

Readministration of IgE Is Required for Repeated Passive Cutaneous Anaphylaxis in Mice

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Key Words

Immunoglobulin E • Mast cells • Passive cutaneous anaphylaxis

Abstract

Background: Homologous passive cutaneous anaphylaxis (PCA) is an established technique that provides a useful tool to study local inflammation due to mast cell activation. Since mast cells are responsive to repeated challenge by IgE receptor crosslinking in vitro, we investigated the responsiveness of mice to repeated PCA activation. **Methods:** Female BALB/c mice were sensitized with intradermal injection of IgE into the ear and provoked through intravenous injection of trinitrophenol-BSA. Repeated provocation was administered, and the outcome was determined by colorimetric measurement of Evans blue dye leakage. **Results:** After local IgE injection, mice could be challenged to demonstrate a PCA reaction at least up to 13 days after IgE sensitization. In contrast, passively sensitized mice did not respond to repeated antigen challenge, i.e. a second provocation administered between 1 and 12 days after the first PCA reaction. However, if these mice were intradermally resensitized with IgE at the same site after the first challenge, they became responsive to the repeated challenge. **Conclusions:** Mast cells become desensitized upon PCA reaction, and resensitization with IgE is critical for mice repeatedly stimulated by PCA activation.

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Introduction

Passive cutaneous anaphylaxis (PCA) described by Ovary [1] is a useful experimental model for studying allergen/IgE interaction leading to the inflammatory mediator release from mast cells and the clinical expression of cutaneous anaphylaxis. Although PCA may be elicited in many animal species, PCA in mice is particularly useful due to their strain diversity and the availability of various types of mouse disease models. Mouse PCA has been widely used for immunopharmacological studies or gene function analysis in allergy.

Mast cells are important effector cells in allergic responses. Degranulation of these cells initiated by cross-linking of IgE bound to its high affinity receptor, FcεRI, with a multivalent antigen results in the release of mediators provoking various clinical manifestations associated with allergy, such as increased secretion, edema and itch [2]. Mast cells are the key cells of allergic reactions and play a major role in the mechanism of PCA, since mice deficient in mast cells do not respond to PCA challenge [3]. Similarly to an allergic inflammation, the PCA reaction is manifested by edema formation that can be quantified by measurement of the protein extravasation, e.g., using Evans blue.

Mast cells activated in vitro have been shown to recover from degranulation, regranulate and be reactive again [4–7]. In the present study, we have investigated the responsiveness of mice to repeated stimulation by PCA.

Methods

Mice

Female BALB/c mice, 8 weeks of age, were purchased from Bomimice (Ry, Denmark) and maintained under specific pathogen-free conditions. The study was approved by the ethical committee in Uppsala.

Reagents

Monoclonal mouse trinitrophenol (TNP)-specific IgE was purified from supernatant of the hybridoma culture (IgE1-b4, ATCC, Manassas, Va., USA) by affinity chromatography using a Sepharose column coupled with a rat anti-mouse κ monoclonal antibody. TNP-BSA (conjugation ratio: 9 TNP groups per BSA) was purchased from Biosearch Technologies, Inc. (Novato, Calif., USA). Evans blue was purchased from Sigma (Steinheim, Germany).

PCA Assay

IgE directed against TNP-BSA was intradermally injected into the right ear of mice lightly anesthetized with avertin. The IgE dosage used was 0.4 μ g in 10 μ l PBS. The left ear was used as a control and injected with 10 μ l PBS. The PCA reaction was induced 24 h later by an intravenous injection of 10 μ g TNP-BSA in 200 μ l of 2% Evans blue in PBS (or otherwise indicated). In some experiments, when mice were provoked twice, Evans blue was omitted in the first provocation. Thirty minutes after provocation, the mice were sacrificed, and the ears were excised and processed by alkali disintegration [8] with a slight modification. In brief, the ear was incubated in 100 μ l 1 N KOH at 37°C overnight and neutralized by the addition of 900 μ l of 0.6 N H₃PO₄:acetone (5:13, v/v). After vigorous vortexing, the mixture was centrifuged at 3,000 rpm for 15 min. The supernatant containing the extravasated dye Evans blue was collected and the absorbance was measured at 620 nm to determine the level of dye extravasation.

Results and Discussion

Typical PCA reactions are shown in figure 1. Dye extravasation levels are displayed in both Evans blue and a TNP-BSA dose-dependent manner. Optimal TNP-BSA dosage was 1–10 μ g/mouse, and 2–4 mg/mouse of Evans blue gave the best measurement of extravasation.

Humans suffering from allergy can experience repeated onsets of the disease, for example, during a pollen season. We hypothesized that mice can also be repeatedly provoked by PCA reaction. However, mice that had been sensitized by an injection of 0.4 μ g/ear of IgE and subjected to a previous round of PCA could not be re-challenged by the same antigen (fig. 2a). This was irrespective of the interval between the first challenge and the second challenge. The intervals tested between the first and the second provocation were 1, 2, 3 and 4 days (fig. 2a), or 13 days (fig. 2b). To exclude the possibility that bound IgE might be detached or degraded after a certain period of time, we tested whether a longer interval between IgE ad-

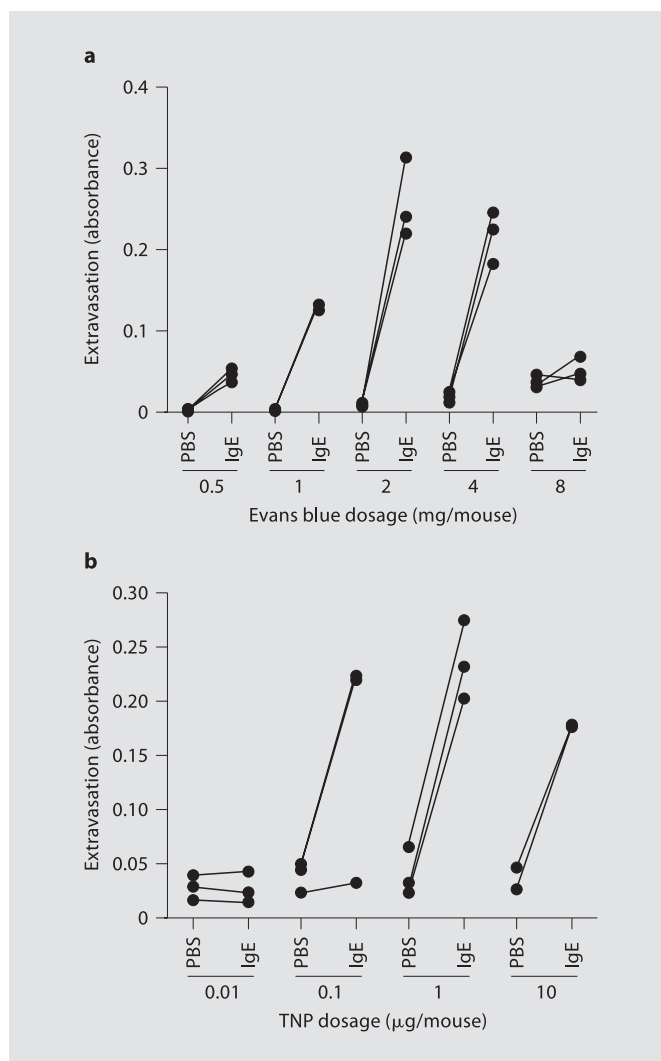
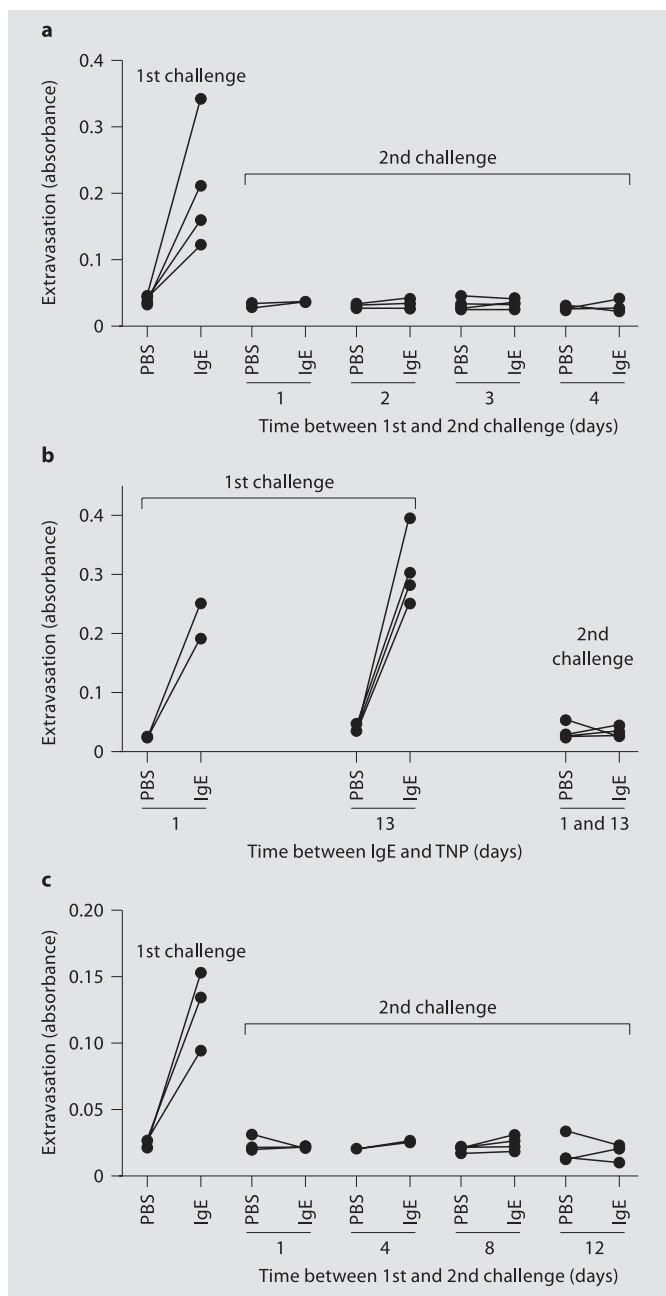


Fig. 1. Dose response of Evans blue and TNP-BSA in PCA. **a** Mouse ears on the right side were sensitized by IgE by intradermal injection (0.4 μ g/ear), and an equal volume of PBS was injected into the ears on the left side. After 24 h, the mice were challenged intravenously through the tail vein with TNP-BSA (10 μ g/mouse) and various amounts of Evans blue as indicated. **b** Mice were challenged intravenously with various amounts of TNP-BSA as indicated, as well as Evans blue (2 mg/mouse). Thirty minutes after the challenge, mouse ears were excised and processed for the measurement of dye extravasation. Each line represents data obtained from 1 mouse (n = 3 mice/group).

ministration and antigen challenge for a single PCA would affect the response of the reaction. As is shown in figure 2b, 13 days after IgE administration, mice were still sensitive to challenge by the antigen TNP-BSA. This is not surprising since IgE is bound to Fc ϵ RI with extremely high affinity [9]. In contrast, if the mice were



challenged 1 day after IgE administration to complete a PCA reaction, then 13 days later, these mice were not sensitive to challenge by the same antigen administration (fig. 2b). If the mice received less IgE injection ($0.04 \mu\text{g}/\text{ear}$) to scale down the first round of provocation, they were still not responsive to a second round of challenge 1–12 days after the first challenge (fig. 2c).

A possible explanation for the unresponsiveness of these mice to repetitive challenge might be due to inter-

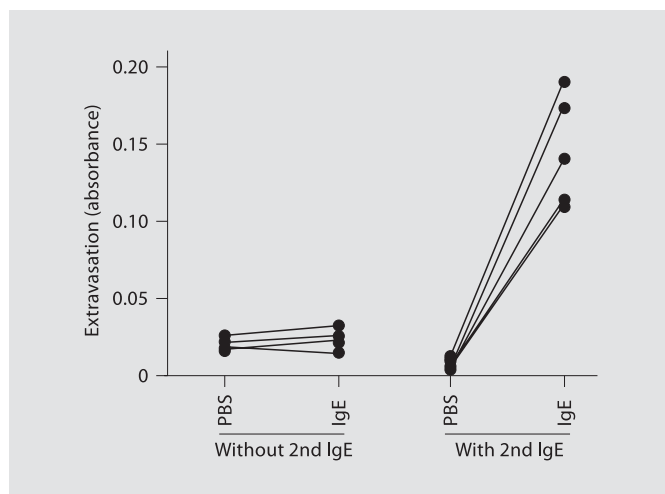


Fig. 2. Unresponsiveness of mouse after repetitive challenge. **a** IgE sensitization was carried out as explained in figure 1. Twenty-four hours after IgE injection, the mice were challenged intravenously with TNP-BSA ($10 \mu\text{g}/\text{mouse}$) alone or together with Evans blue ($2 \text{ mg}/\text{mouse}$). Thirty minutes after the challenge, ears of those mice having received both TNP-BSA and Evans blue were excised and processed for measurement of the level of dye extravasation (1st challenge). For those mice having received TNP-BSA only, a 2nd challenge in a similar way as the first was carried out after various days as indicated, and ears were similarly processed (2nd challenge). **b** Essentially similar to **a**, except that the time interval between IgE sensitization and antigen challenge was extended. **c** Essentially similar to **a**, except that the dosage of the IgE sensitization was $0.04 \mu\text{g}/\text{ear}$. Each line represents data obtained from 1 mouse ($n = 3\text{--}4$ mice/group).

Fig. 3. Restoration of the responsiveness of mice after a 2nd challenge by readministration of IgE. IgE was injected to sensitize mouse ears as explained in figure 1. After 24 h, the mice were challenged intravenously with TNP-BSA ($10 \mu\text{g}/\text{mouse}$). Some mice were resensitized with IgE 9 days after the first challenge. Ten days after the first challenge, all the mice were rechallenged with TNP-BSA and Evans blue. Thirty minutes after the 2nd challenge, mouse ears were excised and processed for measurement of the level of dye extravasation. Each line represents data obtained from 1 mouse ($n = 5$ mice/group).

nalization of the IgE bound to IgE receptors after the previous PCA reaction. Therefore, in the next experiment, we sensitized the mouse ears with IgE ($0.4 \mu\text{g}/\text{ear}$) 9 days after the first challenge and then rechallenged them 1 day after the resensitization, i.e. 10 days after the first IgE sensitization. Resensitized mice responded substantially to the repeated challenge. In contrast, mice that only received IgE sensitization once at the start of the assay were still not responsive to rechallenge (fig. 3). The reason why

we choose to wait 9 days was primarily to let the tissue heal from light damage that the injection causes. Earlier or later time points were never investigated.

It has been described that internalization of cross-linked IgE receptors induces cointernalization of a significant fraction of monomeric IgE-bound receptors [10]. Thus, internalization of not only crosslinked receptors but also receptors with bound monomeric IgE could substantially reduce the degranulation response [11] and thereby diminish the PCA reaction. However, the requirement of resensitization with IgE for repeated challenge in mice stands in contrast with our previous in vitro findings of a mast cell activation model. Both bone marrow-derived mouse mast cells and the mouse mast cell line MCP5/L can be repeatedly activated to release β -hexosaminidase without resensitization with IgE [7]. Furthermore, MCP5/L cells are responsive to repetitive challenge without IgE resensitization to re-upregulate the prosurvival Bcl-2 family gene A1 [12]. However, it is reported that human skin mast cells do require resensitization with IgE in order for them to be responsive to a second IgE receptor-dependent challenge [13]. This apparent discrepancy between the studies could have several explanations, such as different populations of mast cells or the usage of different concentrations of IgE and antigens for the activation. There is also a heterogeneity among IgE molecules that have different effects on IgE receptor internalization, degranulation and cytokine production [14].

Another explanation for the unresponsiveness could be that activated and degranulated mast cells do not survive, and thereby, the number of mast cells in the tissue decreases and the PCA reaction is diminished. Although we and others have described that mast cells survive degranulation and can be activated again [4–7], there are studies showing that the activation-induced survival is dependent on the strength of the IgE receptor crosslinking [15]. However, preliminary data indicate that mast cell numbers are not decreased after a PCA reaction in our model (data not shown).

In summary, BALB/c mice responded to repeated antigen-specific PCA challenge only if resensitized with IgE after the first activation. Desensitization is a complex process that leads to unresponsiveness of a tissue, or a cell, and is used to desensitize allergic patients [16]. However, its exact mechanism is still not clear. Our work has gained some understanding in repeated PCA assay that might provide a better tool for immunopharmacological studies.

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