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The Effect of Thimerosal on Neutrophil Migration

A Comparison with the Effect on Calcium Mobilization and CD11b Expression

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ABSTRACT. The sulfhydryl-reactive compound thimerosal caused a chemotactic stimulation of neutrophil migration at low concentrations and inhibition of chemoattractant-stimulated chemotaxis at high concentrations. Thiosalicylic acid, an analog of thimerosal devoid of mercury, also stimulated migration at low concentrations and caused inhibition at higher concentrations, though the inhibitory effect was less pronounced than that of thimerosal. These results indicate that the stimulatory effect of thimerosal on migration is due to the thiosalicylic acid moiety of the molecule. In contrast with thimerosal which, especially at higher concentrations than required for optimal stimulation of migration, caused an increase in cytosolic free calcium ($[Ca^{2+}]_i$), thiosalicylic acid had no effect on $[Ca^{2+}]_i$ of the neutrophil. This suggests that the presence of mercury is decisive for the calcium-mobilizing effect, but not for stimulation of migration, and that mobilization of calcium and activation of migration are not related. Thimerosal caused a strong increase of CD11b expression in neutrophils in suspension, especially at inhibitory concentrations, while thiosalicylic acid had no effect on CD11b expression. This could mean (but does not prove) that CD11b expression is more related to the calcium-mobilizing effect of thimerosal than to its stimulation of migration. BIOCHEM PHARMACOL **55**;3: 305–312, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. neutrophil; thimerosal; migration; chemotaxis; calcium; CD11b expression

Migration by neutrophils plays a predominant role in both the anti-microbial and the inflammation-promoting activities of these cells by enabling them to reach the site of infection or inflammation. In spite of extensive research, the molecular basis of the migration process remains largely unknown, with the calcium homeostasis during migration being a matter of particular controversy [1–4]. Most chemoattractants cause an increase in cytosolic free calcium ($[Ca^{2+}]_i$)†, but there is no evidence that the ability to cause an increase in $[Ca^{2+}]_i$ is related to the extent of migration. On the contrary, chemotactic migration is inhibited by a number of agents which cause an increase in $[Ca^{2+}]_i$ [5–9].

Thimerosal is an organomercury compound with sulfhydryl-reactive properties. It is clinically used as a topical antiinfective agent because of its antibacterial and antifungal properties. The substance has a profound effect on calcium homeostasis in a number of cells. While it was originally described as an agent having a specific effect on inositol trisphosphate (IP_3)-sensitive calcium stores, recent studies have shown that in addition to IP_3-sensitive stores, ryanodine-sensitive stores are also affected by thimerosal [10, 11]. The effect of thimerosal is biphasic: at low concentrations it causes cytosolic calcium oscillations in endothelial cells, whereas at high concentrations the oscillations are inhibited and a sustained increase in $[Ca^{2+}]_i$ is observed [12].

Thimerosal causes an increase of $[Ca^{2+}]_i$ in neutrophils [13]. It also causes a strong increase in 5-lipoxygenase metabolites when another activator, such as formyl-methionyl-leucyl-phenylalanine (fMLP), is present [13, 14]. Leukotriene formation depended on the presence of extracellular $[Ca^{2+}]_i$, and it was concluded that the enhancing effect of thimerosal on fMLP-induced leukotriene formation was due to its modulating effect on calcium homeostasis.

Because of the effects of thimerosal on calcium homeostasis and because of sulfur-containing compounds were shown in previous studies to be capable of inducing chemotaxis, we decided to study the effect of thimerosal on neutrophil migration. We set out to determine whether the substance could induce chemotaxis by itself and whether thimerosal could affect chemotactic migration activated by other chemoattractants. In addition, we wished to address the question as to whether the effect on migration was connected with the effect on calcium metabolism. To determine the importance of mercury in the thimerosal molecule, we compared the results of thimerosal with those of thiosalicylic acid, a mercury-less analog of thimerosal (Fig. 1).

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† *Abbreviations:* fMLP, formyl-methionyl-leucyl-phenylalanine; $[Ca^{2+}]_i$, cytosolic free calcium concentration; IL-8, interleukin 8; IP_3, inositol trisphosphate; LDH, lactate dehydrogenase; PMA, phorbol myristate acetate; LTB_4: leukotriene B_4.

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