

Stimulation of IgE sensitized human alveolar macrophages by anti-IgE is unaffected by sodium cromoglycate

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Summary

Sodium cromoglycate is an effective prophylactic in the treatment of asthma. However, its mechanism of action is still uncertain. The release of thromboxane B₂ and N-acetyl- β -D-glucosaminidase activity was measured from human alveolar macrophages sensitized with human myeloma IgE. Cells were challenged with rabbit affinity purified anti-IgE (no activity against IgG) in the absence or presence of sodium cromoglycate (10^{-4} – 10^{-8} M). There was no change in the release of either thromboxane B₂ or N-acetyl- β -D-glucosaminidase in the presence of sodium cromoglycate at these concentrations.

Introduction

Sodium cromoglycate (SCG) is established as an effective treatment of the symptoms of asthma [1] as well as an effective inhibitor of various non-specific bronchoconstrictor challenges, such as exercise [2] or the inhalation of irritants [3]. The significance of the observation that SCG inhibits mast cell degranulation in some species [4] has been questioned [5], as SCG appears to have little inhibitory action on mediator release from human pulmonary mast cells [6]. SCG in μ M concentrations has been shown to inhibit some but not all [4,7–9] markers of animal macrophage activation, and in a preliminary report [10] it was stated that SCG could inhibit the release of lysosomal hydrolases from alveolar macrophages after stimulation by IgE-dependent mechanisms. In this study, we have investigated the release of thromboxane B₂ and N-acetyl- β -D-glucosaminidase from human alveolar macrophages stimulated by IgE anti-IgE complexes.

Methods

Alveolar macrophages were harvested from lung lavage fluid obtained from five patients undergoing diagnostic bronchoscopy. Informed patient consent was

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obtained, and the lavage was performed as part of a routine diagnostic service. Cells were incubated with 2 ml of Dulbecco's modified Eagle's essential medium (DMEM) for 1 hr at 37°C in 35 mm plastic wells (Falcon Plastics). The cells were then washed with DMEM at 4°C to remove non-adherent cells, leaving a >95% pure macrophage culture [11]. The cells were then incubated in the presence of 10 000 U human myeloma IgE serum (a kind gift from Dr P. Richies, Westminster Hospital Medical School, London) for 1 hr at 4°C. Following further washing at 4°C, culture dishes were incubated in duplicate in the absence or presence of sodium cromoglycate (10^{-4} – 10^{-8} M) (a kind gift from Dr D.M. Jackson, Fisons plc, U.K.) for 5 min at 4°C before the addition of 100 µg rabbit affinity purified anti-human IgE (no activity against IgG) in all but the control wells. The cells were then incubated for a further 30 min at 4°C, and 15 min at 37°C. The anti-human IgE was a kind gift from Dr D.M. Kemeny (Guy's Hospital Medical School, London, U.K.). Following the final incubation, 900 µl of DMEM was added to 100 µl of 0.1 M Tris-HCl buffer, pH 7.4 and samples were stored at -20°C for subsequent analysis of thromboxane B₂ by radioimmunoassay [12] and N-acetyl-β-D-glucosaminidase activity [11]. The protein content of the dishes was analysed by a modification of the method of Lowry *et al.* [13]. Cells were dried overnight and then dissolved in 2 ml of a 50:50 mixture of 100 mM NaOH and 1% (w/v) sodium dodecyl sulphate. Employing these methods, there were 3.59×10^6 cells/mg of cell protein (range 3.44 – 3.78×10^6). Statistical analysis of results was by two-way analysis of variance [14].

Results

The mean ($\pm$ s.e.m.) values for thromboxane B₂ released by cells not challenged with anti-IgE was 1.7 ± 0.4 ng/mg cell protein, and the mean ($\pm$ s.e.m.) N-acetyl-β-D-glucosaminidase activity was 12.2 ± 4.2 µmol/min/g cell protein. When stimulated with anti-IgE, thromboxane B₂ release was 10.7 ± 3.0 ng/mg and enzyme activity 39.7 ± 13.9 units. The effect of incubation of the cells with SCG is shown in Table 1. There was no inhibition of either thromboxane B₂ or N-acetyl-β-D-glucosaminidase release at any concentration of sodium cromoglycate.

Table 1. The release (mean $\pm$ s.e.m.) of thromboxane B₂ and N-acetyl-β-D-glucosaminidase from human alveolar macrophages

Addition		Thromboxane B ₂ (ng/mg cell protein)	N-acetyl-β-D-glucosaminidase (µmol/min/g cell protein)
Control		1.7 ± 0.4	12.2 ± 4.2
Anti-IgE		10.7 ± 3.0	39.7 ± 13.9
	(10 nM)	9.3 ± 1.2	47.5*
	(100 nM)	11.9 ± 3.4	44.3 ± 16.4
Anti-IgE + sodium cromoglycate	(1 µM)	10.7 ± 3.0	42.2 ± 12.3
	(10 µM)	10.7 ± 3.3	49.1 ± 16.8
	(100 µM)	12.4 ± 3.4	49.0*

Cells were sensitized with 10 000 U human myeloma IgE, and then challenged with the additions shown as described in methods. Values were obtained in duplicate from five patients, except where indicated (*) for $n=2$.

Discussion

Previous reports [15] from this laboratory have described IgE-dependent activation of human alveolar macrophages. There was a simultaneous release of thromboxane B₂, prostaglandin F_{2α}, leukotriene B₄ and N-acetyl-β-D-glucosaminidase. However, there was no increase in the production of active oxygen species, as measured by lucigenin chemiluminescence.

In contrast to reports published previously [10], we have been unable to demonstrate inhibition by SCG of release of thromboxane B₂ or N-acetyl-β-D-glucosaminidase from human alveolar macrophages activated by IgE-dependent mechanisms. This is no ready explanation for the difference in the results presented here and those published by Tonnell and colleagues [10]. The concentration range of SCG shown in Table 1 includes values greatly in excess of those reported to inhibit IgE-dependent release of β-glucuronidase (0.2 μM). However, differences in the experimental design or in the specificities of the anti-IgE antibodies used might result in cellular activation by different mechanisms in these two reports. The anti-IgE employed here has no cross-reactivity with IgG [15].

Previously, Vickova [9] has demonstrated an SCG-dependent reduction in the cytotoxic effects on macrophages that is produced by engulfing silica particles *in vitro* in rabbits or *in vivo* in rats. In addition, 0.4 mmol SCG/kg injected intravenously into mice [7] inhibits formation of specific IgG-producing spleen cells in mice *in vivo*, and doses above 100 μM of SCG reduces the mitogen-induced migration of mouse macrophages *in vitro*. However, other reports [4] have not confirmed a significant modification of antigen-induced inhibition of guinea-pig macrophage motility *in vitro*, at doses of 0.2 mM SCG.

SCG mediates inhibition of both cyclic AMP and cyclic GMP phosphodiesterases in rat peritoneal macrophages with IC₅₀ values of 1.6 and 0.69 mM, respectively [8]. SCG appears between three and ten times less potent than theophylline. Inhibition of phosphodiesterase activity might be used to invoke a possible mechanism for SCG-dependent inhibition of mediator release, involving a transient rise in intracellular cyclic AMP. However, we have been unable to confirm such an inhibitory effect of SCG on mediator release from human alveolar macrophages. In this report, the possible effect of SCG was examined on triggered release of pre-formed enzyme and newly synthesized thromboxane B₂. The negative result does not however exclude the possibility of a selective effect of SCG on other responses of the cell following IgE-dependent activation, e.g., phagocytosis, cellular motility, leukotriene production, etc. Such a selective effect of SCG on cellular responses has not, however, been demonstrated in other cell types.

It is concluded that it is most unlikely that the inhibitory effect of SCG on allergic bronchoconstriction in man results from altered mediator release from alveolar macrophages.

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