

## Property of thimerosal-induced decrease in cellular content of glutathione in rat thymocytes: a flow cytometric study with 5-chloromethylfluorescein diacetate

T. Ueha-Ishibashi <sup>a,b</sup>, T. Tatsuishi <sup>a</sup>, K. Iwase <sup>a</sup>, H. Nakao <sup>a</sup>, C. Umebayashi <sup>a</sup>,  
Y. Nishizaki <sup>a</sup>, Y. Nishimura <sup>a</sup>, Y. Oyama <sup>a,\*</sup>, S. Hirama <sup>c</sup>, Y. Okano <sup>c</sup>

<sup>a</sup> Laboratory of Cellular Signaling, Faculty of Integrated Arts and Sciences, The University of Tokushima, Minami-Jyosanjima 1-1, Tokushima 770-8502, Japan

<sup>b</sup> Clinical Trial Support Center, Kumamoto University Hospital, Kumamoto 860-8556, Japan

<sup>c</sup> Department of Pharmaceutical Care and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan

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### Abstract

There is a concern on the part of public health community that adverse health consequences by thimerosal, a preservative in vaccines for infants, may occur among infants during immunization schedule. Therefore, the effect of thimerosal on cellular content of glutathione was examined on thymocytes obtained from 4-week-old rats using a flow cytometer and 5-chloromethylfluorescein diacetate. Thimerosal at concentrations ranging from 1 to 10  $\mu\text{M}$  reduced the cellular content of glutathione in a concentration-dependent manner, and the complete depletion of cellular glutathione was observed when the cells were treated with 30  $\mu\text{M}$  thimerosal. L-Cysteine significantly attenuated the actions of thimerosal to reduce the glutathione content and to increase the intracellular  $\text{Ca}^{2+}$  concentration. Prolonged incubation (24 h) with 1–3  $\mu\text{M}$  thimerosal induced the apoptosis. The cytotoxic action of thimerosal was greatly augmented when the cells suffered oxidative stress induced by  $\text{H}_2\text{O}_2$ . It may be unlikely that thimerosal exerts potent cytotoxic action under the in vivo condition because the blood concentration of thimerosal after receiving vaccines does not seem to reach micromolar range and nonprotein thiols at micromolar concentrations are present in the blood.

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### 1. Introduction

Thimerosal has been used as an organomercurial preservative in vaccines to prevent contamination with harmful microbes since early 1930s. There is a recent concern on the part of public health community that adverse health consequences by thimerosal may occur among infants during immunization schedule (Ball et al., 2001; van't Veen, 2001; Westphal and Hallier, 2002; Westphal et al., 2003). In laboratory in vitro studies, thimerosal has been used as a sulfhydryl reagent to modify some of membrane and cellular functions

(Bootman et al., 1992; Cai and Sauve, 1997; Marengo et al., 1998; Lang et al., 2000). The action of thimerosal to decrease content of cellular glutathione is probably one of basic actions related to its toxicity because of a following reason. Change in cellular redox status modulates channel and receptor activities in several types of cells (Elliott and Koliwad, 1997; Lipton et al., 1998; Tanaka et al., 1999; Choi and Lipton, 2000; Pessah, 2001). Furthermore, the cell growth and death are related to cellular redox state (Powis et al., 1995; Buttke and Sandstrom, 1995; Hampton and Orrenius, 1998; Mates et al., 2002). Therefore, to elucidate the property of thimerosal-induced action on cellular content of glutathione, we have examined the effect of thimerosal on lymphocytes obtained from rat thymic glands using a flow cytometer with fluorescent dyes.

\* Corresponding author. Tel./fax: +81-88-656-7256.

E-mail address: oyama@ias.tokushima-u.ac.jp (Y. Oyama).