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Large particulate allergens can elicit mast cell-mediated anaphylaxis without exit from blood vessels as efficiently as do small soluble allergens

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ABSTRACT

Anaphylaxis is a rapid-onset, life-threatening allergic reaction in that IgE, mast cells and histamine are commonly involved. It can be experimentally induced in IgE-sensitized animals by intravenous injection of corresponding allergens, and the sign of anaphylactic reaction can be detected within minutes after allergen challenge. However, it remains puzzling why the anaphylactic reaction can be initiated *in vivo* so quickly, considering that allergens are delivered into the blood circulation while mast cells reside within peripheral tissues but not in the blood circulation. To address this issue, we compared two different forms of the same allergen, small soluble and large particulate ones, in their ability to induce anaphylaxis in IgE-sensitized mice. In contrast to our expectation, particulate allergens could induce anaphylaxis as quickly and efficiently as did soluble allergens, even though they remained inside of blood vessels. In vivo imaging analysis suggested the direct interaction of intravascular particulate allergens and perivascular mast cells across the capillary wall. Taken together with previous report that perivascular mast cells can capture IgE in the blood circulation by extending cell processes across the vessel wall, our findings imply that blood-circulating allergens, regardless of their size, can stimulate mast cells without exit from blood vessels, by means of cross-linking IgE on mast cell processes inserted into the vessel lumen, and hence initiate anaphylactic reaction so quickly.

1. Introduction

Anaphylaxis is a rapid-onset and potentially fatal allergic reaction. It is well documented that mast cells and IgE are critically involved in anaphylaxis [1–3]. IgE binds to the cell surface of mast cells through the high-affinity IgE receptor FcεRI [4]. When corresponding allergens bind to and cross-link IgE on mast cells, chemical mediators such as histamine are released from activated mast cells, leading to anaphylaxis with increased vascular permeability [4–6]. Two major protocols are available for inducing systemic anaphylaxis in animals, active and passive ones in terms of sensitization [7]. In both models, soluble proteins such as bovine serum albumin (BSA) and ovalbumin (OVA) are commonly used as allergens, and the sign of anaphylaxis, including hypothermia, becomes evident within minutes after the challenge of allergens to sensitized animals [7].

It remains poorly documented why anaphylaxis can be elicited within a short period of time after intravenous administration of allergens. In contrast to blood-circulating basophils, mast cells reside in peripheral tissues, particularly in perivascular regions [4]. One may assume that intravenously administered allergens diffuse quickly out of vessels and stimulate IgE-sensitized mast cells

Abbreviations: OVA, ovalbumin; BSA, bovine serum albumin; TNP, 2,4,6-trinitrophenol; Immunoglobulin E, IgE; Mcpt8, mouse mast cell protease-8; DTR, diphtheria toxin receptor; PCA, passive cutaneous anaphylaxis; ESAM-1, endothelial cell-selective adhesion molecule-1.

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located nearby outside of vessels. However, this scenario does not seem to be consistent with the observation of Evans blue dye extravasation. Evans blue dye is widely used to assess the vascular permeability in animal models [8–10]. This dye binds albumin when delivered into the blood circulation [11]. Under physiological conditions, the endothelium is impermeable to albumin, and therefore the dye bound to albumin remains restricted within blood vessels. When the vascular permeability increased under pathological conditions such as anaphylaxis, the extravasation of albumin-bound dye occurs, resulting in blue colorization of surrounding tissues [8–10]. Thus, the above-mentioned scenario may become operative once anaphylaxis is elicited, but does not seem to account for the initial step triggering anaphylaxis. Intriguingly, Cheng et al. recently reported that perivascular mast cells can capture IgE in the blood circulation by extending cell processes across the vessel wall [12]. This prompted us to examine the possibility that allergens delivered into the blood circulation may stimulate IgE-sensitized mast cells even without exit from blood vessels, leading to acute-onset of anaphylactic reaction.

In the present study, we examined this possibility by using soluble allergen-conjugated polystyrene microspheres (3 μm in diameter) as particulate allergens that were expected too large to leak out from blood vessels, and examined their ability to induce anaphylaxis in IgE-sensitized mice.

2. Materials and methods

2.1. Mice

C57BL/6 wild-type mice (7–8 weeks old) were purchased from the SLC (Shizuoka, Japan). Mast cell-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ [13], basophil-deficient *Mcpt8*^{DTR} [14] and histidine decarboxylase (HDC)-deficient [15] C57BL/6 mice were as described previously and maintained under specific-pathogen-free conditions in our animal facilities. All animal studies were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University.

2.2. Preparation of particulate allergens

Plain or fluorescent Polybead® Carboxylate Microspheres (3.0 μm in diameter, Ploysciences, Inc) were treated with activation solution (20 mM EDAC, 50 mM 2-[N-moropholino] ethanesulfonic acid (MES), 100 mM N-hydroxysuccinimide (Sigma–Aldrich) in deionized water) at room temperature for 30 min. After washing with deionized water, activated beads (1% w/v) were mixed with equal volume of 1 mg/ml hapten 2,4,6-trinitrophenol-conjugated OVA (TNP_{12}-OVA) or OVA containing 50 mM MES, and incubated overnight, followed by addition of 41.4 μM of ethanolamine to stop the reaction. Allergen-conjugated beads (TNP-OVA-beads and OVA-beads) were washed ten times with 1% BSA in PBS to remove unbound allergens and ethanolamine, and stored at 4 °C.

2.3. Bone marrow-derived mast cells and degranulation assay

Bone marrow-derived mast cells (BMMC) were prepared and cultured for 4 weeks as described previously [16]. Degranulation of BMMCs cells were determined by measurement of β-hexosaminidase release as previously described [17] with some modifications. Briefly, BMMCs (2.0×10^6 cells/0.5 ml/well) were sensitized with TNP-specific IgE (IGELb4, 1 μg/ml) for overnight, and then stimulated with 300 ng/ml soluble allergens or 1.0×10^6 of allergen-conjugated beads. The activity of β-hexosaminidase in culture supernatants and cell lysates was measured, and the % release of β-hexosaminidase was calculated as described [17]. In experiments shown in Fig. 1B, soluble or particulate antigens were added to the upper chamber of an inserted membrane filter (Greiner) while BMMCs were placed in the lower chamber. Culture media were collected from both upper and lower chambers after 1 h-culture, and analyzed for β-hexosaminidase activity.

2.4. Basophil depletion in vivo

Mcpt8^{DTR} mice were treated with intravenous administration of diphtheria toxin (DT, 25 μg/kg body weight) or control PBS one day before experiments.

2.5. Passive systemic anaphylaxis

Mice were systemically sensitized with intravenous administration of 50 μg TNP-specific IgE. One day after IgE injection, mice were intravenously challenged with soluble allergens (50 μg) or particulate allergens (1×10^7). Their rectal temperatures were monitored by digital thermometer (Shibaura Electronics Co., Ltd. Saitama, Japan).

2.6. Passive cutaneous anaphylaxis (PCA)

Mice were locally sensitized with an intradermal injection of 20 ng of TNP-specific IgE into the right ear, and the same volume of PBS was injected into the right ear as a control. One day later, they were intravenously challenged with soluble allergens (50 μg) or particulate allergens (1×10^7) in 100 μl of PBS containing 0.5% Evans blue dye. Thirty minutes later, the dye was extracted from each dissected ear in 0.7 ml of formamide at 63 °C for overnight. The amount of dye extracted was determined spectrophotometrically at 620 nm and calculated from a standard curve established with known amounts of dye.

2.7. Fluorescent intravital imaging

Mice were anesthetized with isoflurane gas (5% induction and 1.5% maintenance via a nose cone), and their body temperature was maintained at 37 °C with a heating blanket. To visualize mast cells and blood vessels, mice were treated with allophycocyanin (APC)-conjugated anti-c-kit (8 μg) and phycoerythrin (PE)-conjugated anti-ESAM-1 (8 μg). Intravital images of ear skin during PCA were

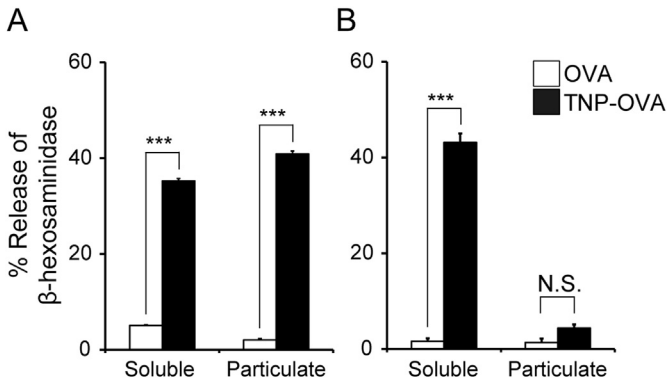

Fig. 1. Particulate allergens can stimulate IgE-sensitized mast cells directly but not through a membrane filter, unlike soluble allergens. BMMCs sensitized with TNP-specific IgE were incubated for 1 h with soluble allergens (TNP-OVA or control OVA) or allergen-conjugated beads (TNP-OVA-beads or control OVA-beads) in the same chamber (A) or in different chambers separated by a membrane filter with 0.4 μm pores (B), followed by β-hexosaminidase release assay (mean ± SEM, n = 3). Data shown are representative of three independent experiments. N.S., not significant. ***$p < 0.001$.