

Dear Editor

Modulation of Human Basophil Degranulation by Geranylgeranyl Compounds

Recent experimental studies have shown that the basophil is critical for a subtype of anaphylaxis or IgE-mediated very late-phase skin inflammation.¹ Motility and activation of basophils are known to be regulated by various endogenous and/or exogenous substances. The panel of known such basophil-directed molecules is expanding. One important candidate may be geranylgeranyl compounds, which were initially demonstrated to be protectors of the gastric mucosa.² These compounds have also demonstrated protective actions in situations that are often hazardous to the host. In rodent studies, geranylgeranylacetone (GGA) suppressed the development of several inflammatory reactions and enhanced tissue regeneration *in vivo*.^{2,3} However, the effects of GGA on allergic effectors and immunologic reactions have not been fully clarified.

In this study, we assessed the pharmacological actions of GGA and related compounds on basophil degranulation, detected as release of histamine. Basophils were obtained from non allergic volunteers by dextran sedimentation of whole blood. Cells were preincubated with GGA (Wako Pure Chemicals, Osaka, Japan) for 15 min at 37°C, washed and then stimulated with a secretagogue for 45 min.⁴

Degranulation of basophils by polyclonal anti-IgE antibody (MBL, Nagoya, Japan) and by phorbol myristate acetate (PMA) (Sigma, St. Louis, MO, USA) was significantly enhanced by preincubation of cells for 15 min with GGA at 1.4 or 2.7 mM (Fig. 1a). Similar results were observed for highly pure basophils (purity >95%; prepared by Percoll gradient centrifugation followed by negative MACS selection), indicating that GGA acts directly on basophils. On the other hand, GGA showed no clear effect on basophil degranulation by a chemokine, monocyte chemoattractant protein (MCP)-1 or Ca ionophore A23187. As shown in Figure 1b, up to 15 min of incubation of basophils with GGA alone at 2.7 mM did not induce release of histamine. However, 30 min or longer incubation resulted in higher, 15 to 20% histamine release, suggesting that GGA at this concentration might damage basophils in a time-dependent manner. The extent of enhancement of IgE-mediated basophil degranulation by GGA was mild compared to that by IL-3, and GGA did not show additive augmentation of histamine release by IL-3-treated basophils (Fig. 1c). As shown in Figure 1d, another geranylgeranyl compound, geranylgeraniol (Sigma), also upregulated basophil histamine release evoked by anti-IgE antibody

or PMA. On the other hand, compounds having smaller structures, such as geranylacetone and farnesol, showed no effect on basophil degranulation (data not shown). Statins inhibit intracellular geranylgeranylation, resulting in suppression of the activation profiles of inflammatory cells,⁵⁻⁷ and basophil degranulation in response to anti-IgE antibody or PMA was significantly suppressed by preincubation with simvastatin at 50 μ M (Fig. 1e).

Geranylgeranyl compounds are reported to be involved in intracellular signal cascades in various cells, including mast cell activation evoked by IgE crosslinkage.⁵⁻⁷ Our present finding that exogenously added GGA can enhance basophil activation suggests that GGA enters basophils and then behaves as a substrate in the cell activation pathway. Simvastatin, which can inhibit intracellular geranylgeranylation, suppressed basophil degranulation triggered by anti-IgE antibody and PMA, but not A23187. This finding coincides with our results that GGA augmented basophil degranulation evoked by anti-IgE antibody and PMA, but not A23187 or MCP-1, suggesting that protein kinase C or related molecule(s) may be the target of GGA. It appears that geranyl and farnesyl compounds are not involved in the above pathway in basophils.

GGA's various novel actions are being unveiled through recent experimental approaches. Mostly based on murine studies, this compound is able to suppress vicious inflammatory disorders of the skin or visceral organs, including pulmonary inflammation induced by gefitinib, an inhibitor of epidermal growth factor receptor-mediated signals.³ It is thought that one of main *in vivo* effects of GGA is induction of tissue-stabilizing heat shock proteins.^{2,3} *In vivo* studies have so far tested GGA at μ M to mM concentrations and have found increases in those proteins and changes in cell fate.^{8,9} Our present findings suggest that GGA at relatively high concentrations can also exert acute effects on cellular functions and enhance the activation of basophils evoked by certain secretagogues. Although the *in vivo* significance of our findings and the whole aspect of the biologic actions of GGA remain unclear, elucidation of the precise roles of this compound in allergies and other tissue-damaging disorders is of special interest in light of its characteristic host-protecting properties.

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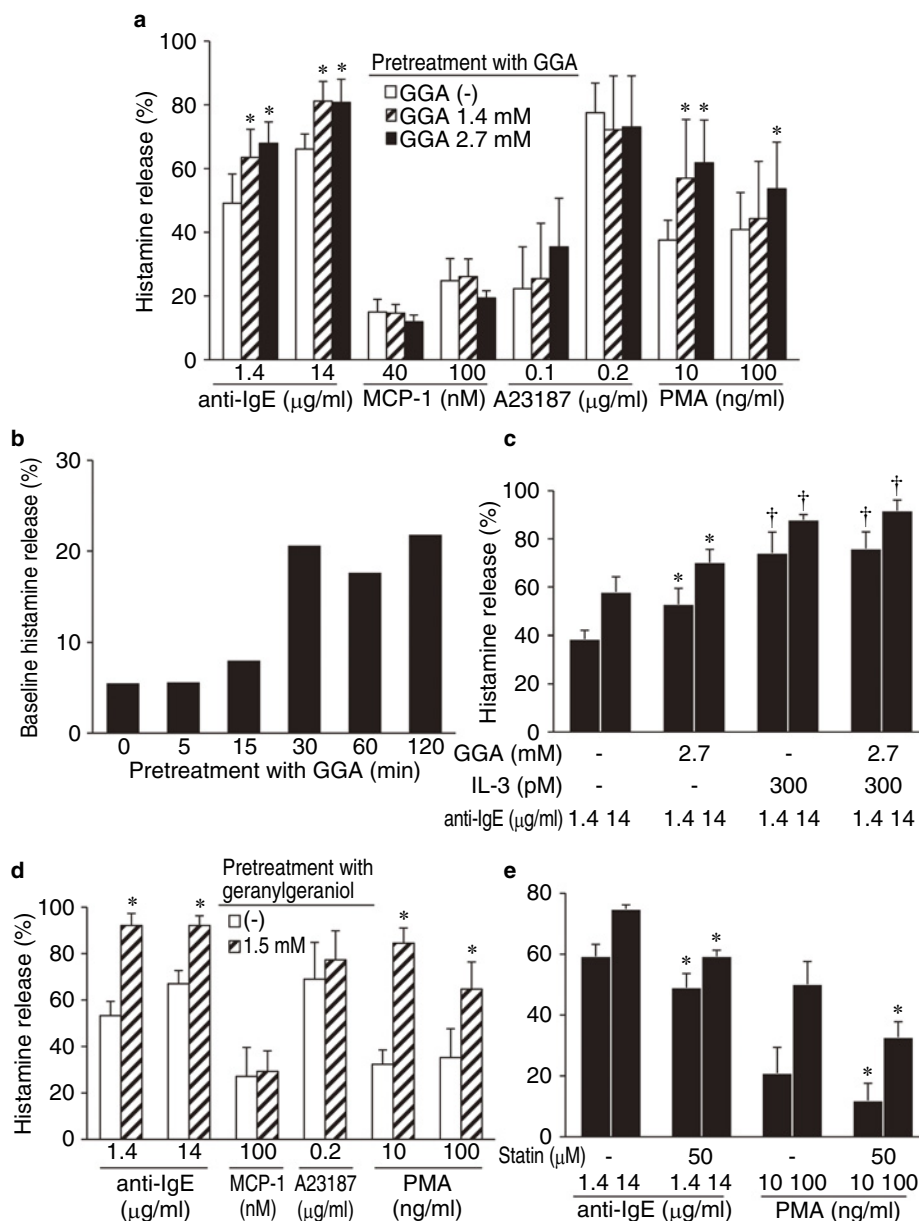


Fig. 1 Modulation of basophil histamine release by geranylgeranyl and related compounds. **(a)** Basophil preparations were preincubated with and without GGA and then stimulated with various secretagogues. GGA at 1.4 and 2.7 mM corresponds to 0.5 and 1 μl/ml, respectively. Histamine release was expressed as a percentage of the total cellular histamine after subtracting spontaneous release (usually <5%). Data are the mean ± SEM of three to six separate experiments. * $p \leq 0.05$, versus corresponding values of cells not preincubated with GGA. **(b)** Baseline histamine release by basophils after treatment with GGA. Cells were preincubated with GGA at 1 μl/ml for the indicated times, washed and then incubated without any secretagogue for 45 min. Data shown are representative of three separate experiments that generated similar results. **(c)** Priming effects of GGA and IL-3 on basophil degranulation. Cells were preincubated with and without GGA for 15 min, washed and then incubated with and without IL-3 before stimulation with anti-IgE antibody. Data are the mean ± SEM ($n = 3$). * $p < 0.05$ versus corresponding value of cells not preincubated with GGA. † $p < 0.05$ versus corresponding value of IL-3-untreated cells. **(d)** Effect of geranylgeraniol on basophil histamine release. The indicated concentration of this compound (0.5 μl/ml, 1.5 mM) did not induce non-specific histamine release. Data are the mean ± SEM ($n = 3$). * $p < 0.05$ versus corresponding value of cells not preincubated with geranylgeraniol. **(e)** Simvastatin suppressed basophil degranulation. Basophils were preincubated with and without simvastatin for 15 min at 37°C, washed and then stimulated with anti-IgE antibody or PMA for 45 min. Data are the mean ± SEM ($n = 4$). * $p < 0.05$, versus corresponding value of cells untreated with simvastatin.

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