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Biochemical and molecular basis of thimerosal-induced apoptosis in T cells: a major role of mitochondrial pathway

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The major source of thimerosal (ethyl mercury thiosalicylate) exposure is childhood vaccines. It is believed that the children are exposed to significant accumulative dosage of thimerosal during the first 2 years of life via immunization. Because of health-related concerns for exposure to mercury, we examined the effects of thimerosal on the biochemical and molecular steps of mitochondrial pathway of apoptosis in Jurkat T cells. Thimerosal and not thiosalcylic acid (non-mercury component of thimerosal), in a concentration-dependent manner, induced apoptosis in T cells as determined by TUNEL and propidium iodide assays, suggesting a role of mercury in T cell apoptosis. Apoptosis was associated with depolarization of mitochondrial membrane, release of cytochrome c and apoptosis inducing factor (AIF) from the mitochondria, and activation of caspase-9 and caspase-3, but not of caspase-8. In addition, thimerosal in a concentration-dependent manner inhibited the expression of XIAP, cIAP-1 but did not influence cIAP-2 expression. Furthermore, thimerosal enhanced intracellular reactive oxygen species and reduced intracellular glutathione (GSH). Finally, exogenous glutathione protected T cells from thimerosal-induced apoptosis by upregulation of XIAP and cIAP1 and by inhibiting activation of both caspase-9 and caspase-3. These data suggest that thimerosal induces apoptosis in T cells via mitochondrial pathway by inducing oxidative stress and depletion of GSH.
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Introduction

Apoptosis is a physiological form of cell suicide that plays a role in embryogenesis, metamorphosis, cellular homeostasis, and as a defensive mechanism to remove infected, mutated, or damaged cells. In the immune system, apoptosis plays an important role in the selection of T cell repertoire, deletion of self-reactive lymphocytes, natural killer (NK)- and T cell-mediated cytotoxicity, and termination of response at the end of an immune response.[1,2] There are two major pathways of apoptosis: the death receptor pathway and the mitochondrial pathway.[3–11] There is recent evidence to suggest that these two pathways may be linked in certain cell types.[1,10,11] In both pathways, a series of molecular and biochemical steps leads to the activation of common effector or executioner cysteine proteases, the caspases resulting in the cleavage of a number of nuclear and cytoplasmic substrates, including those responsible for the maintenance of nuclear integrity, cell cycle progression, and DNA repair.

Thimerosal (also known as Thimersel, merthiolate or sodium ethylmercuri-thiosalicylate) is a water-soluble derivative of thiosalicylic acid with antimicrobial activity. Thimerosal is used as an antimicrobial agent and a preservative in cleaning solutions for eye lenses, cosmetics, and vaccines. It is estimated that an infant could be exposed to as high as 187.5 μg of ethylmercury from the routine immunization schedule.[12] Because of health-related concern from exposure to Hg from thimerosal-containing vaccines, the American Academy of Family Physicians, the American Academy of Pediatrics, the Advisory Committee on Immunization Practices (ACIP), and the United States Public Health Service have published their recommendations to remove and greatly reduce thimerosal from vaccines a soon as possible.[12] Hg has been shown to induce a number of immunological and neurotoxic changes, including increased production of Th2 cytokines, increased levels of IgE, decreased activity of T cells and NK cell, suppression of IgG, production of autoantibodies to a variety of self antigens (eg, neural antigens), and apoptosis in microglia and astrocytes.[13–21] The majority of these studies have been performed both *in vitro* and *in vivo* in experimental animals. Because of likely exposure to high levels of Hg via vaccines containing thimerosal and its possible effects on the immune system, we examined the effect of thimerosal in concentrations well within exposure on biochemical and molecular steps of T cell apoptosis.

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