-2 cells exposed with low-concentration asbestos for long term showed higher viability after treatment with high concentration of asbestos than original MT-2 cells, and they were designated as MT-2Rst. However, it is still unclear how asbestos induces apoptosis of MT-2 cells and molecular basis of resistance of MT-2Rst cells to high concentration of asbestos.

Methods We analysed acute effect of asbestos on mitochondria using mitochondrial membrane potential indicator JC-1 dye and immunoblot analysis using antibodies recognising DNA damage markers.

Result It was found that asbestos fibres induced loss of mitochondrial membrane potential and phosphorylation of Histone H2AX, a marker protein of DNA damage.

Discussion These data suggest that asbestos fibres induces apoptosis of MT-2 cells through the mitochondrial dysfunction and DNA damage, and these apoptotic pathways are modified in MT-2Rst cells.

776 NANOTOXICITY OF TITANIUM NANOSHEETS FOR HUMAN IMMUNE CELLS

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Introduction The characteristic toxicity of nano-scaled materials, that is nanotoxicity, is a recent problem arising in association with nanotechnology. Titanium nanosheets (TNS) are known as 2D materials composed of titanium and oxygen with very thin structure and expected to be valuable for industrial usage. The present study examined the effect of exposure to TNS on human immune cells.

Methods Human peripheral blood mononuclear cells (PBMC) or magnetically isolated $CD14^+$ monocyte or $CD4^+$ T cells were cultured with TNS. Apoptosis was assayed by flow cytometry with annexin V staining. Intracellular