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 epithelial 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