Thimerosal induces TH2 responses via influencing cytokine secretion by human dendritic cells

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Abstract: Thimerosal is an organic mercury compound that is used as a preservative in vaccines and pharmaceutical products. Recent studies have shown a TH2-skewing effect of mercury, although the underlying mechanisms have not been identified. In this study, we investigated whether thimerosal can exercise a TH2-promoting effect through modulation of functions of dendritic cells (DC). Thimerosal, in a concentration-dependent manner, inhibited the secretion of LPS-induced proinflammatory cytokines TNF-α, IL-6, and IL-12p70 from human monocyte-derived DC. However, the secretion of IL-10 from DC was not affected. These thimerosal-exposed DC induced increased TH2 (IL-5 and IL-13) and decreased TH1 (IFN-γ) cytokine secretion from the T cells in the absence of additional thimerosal added to the coculture. Thimerosal exposure of DC led to the depletion of intracellular glutathione (GSH), and addition of exogenous GSH to DC abolished the TH2-promoting effect of thimerosal-treated DC, restoring secretion of TNF-α, IL-6, and IL-12p70 by DC and IFN-γ secretion by T cells. These data suggest that modulation of TH2 responses by mercury and thimerosal, in particular, is through depletion of GSH in DC. *J. Leukoc. Biol.* 81: 474–482; 2007.

Key Words: *APC · heavy metal · immune modulation*

INTRODUCTION

Exposure to mercury is widespread in the world, and inorganic mercury, ethylmercury, and methylmercury are the predominant chemical species. The primary sources of exposure to mercury are amalgam, mercury vapors, vaccination, and seafood consumption [1–3]. Thimerosal (ethylmercurithiosalicylate) is an organic mercury compound that has been used as a preservative in vaccines, intramuscular immune globulin preparations, skin test antigens, antivenoms, ophthalmic and nasal products, and tattoo inks [1–3]. It has 49.6% mercury by weight, and following its administration, its metabolite, ethylmercury, dissociates from thiosalicylic acid and binds to blood or other tissue. The extensive use of vaccines in today's society has led to concerns about immunization safety. Today, children receive more total number of vaccinations given together during the first two years of life, leading to exposure to quantities of mercury that exceeds the safety guidelines through thimerosal in vaccines. There is an increasing concern about association between the exposure to mercury (via vaccination) and the development of neurodevelopmental disorders, especially autism and learning disabilities [3–8]. This has led to thimerosal being withdrawn from pediatric vaccines in the United States starting in 1999 (Centers for Disease Control and Prevention, 1999). Nevertheless, thimerosal is still used in influenza, diphtheria toxoid and acellular pertussis, and tetanus toxoid vaccines. The majority of the studies are directed toward understanding the neurotoxic effect of thimerosal, and few studies deal with its effect on the immune system.

The effect of mercury on the immune system has been studied mostly in rodents. These studies have revealed that subtoxic doses of mercury exposure in genetically susceptible H-2 mice strains result in the development of systemic autoimmunity characterized by lymphoproliferation with polyclonal B cell activation and hyper-γ-globulinemia, production of autoantibodies targeting the 34-kDa nucleolar protein fibrillarin, and development of immune-complex deposits [9–15]. The different forms of mercury differ in the type and range of immune disorders, and ethylmercury (thimerosal) and inorganic mercury are similar in that they cause systemic autoimmunity, characterized by a marked increase of IgE and systemic immune-complex deposits [16, 17]. Antifibrillarin autoantibodies (AFA) and maximum levels of serum IgE are present as early as 10 days after exposure to ethylmercury in the mice [16]. Similar to the autoimmune disease induced by inorganic mercury, thimerosal induces a distinctly increased expression of IL-4 mRNA and a large increase in TH2-dependent, Ig-secreting cells and serum Igs [18]. The increase in IL-4 has been attributed to a direct induction of IL-4 gene expression in lymphocytes by mercury [19]. Methylmercury, conversely, induces only modest titers of AFA and none of the above symptoms [16, 17, 20]. One of the possible explanations is that ethylmercury is converted much faster into inorganic mercury compared with methylmercury, leading to an earlier and more potent effect on the immune system. The immunosuppressive effects of ethyl and methyl mercury are similar and more potent than inorganic mercury in that they both cause reduction in the number and proliferative capacity of splenic T and B lymphocytes [17, 20]. Studies in humans document

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