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Effects of Asbestos Exposure on Induction of Cytotoxic T Lymphocytes in Mixed Lymphocyte Reactions and Cytotoxic Potential of CD8⁺ Lymphocytes in Asbestos-Exposed People

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Introductions

Exposure to asbestos can cause malignant mesothelioma (MM) and lung cancer (Refs. 1-3). These diseases need a long period to develop after exposure to asbestos, suggesting that asbestos might gradually impair anti-tumor immunity. We have revealed several findings including impaired function of natural killer (NK) cells (Refs. 4-5). Tumor-specific cytotoxic T lymphocytes (CTL) differentiated from naïve CD8⁺ T cells and NK cells play a critical role in the anti-tumor immune response. However, until now, effect of asbestos fibers on CTL has not been examined. In the present study, we investigated the effect of asbestos-exposure on differentiation of CTL and functional properties of CD8⁺ cells from asbestos-exposed people with pleural plaque (PL).

Materials and Methods

Blood samples

PBMCs were prepared from the blood of healthy volunteers (HV) and PL-positive people. All of blood samples were taken from people from whom informed consent had been obtained. The Institutional Ethics Committees of Kawasaki Medical School and Okayama Rosai Hospital approved the project

Induction of human CTL upon exposure to asbestos

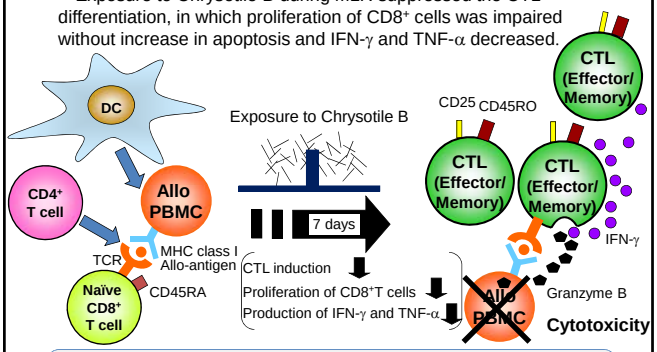
CTLs were induced using primary and allogenic mixed lymphocyte reactions (MLR), in which PBMCs were cultured with irradiated allogenic those. After 7 days of MLR, PBMCs were collected and used to assay cytotoxicity as effectors (E) against the targets, Dio fluorescence-labeled allogenic PBMCs (T) by using flow cytometry (FCM). The PBMCs collected were also analyzed for phenotypic and functional markers of CD8⁺ T cells with fluorescence-labeled antibodies (Table 1), and cell proliferation of CD8⁺ cells by using FCM. The supernatants of PBMCs collected were examined for productions of cytokines (IL-10, IFN-γ, TNF-α, and IL-2) using by BD™ Cytometric Bead Array.

Results

PBMCs exposed to CB, but not CR, showed a marked decrease in cytotoxicity. They also showed decreases in granzyme B⁺ cells, IFN-γ⁺ cells, CD45RO⁺ effector/memory cells and CD25⁺ activated cells in CD8⁺ cells compared with PBMCs after MLR without CB. CB exposure suppressed the proliferation in CD8⁺ cells without increase in annexin V⁺ apoptotic cells in CD8⁺ and CD4⁺ cells. The productions of IL-10, IFN-γ, and TNF-α, but not IL-2 were also suppressed by CB exposure. There was no difference in the percentage of IFN-γ⁺ cells in stimulated CD8⁺ lymphocytes between PL group and HV. In contrast, the percentages of granzyme B⁺ and perforin⁺ cells were higher in PL group than those in HV.

Conclusion

Exposure to Chrysotile B during MLR suppressed the CTL differentiation, in which proliferation of CD8⁺ cells was impaired without increase in apoptosis and IFN-γ and TNF-α decreased.



Contrary to expectation, granzyme B⁺ and perforin⁺ cells in CD8⁺ lymphocytes stimulated with PMA/ionomycin were not lower in PL-positive people than those in with HV.

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