

# Abstracts of the 2006th Encouragement Award's Memorial Lectures of the 74th Annual Meeting of the Medical Association of Nippon Medical School

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## Abstracts of the 2006th Encouragement Award's Memorial Lecture (1)

### New Mechanism of Organophosphorus Pesticide-induced Immunotoxicity

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Organophosphorus pesticides (OPs) are widely used throughout the world as insecticides in agriculture and for eradicating termites around homes. Many OPs remain on the market in Japan. The main toxicity of OPs is neurotoxicity, which is caused by the inhibition of acetylcholinesterase. OPs also affect immune responses, including antibody production, interleukin-2 production, T cell proliferation, decrease of CD5 cells, and increases of CD26 cells and autoantibodies, inhibition of natural killer (NK), lymphokine-activated killer (LAK), and

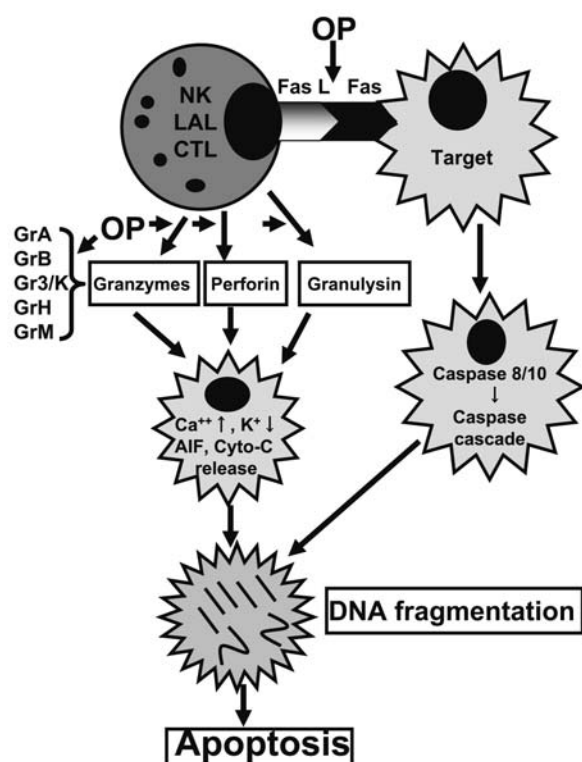


Fig.1 OPs impair the granule exocytosis pathway (perforin/granzyme/granulysin pathway) and the FasL/Fas pathway of NK, LAK and CTL cells. → : inhibition.

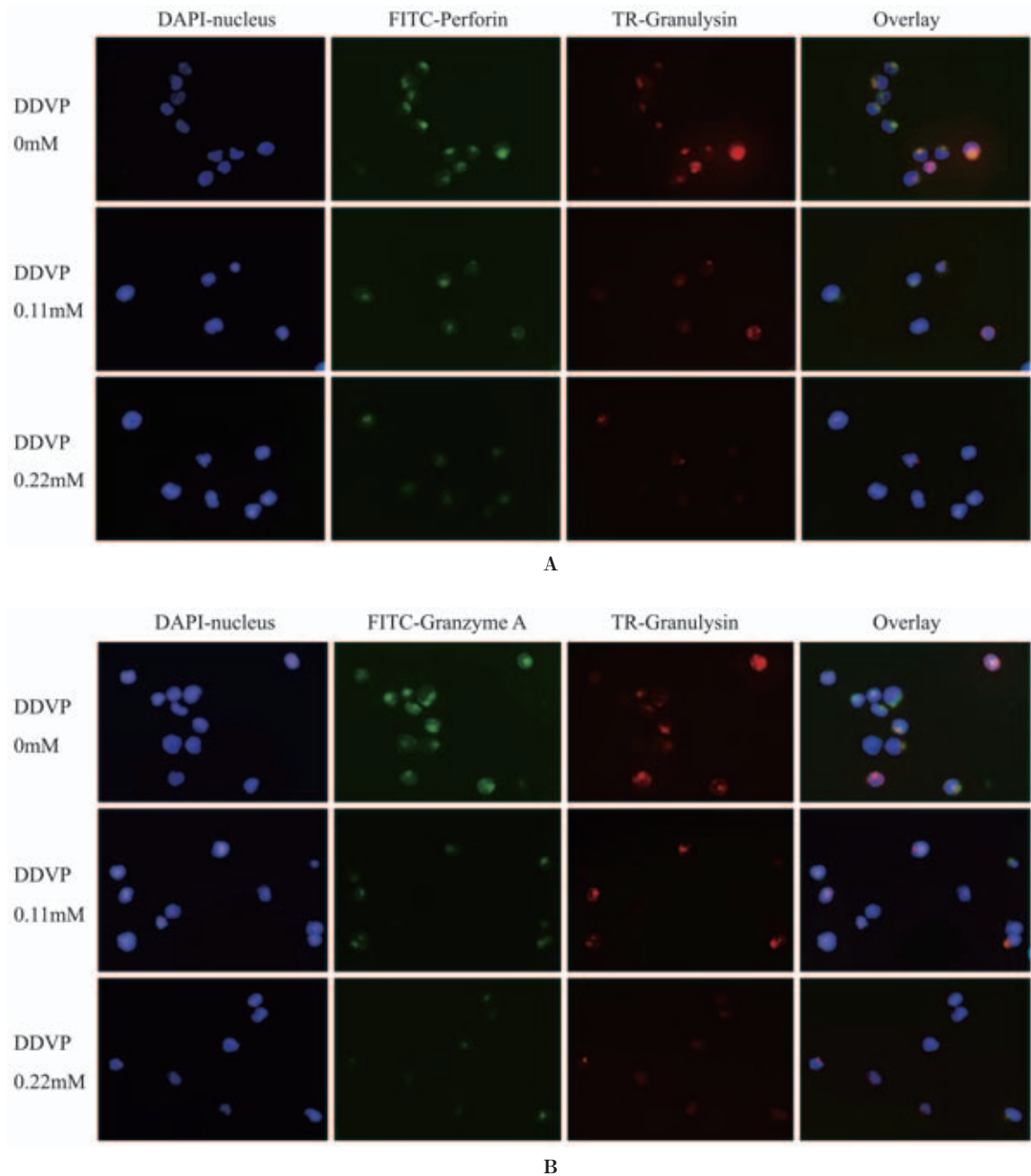


Fig. 2 Effect of DDVP at 0.11 and 0.22 mM on intracellular perforin/granulysin (A) and granzyme A/granulysin (B) in NK-92CI cells after in vitro treatment for 15 h. The NK-92CI cells were fixed/permeabilized with Cytotfix/cytoperm solution, and then double-staining of perforin/granulysin and granzyme A/granulysin was performed. The intracellular perforin and granzyme A were stained with fluorescein isothiocyanate -anti-human perforin and granzyme A, respectively. Intracellular granulysin was first stained with a rabbit anti-human granulysin polyclonal antibody, then stained with a TR-goat anti-rabbit IgG.

cytotoxic T lymphocyte (CTL) activities. However, there have been few papers investigating the mechanism of OP-induced immunotoxicity, especially, the mechanism of OP-induced inhibition of cytolytic activity of killer cells. This study reviews the new mechanism of OP-induced inhibition of activities of NKs, LAKs, and CTLs. It has been reported that NKs, LAKs, and CTLs induce cell death in tumors or virus-infected target cells by two main mechanisms. The first mechanism, called the granule exocytosis pathway, involves the direct release of cytolytic granules that contain the pore-forming protein perforin, several serine proteases termed granzymes,

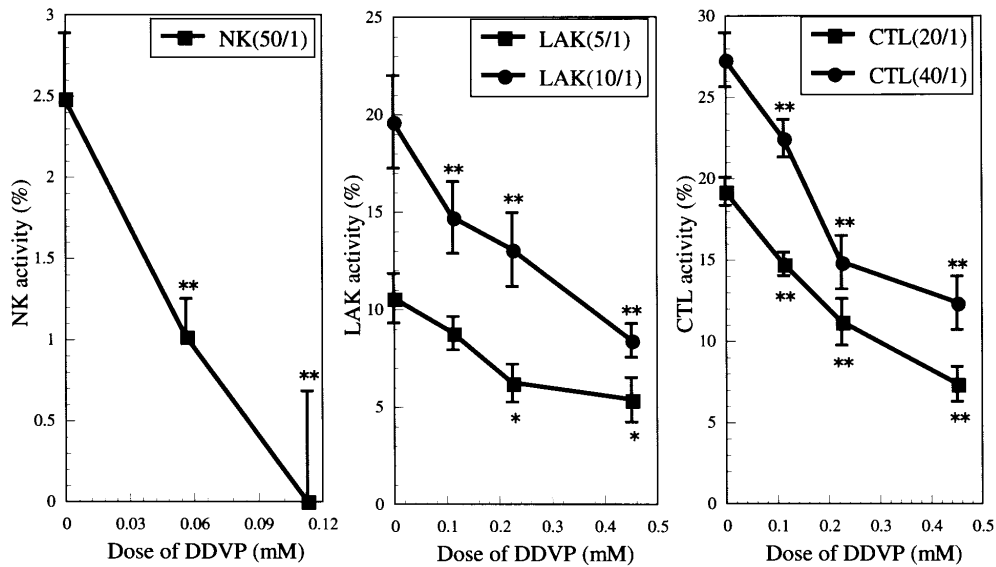


Fig. 3 Effect of DDVP on activities of NKs, LAKs and CTLs of PKO mice *in vitro*.

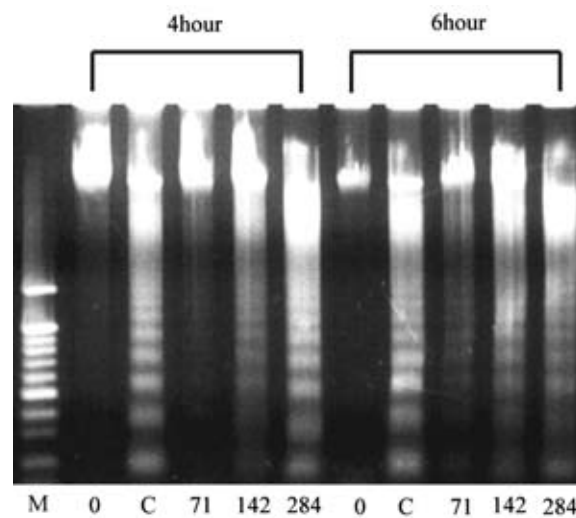


Fig. 4 DNA fragmentation induced by the OP chlorpyrifos in U937 cells determined with agarose gel electrophoresis. M: marker of the DNA ladder, C: a positive control, camptotecin at 6 μM. The concentrations of chlorpyrifos were 0, 71, 142, and 284 μM. Data shown are representative of three similar experiments.

and granulysin by exocytosis to kill target cells. The second mechanism is mediated by the Fas ligand (FasL)/Fas pathway, in which FasL (CD95L), a surface membrane ligand of the killer cell, cross links with the target cell's surface death receptor Fas (CD95) to induce apoptosis of the target cells. While performing immunotoxicologic investigations of several organophosphorus compounds, we found that diisopropyl methylphosphonate (DIMP) and diethyl methylphosphonate (DEMP), the by-products generated during the synthesis of sarin used in the Tokyo subway attacks, significantly inhibited human and murine NK and murine CTL activities *in vitro*. Both DIMP and Demp have also been shown to be potent inhibitors of serine esterases, such as acetylcholinesterase and serum cholinesterase, which are similar to OPs in toxicity. Thus, we speculate that OPs may also inhibit NK and CTL activities, as do DIMP and Demp. To clarify whether OPs also affect NK and CTL activities, we first investigated the effect of 5 OPs: dimethyl 2,2-dichlorovinyl phosphate (DDVP, dichlorvos), dimethyl 2,2,2-trichloro-hydroxyethylphosphonate (DEP), dimethoate (DMTA), acephate, and S-2-

ethylsulfanyl-1-methylethyl O,O-dimethyl phosphorothioate (ESP) on human NK activity. We found that all 5 OPs significantly decreased human NK activity in a dose-dependent manner. We also found that OPs significantly inhibited the activities of human LAKs and of murine NKs, CTLs, and LAKs. We then explored the mechanism of OP-induced inhibition of cytolytic cells and found that OPs inhibit activities of NKs, LAKs, and CTLs by at least the following three mechanisms. 1) OPs impair the granule exocytosis pathway of NK, LAK and CTL cells (**Fig. 1**) by inhibiting the activity of granzymes and by decreasing the intracellular level of perforin, granzyme A, and granulysin, which was mediated by inducing degranulation of NK cells (**Fig. 2**), and by inhibiting the transcription of mRNAs of perforin, granzyme A, and granulysin. 2) OPs impair the FasL/Fas pathway of NK, LAK and CTL cells (**Fig. 1, Fig. 3**), as investigated by using perforin-knockout mice, in which the granule exocytosis pathway of NK cells does not function and only the FasL/Fas pathway remains functional. 3) OPs induce apoptosis of immune cells as determined by analysis of annexin-V staining and the intracellular level of active caspase-3 by flow cytometry and by DNA fragmentation analysis (**Fig. 4**).

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