



Suppression by Thimerosal of *Ex-Vivo* CD4⁺ T Cell Response to Influenza Vaccine and Induction of Apoptosis in Primary Memory T Cells

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Abstract

Thimerosal is a preservative used widely in vaccine formulations to prevent bacterial and fungal contamination in multidose vials of vaccine. Thimerosal was included in the multidose non-adjuvanted pandemic 2009 H1N1 vaccine Panenza. In the context of the analysis of the *ex-vivo* T cell responses directed against influenza vaccine, we discovered the *in vitro* toxicity Panenza, due to its content in thimerosal. Because thimerosal may skew the immune response to vaccines, we investigated in detail the *ex-vivo* effects of thimerosal on the fate and functions of T cells in response to TCR ligation. We report that *ex-vivo* exposure of quiescent or TCR-activated primary human T cells to thimerosal induced a dose-dependent apoptotic cell death associated with depolarization of mitochondrial membrane, generation of reactive oxygen species, cytochrome c release from the mitochondria and caspase-3 activation. Moreover, exposure to non-toxic concentrations of thimerosal induced cell cycle arrest in G0/G1 phase of TCR-activated T cells, and inhibition of the release of proinflammatory cytokines such as IFN gamma, IL-1 beta, TNF alpha, IL-2, as well as the chemokine MCP1. No shift towards Th2 or Th17 cells was detected. Overall these results underline the proapoptotic effect of thimerosal on primary human lymphocytes at concentrations 100 times less to those contained in the multidose vaccine, and they reveal the inhibitory effect of this preservative on T-cell proliferation and functions at nanomolar concentrations.

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Introduction

Thimerosal is a preservative used widely in vaccine formulations to prevent bacterial and fungal contamination in multidose vials of vaccine [1] [2]. Thimerosal, named also thiomersal or merthiolate in clinical studies, is an ethylmercury-containing pharmaceutical compound that contains 49.6% mercury by weight and metabolizes into ethylmercury (etHg) and thiosalicylate [3]. Thimerosal has served as a preservative in vaccines since 1930, but in the late 1990 concerns came as more thimerosal-containing vaccines were added to the recommended infant and child immunization schedule [4]. Research on the specific *in vivo* toxicity of low doses of etHg relevant to vaccines has only recently been performed [5] [6,7]. *In vitro*, thimerosal has been shown to cause a number of neurotoxic changes, including neuronal mitochondrial cell death characterized by the release of cytochrome c and apoptosis inducing factor from mitochondria to cytosol [8], DNA breaks and caspase 3 activation in cultured human neuronal cells [9], and mitochondrial-mediated apoptosis in a human neuroblastoma cell

line following exposure to μM concentrations of thimerosal [10]. The deleterious effects of thimerosal were also reported on HeLa S epithelial cells, inducing an oxidative stress and cell death that were completely suppressed by pretreating the cells with N-acetylcysteine (NAC), a radical scavenger [11]. Thimerosal could also cause S phase arrest followed by mitochondrial apoptosis in murine myoblast cells that occurred via inhibition of the PI3K/Akt/survivin signaling pathway [12]. Surprisingly, little is known about the impact of thimerosal on immune cell functions. Trompezinski *et al.* found that it induced an oxidative stress in monocyte-derived dendritic cells [13], and Agrawal *et al.* reported that thimerosal could exercise a Th2 promoting effect through modulation of functions of dendritic cells [14]. At the T cell level, thimerosal was reported to induce caspase-dependent mitochondrial apoptosis in the human leukemic Jurkat T cells [15,16].

In the context of the clinical trial MICIVAX, designed to compare the efficacy and safety of influenza vaccine in patients with inflammatory bowel disease (IBD) receiving immunosuppressive therapy with patients not receiving immunosuppressants, we