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LETTER TO THE EDITOR

Inhibition by Tranilast of the Synergistic Induction of Degranulation and IL-13 Expression by IL-33 and FcεRI Cross-linking in Mast Cells

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ABSTRACT

Purpose: To assess the effects of tranilast on degranulation and IL-13 production in mast cells activated by IL-33 and cross-linking of FcεRI.

Methods: Bone marrow-derived mast cells (BMMCs) were sensitized with anti-DNP IgE. Sensitized cells were pretreated with tranilast and further stimulated with DNP-BSA, IL-33, or a combination of DNP-BSA and IL-33. Degranulation and the level of IL-13 release and mRNA expression in BMMCs were measured.

Results: Simultaneous stimulation with DNP-BSA and IL-33 resulted in marked increase in β-hexosaminidase release. Tranilast significantly inhibited degranulation of BMMCs in the condition of combined treatment of DNP-BSA and IL-33. Combination of DNP-BSA and IL-33 induced a pronounced increase in the IL-13 release and mRNA expression. Tranilast significantly inhibited IL-13 release and mRNA expression in BMMCs stimulated by DNP-BSA and IL-33.

Conclusions: Tranilast has efficacy on the inhibition of degranulation and IL-13 production in BMMCs induced by the combination of DNP-BSA and IL-33.

Vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC) are severe ocular allergic diseases that are characterized by the formation both of giant papillae at the tarsal conjunctiva and of corneal lesions that threaten vision. Both the giant papillae and corneal lesions develop as a consequence of interactions between immune cells, such as T helper 2 (Th2) cells, mast cells, and eosinophils and tissue structural cells such as fibroblasts. These interactions are mediated by cytokines, among which the Th2 cytokine interleukin (IL)-13 plays a key role.¹ Giant papillae are formed as the direct result of excessive outgrowth of conjunctival fibroblasts and deposition of extracellular matrix. We have previously shown that IL-13 stimulates the proliferation of conjunctival fibroblasts, and protects these cells from apoptotic cell death.¹ In response to stimulation with IL-13, conjunctival fibroblasts also synthesize extracellular matrix proteins and tissue inhibitors of metalloproteinases (TIMPs), as well as downregulate

the production of matrix metalloproteinases (MMPs). IL-13 contributes to the formation of corneal lesions by stimulating the expression of chemokines and adhesion molecules specific for eosinophils in human keratocytes. The recently discovered cytokine IL-33 has also been implicated as a key factor in the pathogenesis of VKC/AKC. IL-33 was identified as a member of the IL-1 family of cytokines and induces Th2 cytokine production in a variety of cell types including Th2 cells, mast cells, basophils, and eosinophils. The expression of IL-33 was shown to be increased in vascular endothelial cells and epithelial cells of giant papillae in individuals with AKC.² These various observations suggest that the IL-33 together with IL-13 can be potential therapeutic targets in VKC/AKC.

With the use of mast cell-deficient and mast cell-reconstituted mice, we have shown that conjunctival mast cells play an important role not only in the early-phase reaction but also in late-phase inflammation

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KF and AF designed the research; KT performed the experiments; all authors contributed to the content of the article.

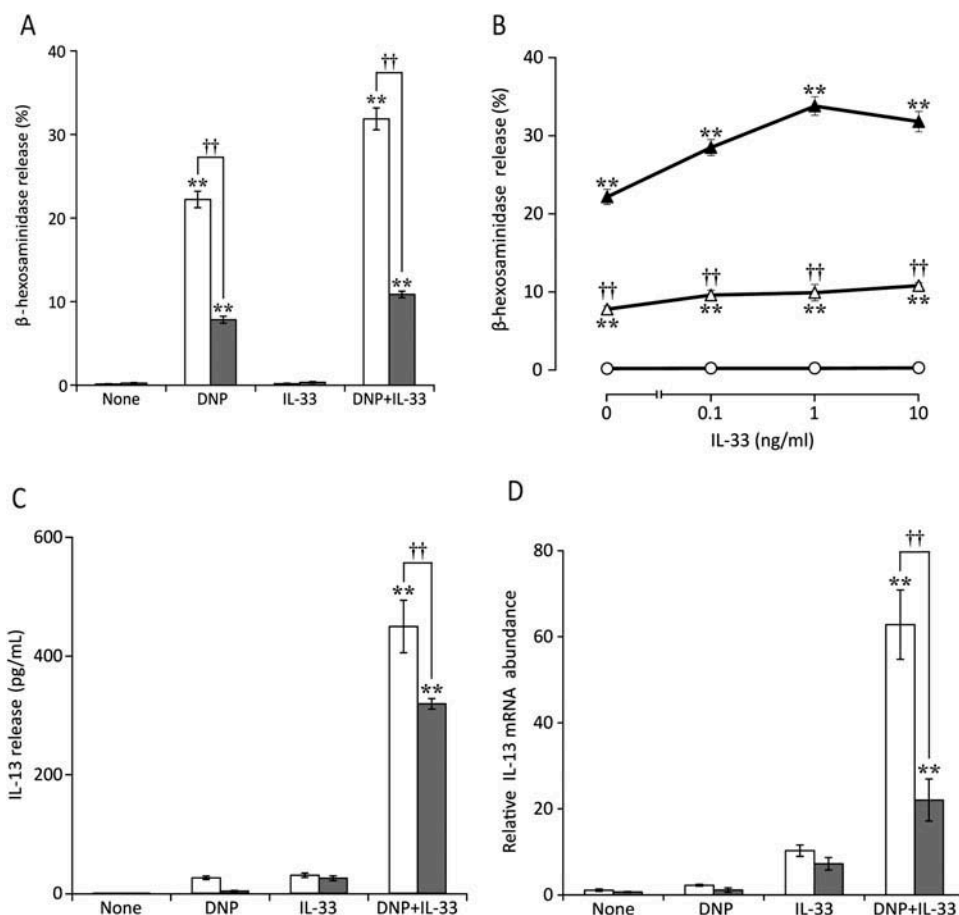


FIGURE 1. Effect of tranilast on BMMC degranulation induced by Fc ϵ RI cross-linking and IL-33. (A) Cells sensitized with anti-DNP IgE were incubated at 37°C first for 10 min in the absence (open bars) or presence (closed bars) of tranilast (200 μ M) and then for 30 min in the additional absence or presence of DNP-BSA (10 ng/mL), IL-33 (10 ng/mL), or both agents. The activity of β -hexosaminidase in the culture supernatants was then measured. (B) Cells sensitized with anti-DNP IgE were incubated first for 10 min in the absence (closed triangles, open circles) or presence (open triangles) of tranilast (200 μ M) and then for 30 min in the additional presence of the indicated concentrations of IL-33 and either the absence (open circles) or presence (closed triangles, open triangles) of DNP-BSA (10 ng/mL). The activity of β -hexosaminidase in the culture supernatants was then measured. All data are means $\pm$ SEM from three independent experiments. ** p <0.01 (Tukey–Kramer test) vs the corresponding value for cells incubated without addition of DNP-BSA and IL-33; ^{++}p <0.01 (Tukey–Kramer test) for the indicated comparisons (A) or vs the corresponding value for cells incubated with DNP-BSA but without tranilast (B). Effects of tranilast on IL-13 release and IL-13 mRNA abundance in BMMCs stimulated by Fc ϵ RI cross-linking and IL-33. Cells sensitized with anti-DNP IgE were incubated at 37°C for 10 min in the absence (open bars) or presence (closed bars) of tranilast (200 μ M) and then for 20 h (C) or 6 h (D) in the additional absence or presence of DNP-BSA (10 ng/mL), IL-33 (10 ng/mL), or both agents. The concentration of IL-13 in the culture supernatants (C) and the cellular abundance of IL-13 mRNA normalized by the amount of β -actin mRNA (D) were then determined. All data are means $\pm$ SEM from three independent experiments. ** p <0.01 (Tukey–Kramer test) vs the corresponding value for cells incubated without addition of DNP-BSA and IL-33; ^{++}p <0.01 (Tukey–Kramer test) for the indicated comparisons.

during ocular allergy.³ Mast cells have also been found to be an important source of IL-13 during allergic inflammation, with these cells secreting large amounts of IL-13 in response to co-stimulation with IL-33 and immunoglobulin E (IgE). In Japan, tranilast is a mast cell stabilizer and is administered in eyedrops or orally for the treatment of allergic diseases. We have now examined the effects of tranilast on degranulation and IL-13 production in mast cells activated by IL-33 and cross-linking of Fc ϵ RI; the high-affinity IgE receptor.

Bone marrow-derived mast cells (BMMCs) were obtained by culture of bone marrow cells from C57BL/6

mice for >4 weeks in RPMI 1640 medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum, IL-3 (PeproTech, Rocky Hill, NJ), and stem cell factor (PeproTech) as described previously.³ BMMCs were sensitized with monoclonal IgE specific for 2,4-dinitrophenol (DNP) (Sigma, St. Louis, MO) and were then exposed to tranilast (Cayman Chemical, Ann Arbor, MI) in RPMI 1640 for 10 min at 37°C before stimulation with DNP-conjugated bovine serum albumin (BSA) (LSL, Tokyo, Japan), IL-33 (R&D Systems, Minneapolis, MN), or a combination of DNP-BSA and IL-33. Degranulation of BMMCs was evaluated by measurement of the activity

of β -hexosaminidase released into the culture medium. The abundance of IL-13 mRNA in BMMCs was determined by reverse transcription and real-time polymerase chain reaction analysis with the use of an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA).³ The amount of IL-13 released into culture medium was quantified with an enzyme-linked immunosorbent assay (R&D Systems).

Treatment of BMMCs with DNP-BSA (10 ng/mL) alone, but not with IL-33 (10 ng/mL) alone, increased β -hexosaminidase release (Figure 1A). Consistent with previous results,⁴ simultaneous stimulation with both DNP-BSA and IL-33 resulted in a synergistic increase in β -hexosaminidase release. This effect of IL-33 on degranulation of IgE-stimulated mast cells was concentration dependent, being maximal at 1.0 ng/mL (Figure 1B). Tranilast (200 μ M) significantly inhibited degranulation of BMMCs stimulated either with DNP-BSA alone or with the combination of DNP-BSA and IL-33 (Figure 1A,B). We also examined the effect of tranilast on the release of IL-13 by BMMCs. Although incubation of BMMCs with DNP-BSA or IL-33 alone did not substantially affect the release of IL-13, the combination of both agents induced a pronounced increase in IL-13 release, consistent with previous observations.⁴ Tranilast significantly inhibited IL-13 release from BMMCs stimulated with DNP-BSA and IL-33 (Figure 1C). Furthermore, whereas DNP-BSA or IL-33 alone induced only a relatively small (2-fold and 10-fold, respectively) increase in the abundance of IL-13 mRNA in BMMCs, the combination of both agents induced a 63-fold increase in the amount of IL-13 mRNA and this effect was inhibited by ~65% in the presence of tranilast (Figure 1D).

We have here shown that tranilast inhibited degranulation and IL-13 production in BMMCs stimulated with the combination of DNP-BSA and IL-33. Tranilast was previously found to inhibit the expression of IL-13 in the lung and to suppress airway remodeling in a mouse model of chronic asthma.⁵ Tissue remodeling is a

complex process that encompasses basal zone hyperplasia, epithelial-mesenchymal transition, fibrosis, angiogenesis, and hypertrophy and hyperplasia of fibroblasts and smooth muscle cells. Our present results therefore do not elucidate the entire mechanism of action of tranilast in vivo, but the inhibition of IL-13 synthesis in mast cells by this drug might be expected to be an important contributing factor to its therapeutic effects.

In summary, our results suggest that the therapeutic effects of tranilast might be attributable to inhibition of late-phase allergic inflammation mediated by IL-33, in particular to suppression of IL-13 release by mast cells, as well as to its activity as a mast cell stabilizer.

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REFERENCES

1. Kumagai N, Fukuda K, Fujitsu Y, et al. Role of structural cells of the cornea and conjunctiva in the pathogenesis of vernal keratoconjunctivitis. *Prog Retin Eye Res.* 2006;25:165–187.
2. Matsuda A, Okayama Y, Terai N, et al. The role of interleukin-33 in chronic allergic conjunctivitis. *Invest Ophthalmol Vis Sci.* 2009;50:4646–4652.
3. Fukuda K, Ohbayashi M, Morohoshi K, et al. Critical role of IgE-dependent mast cell activation in a murine model of allergic conjunctivitis. *J Allergy Clin Immunol.* 2009;124:827–833.
4. Silver MR, Margulis A, Wood N, et al. IL-33 synergizes with IgE-dependent and IgE-independent agents to promote mast cell and basophil activation. *Inflamm Res.* 2010;59:207–218.
5. Kim SJ, Kim JW, Kim YH, et al. Effects of tranilast and pentoxifylline in a mouse model of chronic asthma using house dust mite antigen. *J Asthma.* 2009;46:884–894.