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Letter to the Editor

The role of adenosine for IgE receptor-dependent degranulation of human peripheral basophils and skin mast cells

Dear Editor

Basophils and mast cells (MCs) play important roles in IgE antibody-associated allergic disorders, such as urticaria, pollen allergy and food allergy. Basophils and MCs express high-affinity IgE receptors (FcεRI) on their surface.^{1,2} Crosslinkage of IgE on the cell surface by antigen (allergen) results in the release of preformed mediators, such as histamine, from secretory granules, followed by generation of newly synthesized mediators including the products of arachidonic acid metabolism and cytokines. Although the mechanisms of basophil and MC activation have been well investigated, the attenuation pathway of the activation of these cells has remained unclear.

Adenosine has been implicated as an endogenous distress signal to balance inflammatory responses.^{3,4} It is rapidly produced from adenosine triphosphate (ATP) by enzymatic phosphohydrolysis, and can activate all four G protein-coupled adenosine receptors (A1, A2a, A2b and A3). Gomez *et al.* reported that treatment with high concentrations (10^{-4} – 10^{-3} M) of adenosine suppressed FcεRI-induced degranulation of human lung and skin mast cells.⁵ On the other hand, other studies have shown that adenosine potentiates IgE-dependent degranulation of LAD2, a human mast cell line,⁶ and mouse bone marrow derived mast cells (BMMCs).⁷

Moreover, target receptors of adenosine for the suppression of MC activation and the effects of adenosine on human peripheral basophils have not been examined. In this study, we investigated the role of adenosine and its receptor on IgE receptor-dependent activation of human peripheral basophils and human skin mast cells (hsMCs).

We first investigated the expression of adenosine receptors in human peripheral basophils and hsMCs. Basophils were magnetically isolated from human peripheral blood, using the EasySep™ Human Basophil Enrichment Kit (Supplementary Methods). HsMCs were isolated from human skin with collagenase, hyaluronidase and DNase followed by cell culture for 1–2 months with human stem cell factor (hSCF) (Supplementary Methods).⁸ Figure 1 shows the relative expression level of adenosine receptors in human peripheral basophils and hsMCs at mRNA level. While all adenosine receptors were expressed in hsMCs, A2a, A2b and A3, but no A1 receptors were expressed in basophils at the mRNA level.

We then investigated the effect of adenosine on the activation of human peripheral basophils and hsMCs. The treatment of human peripheral basophils with adenosine partially inhibited the release of histamine in response to anti-IgE (Fig. 2A). Degranulation of hsMCs sensitized with human IgE antibodies in response to anti-IgE, represented by β-hexosaminidase and histamine release, was

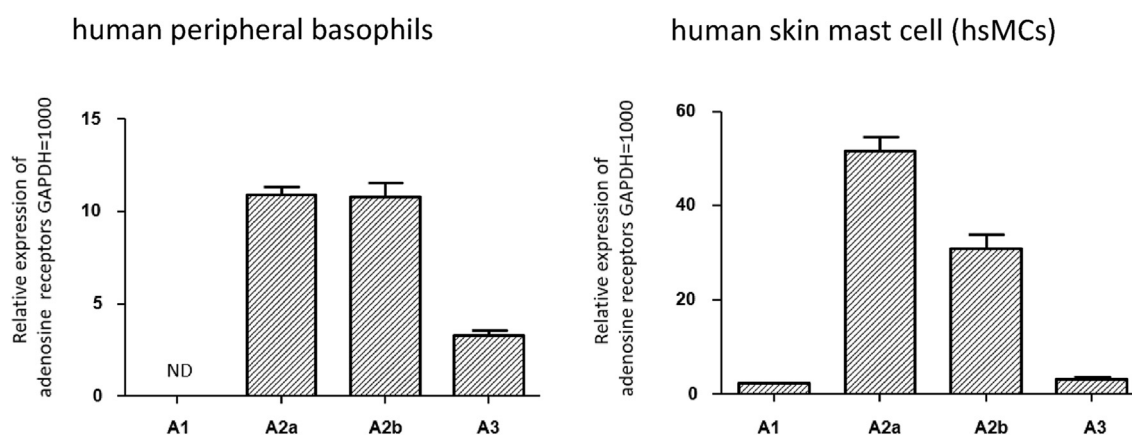


Fig. 1. Relative expression levels of adenosine receptors in human peripheral basophils and human skin mast cells (hsMCs). A1, A2a, A2b and A3 receptor mRNA levels in human peripheral basophils and hsMCs were analyzed by real-time PCR. Data were normalized to GAPDH mRNA levels. Values are shown as the mean $\pm$ SEM ($n = 3$). ND, not detected. The primers were used for PCR reactions as listed in Supplementary Methods.

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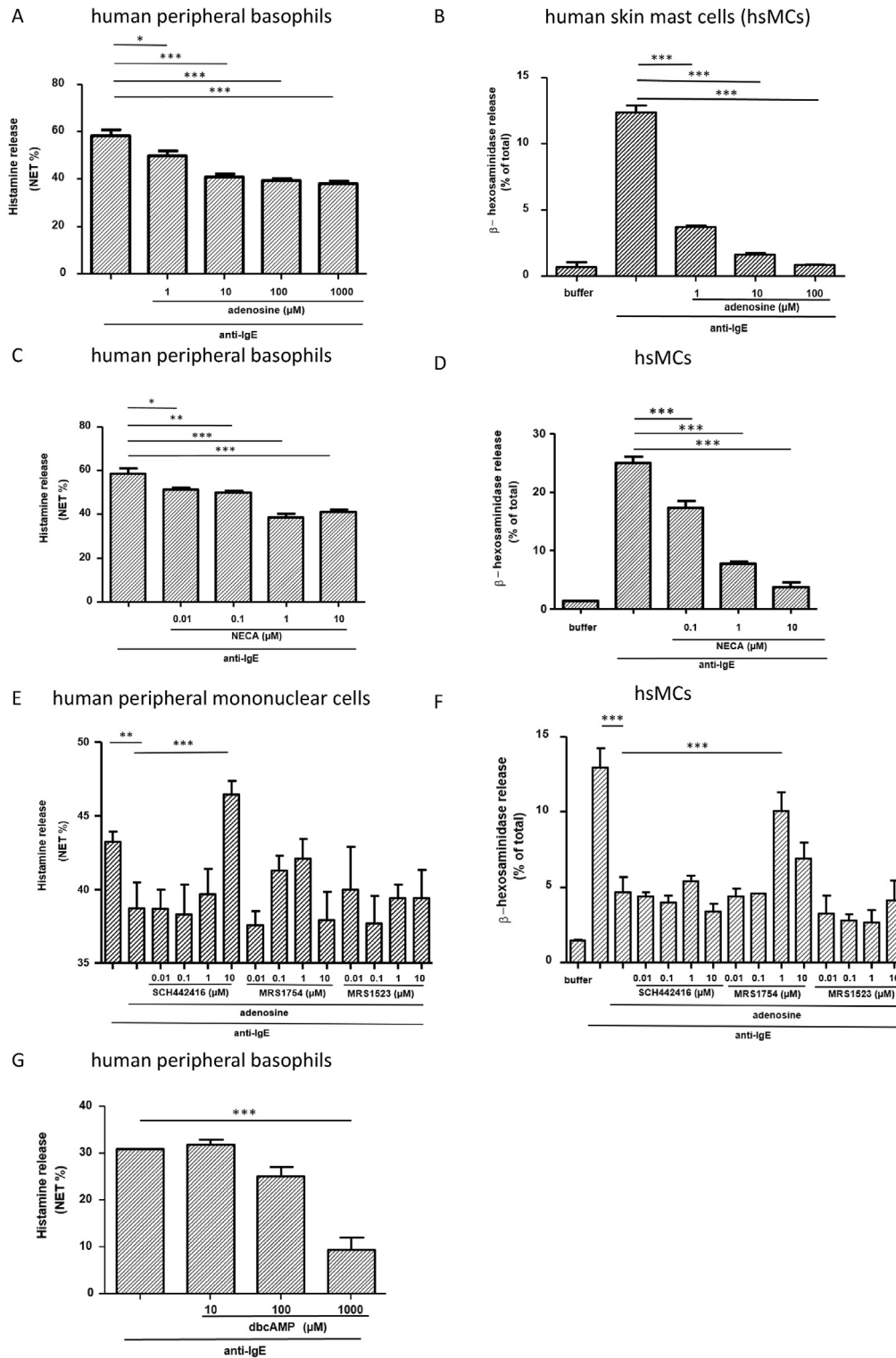


Fig. 2. Effect of adenosine, NECA or dbcAMP in the presence or absence of adenosine receptor antagonists on the IgE receptor-dependent activation of human peripheral basophils and hsMCs. **(A, B)** Adenosine suppressed the activation of basophils and hsMCs in response to anti-IgE at the concentration of 83.3 ng/ml for human peripheral basophils and 166.6 ng/ml for hsMCs, respectively. Results are expressed as mean $\pm$ SE from three to five separate determinations performed in triplicate. Adenosine alone had no effect on histamine release from basophils and β -hexosaminidase release from hsMCs without anti-IgE stimulation (data not shown). **(C, D)** NECA, a high affinity adenosine receptor agonist, inhibited the IgE receptor-dependent activation of basophils and hsMCs in a concentration-dependent manner. **(E)** Treatment with adenosine A2a receptor antagonist, SCH442416 inhibited adenosine (1000 μ M)-induced suppression of peripheral blood mononuclear cells (PBMC) activation. **(F)** Treatment with adenosine A2b receptor antagonist abolished adenosine (10 μ M)-induced suppression of hsMCs activation. **(G)** dbcAMP inhibited IgE receptor-dependent histamine release of human peripheral basophils in a concentration-dependent manner. The difference among each of the points was tested using a one-way ANOVA followed by Tukey's test and considered significant with $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***). Degranulation of hsMCs and histamine release from basophils were evaluated as described in [Supplementary Methods](#).

more effectively suppressed by the treatment with adenosine in a concentration-dependent manner (Fig. 2B, Supplementary Fig. 1). Moreover, 5'-N-ethylcarboxamidoadenosine (NECA), a high affinity adenosine receptor agonist, also inhibited the IgE receptor-dependent degranulation of basophils and hSMCs (Fig. 2C, D, and Supplementary Fig. 1). We next studied the role of receptors for adenosine on degranulation of basophils and hSMCs. Treatment with SCH442416, adenosine A2a receptor antagonist, but not MRS1523, A3 antagonist, inhibited the suppressive effect of adenosine on the release of histamine from peripheral blood mononuclear cells (PBMC). MRS1754, adenosine A2b antagonist, at 0.1 and 1 μ M tended to inhibit the effect of adenosine, but that at 10 μ M did not show such an activity (Fig. 2E). Adenosine receptor antagonists did not affect on histamine release from PBMC with or without anti-IgE in the absence of adenosine (Supplementary Fig. 2A, B). On the other hand, the suppressive effect of adenosine on hSMCs was abolished by MRS1754 at 1 μ M (Fig. 2F). MRS1754 had no effect on the spontaneous release of β -hexosaminidase from hSMCs (Supplementary Fig. 3), but anti-IgE-induced release of β -hexosaminidase from hSMCs was partially blocked by a high concentration of MRS1754, 10 μ M (Supplementary Fig. 3). Therefore, the weak effect of 10 μ M MRS1754 on the suppressive effect of adenosine on hSMCs might be due to a toxic effect of high concentration of MRS1754 on hSMCs. SCH442416 and MRS1523 affected neither spontaneous nor IgE-induced degranulation of hSMCs (data not shown). Thus, adenosine appears to suppress IgE receptor-dependent activation of human peripheral basophils and hSMCs via A2a and/or A2b receptors.

Since both adenosine A2a- and A2b-receptors are G α s-coupled receptors, the binding of adenosine to these receptors should result in an increase of cyclic AMP (cAMP) concentration followed by the activation of protein kinase A (PKA) in the cells.⁹ Moreover, Eskandari *et al.* reported that dibutyryl-cAMP (dbcAMP), an analog of cAMP that stimulates PKA, inhibited the degranulation of hSMCs by anti-IgE.¹⁰ We further studied the effect of PKA activation by dbcAMP on degranulation of basophils and hSMCs. As expected, pre-treatment by dbcAMP inhibited both anti-IgE induced release of histamine from basophils (Fig 2G) and β -hexosaminidase release from hSMCs (data not shown) in a concentration-dependent manner. Our findings imply that the application of adenosine A2a and A2b receptor agonists could represent effective therapeutic measures for patients with immediate type allergies. Further studies of each adenosine receptor by gene knock-down experiments would clarify more detailed mechanism of regulation of human basophils and mast cells activation by antigens and adenosine.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.alit.2018.03.007>.

Conflict of interest

The authors have no conflict of interest to declare.

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References

- Galli SJ. Mast cells and basophils. *Curr Opin Hematol* 2000;7:32–9.
- Yanase Y, Hide I, Mihara S, Shirai Y, Saito N, Nakata Y, et al. A critical role of conventional protein kinase C in morphological changes of rodent mast cells. *Immunol Cell Biol* 2011;89:149–59.
- Idzko M, Ferrari D, Eltzschig HK. Nucleotide signalling during inflammation. *Nature* 2014;509:3–10.
- Junger WG. Immune cell regulation by autocrine purinergic signaling. *Nat Rev Immunol* 2011;11:201–12.
- Gomez G, Zhao W, Schwartz LB. Disparity in Fc ϵ RI-induced degranulation of primary human lung and skin mast cells exposed to adenosine. *J Clin Immunol* 2011;31:479–87.
- Leung CT, Li A, Banerjee J, Gao ZG, Kambayashi T, Jacobson KA, et al. The role of activated adenosine receptors in degranulation of human LAD2 mast cells. *Purinergic Signal* 2014;10:465–75.
- Yoshida K, Ito M, Matsuoka I. Divergent regulatory roles of extracellular ATP in the degranulation response of mouse bone marrow-derived mast cells. *Int Immunopharmacol* 2017;43:99–107.
- Fujisawa D, Kashiwakura J, Kita H, Kikukawa Y. Expression of Mas-related gene X2 on mast cells is upregulated in the skin of patients with severe chronic urticaria. *J Allergy Clin Immunol* 2014;134:622–33.
- Fredholm BB, Ijzerman AP, Jacobson KA, Klotz KN, Linden J. Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001;53:527–52.
- Eskandari N, Bastan R, Peachell PT. Regulation of human skin mast cell histamine release by PDE inhibitors. *Allergol Immunopathol* 2015;43:37–41.

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Supplementary Methods

1. Reagents

EasySep™ Human Basophil Enrichment Kit was from STEMCELL Technologies (Vancouver, Canada). Ficoll-Paque Plus was from GE Healthcare Japan Corporation (Tokyo, Japan). X-vivo-15 was from Lonza (Walkersville, MD, USA). Human SCF were purchased from R&D Systems Inc (Minneapolis, MN, USA). The RNeasy Mini Kit and the Quanti Tect Reverse transcription Kit were from Qiagen (Valencia, CA, USA). An anti-DNP IgE and adenosine were from Sigma-Aldrich Japan (Tokyo, Japan). Reverse-phase HPLC was from Shimadzu (Kyoto, Japan).

2. Isolation of basophils from human peripheral blood

Human peripheral blood basophils were isolated from fresh heparinized blood of healthy donors using Ficoll-Paque Plus density gradient separation followed by magnetic depletion of non-basophils using the EasySep™ Human Basophil Enrichment Kit. Purity of isolated basophils was > 90%.

3. Isolation and culture of human skin derived mast cells

Human skin derived mast cells (hsMCs) were isolated from human skin. Skin was cut into fragments and incubated in a solution of HBSS containing type 2 collagenase (1.5 mg/ml), hyaluronidase (0.7 mg/ml), type I DNase (0.3 mg/ml), 1% FCS and 1mM CaCl₂ for 2hr at 37 °C. The dispersed cells were separated from residual tissues by filtration through a 70 mesh sieve and suspended in X-vivo-15 media. Isolated mast cells were cultured in X-vivo-15 media with 100 ng/ml recombinant human SCF for 1-2 months. Purity of hsMCs was > 70% as assessed by alcian blue/safranin stain and toluidine blue stain.

4. Real-time PCR

Total RNA was extracted from cells (basophils and hsMCs) using the RNeasy Mini kit and cDNA was generated using the Quanti Tect Reverse transcription kit. Expression of GAPDH was measured as an internal control to calibrate gene expression. Levels of mRNA expression of A1, A2a, A2b and A3 receptor were evaluated using ABI 7300 Real-time PCR system by TaqMan gene expression assay (Hs00181231_m1 for A1 receptor, Hs00169123_m1 for A2a receptor, Hs00386497_m1 for A2b receptor, Hs00252933_m1 for A3 receptor, Hs02758991_g1 for GAPDH).

5. Degranulation assay

Degranulation of hsMCs was evaluated by measuring the release of β -hexosaminidase, a granule marker that hydrolyzes *p*-nitrophenyl-*N*-acetyl- β -glucosamine to the chromophore, *p*-nitrophenol as previously described.² Briefly, hsMCs were sensitized with 50 ng/ml anti-DNP IgE. Next day, hsMCs were resuspended in piperazine-N- N'-bis (2-ethanesulfonic acid) (PIPES) buffer containing 119 mM NaCl, 5 mM KCl, 1.0 mM CaCl₂, 0.4 mM MgCl₂, 5.6 mM glucose, 25 mM PIPES and 1 mg/ml bovine serum albumin (BSA; pH 7.2). The cells were allowed to settle onto a 96-well plate. Cells were washed and incubated in the presence or absence of adenosine for 15 min followed by the addition of 166.6 ng/ml DNP-human serum albumin for 30 min. Degranulation was evaluated by measuring optical density of the chromophore.

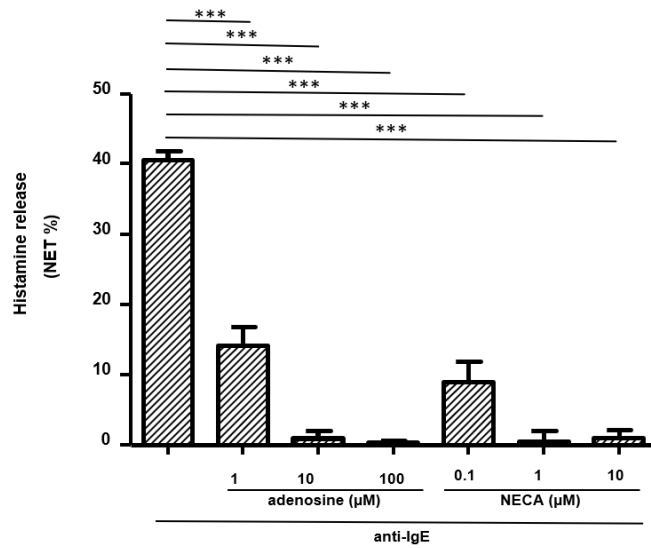
6. Histamine release test

Histamine release tests with human basophils were performed as described previously with

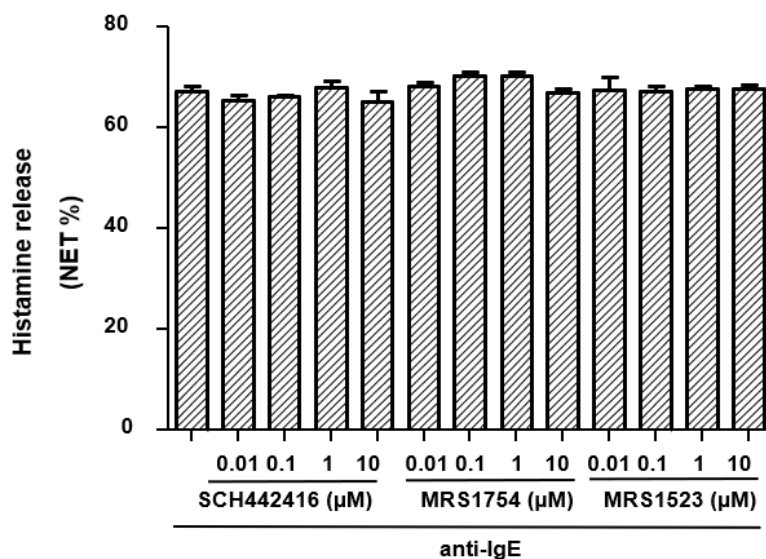
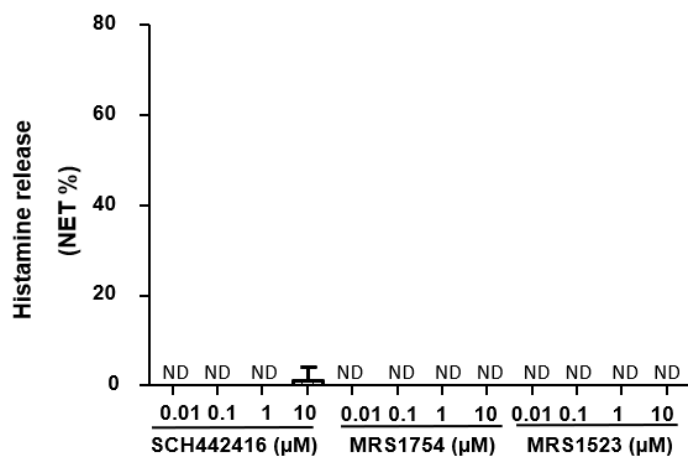
goat anti-IgE antibody as positive control.¹¹ Histamine was extracted and measured by reverse-phase HPLC.

Reference for Supplementary Methods

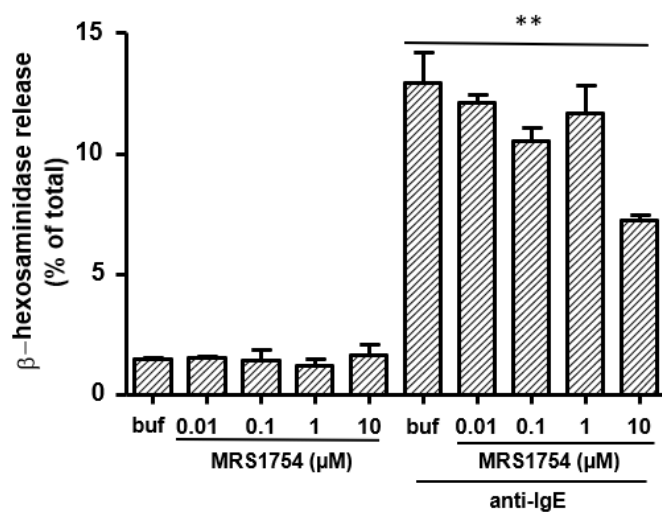
11. Yanase Y, Hiragun T, Yanase T, Kawaguchi T, Ishii K, Hide M. Evaluation of peripheral blood basophil activation by means of surface plasmon resonance imaging. *Biosens Bioelectron* 2012; **32**: 62-68.



Supplementary Fig. 1. Effects of adenosine and NECA on IgE receptor-dependent histamine release from hsMCs. Adenosine and NECA suppressed histamine release from hsMCs in response to anti-IgE at the concentration of 166.6 ng/ml.

A**B**

Supplementary Fig. 2. Effects of adenosine antagonists on the release of histamine from PBMC in the presence (A) or absence (B) of anti-IgE. Adenosine antagonists had no effect on histamine release in response to anti-IgE at the concentration of 83.3 ng/ml.



Supplementary Fig. 3. Effect of MRS1754 on the release of β -hexosaminidase from hsMCs in the presence or absence of anti-IgE. MRS1754 had no effect on spontaneous release of β -hexosaminidase from hsMCs without stimulation. A high concentration of MRS1754, 10 μ M, partially suppressed the release of β -hexosaminidase induced by anti-IgE (166.6 ng/ml).