

ORIGINAL ARTICLE

Basic Mechanisms in Allergic Disease

Rush desensitization with a single antigen induces subclinical activation of mast cells and protects against bystander challenge in dually sensitized mice

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Summary

Background: Rush desensitization can provide short-term tolerance to individuals who are allergic to certain medications in instances where other therapeutic interventions are limited. While rush desensitization (DS) is typically successful in preventing adverse type I hypersensitivity reactions, the mechanism of allergic protection remains unknown. Given the rise in prevalence of individuals displaying multiple allergies, understanding the impact of rush DS on “bystander” allergens, or additional allergens to which an individual is sensitized, could help inform clinical recommendations.

Objective: To evaluate the effect of rush DS on bystander sensitization.

Materials and Methods: We used a murine model of rush DS, whereby BALB/c mice were sensitized to ovalbumin (OVA) and desensitized through repeated intraperitoneal injections of OVA. Using a local anaphylaxis assay, we measured ear swelling by Evans blue extravasation following intradermal challenge. In studies to measure the impact on bystander antigens, a modified protocol was used in which mice were dually sensitized to OVA and Keyhole limpet hemocyanin (KLH), and desensitized to either OVA or KLH prior to allergic challenge.

Results: The immunological effects of rush DS were independent of changes in Th1 and Th2 cytokine production and circulating OVA-IgE levels. Instead, rush DS resulted in subclinical degranulation of mast cells prior to challenge. In our dual sensitization model, rush DS with a single antigen conferred protection against allergic challenge to a secondary antigen. Bystander protection required prior sensitization, as DS with an irrelevant antigen did not impact allergic responsiveness.

Conclusions and Clinical Relevance: We reveal that a key mechanism of rush DS protection against allergic responsiveness may be the subclinical degranulation of mast cells. Therefore, performing rush DS to a single antigen to which one is IgE-sensitized may be sufficient to desensitize to multiple allergens. Future studies could lead to streamlined protocols of rush DS for patients with multiple allergies.

1 | INTRODUCTION

Medication allergies pose a serious challenge in clinical settings and are of increasing concern as they have been on the rise in recent years.¹ Common medication allergies include those to antibiotics, chemotherapeutics, and initial doses for allergy immunotherapy.² Medication allergies can severely impede appropriate treatment for atopic individuals, particularly when therapeutic options are already limited. Adverse reactions to medications can result in type I hypersensitivity reactions, which can range in severity and include life-threatening outcomes such as anaphylactic shock.

In cases where administration of a certain medication is critical, rush desensitization (DS; or rapid desensitization) may be performed to allow treatment to occur. Rush DS confers temporary tolerance through the administration of doses of increasing amounts of allergen over a relatively brief period of time, typically no more than 1–3 days.³ However, certain rush DS schedules can allow an allergic individual to tolerate a therapeutic dose of medication within a matter of hours.^{4,5} Nevertheless, even controlled exposure to allergen can potentially result in adverse events such as anaphylaxis.⁶ Thus, despite the general effectiveness of rush DS, it still comes with serious risks; and because the mechanism remains unclear, the ability to optimize schedules is limited.⁷ For instance, the requirements for rush DS to effectively treat polysensitized individuals are unknown.

Several *in vitro* models of rush DS have been described.^{8,9} In this study, we used a cutaneous model of local anaphylaxis to evaluate the allergic responsiveness *in vivo* of mice that underwent rush DS. In the model, which was previously described by our laboratory, mice are sensitized to the model antigen ovalbumin (OVA) and challenged by intradermal injection in the ear.^{10,11} Allergic responsiveness was measured by the extent of swelling or vascular permeability in the ear that occurs in response to allergen challenge. Based on a previous rush DS protocol published by our laboratory, the rush DS schedule used in this study was modified to achieve clinical desensitization within a single day.¹¹

We found that rush DS resulted in a significant reduction in allergic responsiveness to local allergen challenge, with no changes in Th1 and Th2 pathways. Additionally, the regulatory cytokine IL-10 was not required for the protective effects of rush DS in our model, as determined through experiments with knockout mice. However, plasma levels of murine mast cell protease 1 (mMCP-1), a pre-formed mast cell granule protein, and ear histology demonstrated that mast cells degranulated throughout the rush DS process.

Our studies on the impact of rush DS on bystander allergens also revealed that protection against allergic challenge can be achieved through single antigen DS in mice sensitized to two allergens. Loss of reaction to allergen challenge was dependent on previous sensitization to the allergen used for desensitization, as rush DS with an irrelevant antigen did not affect responsiveness to local allergen challenge. Furthermore, loss of allergic responsiveness was not accounted for by cross-reactivity of allergens and was independent of T cell proliferation or cytokine production. Our data suggest that subclinical

depletion of inflammatory mediators through allergen-specific degranulation is a potential mechanism by which loss of allergic responsiveness is achieved during rush DS. Subclinical degranulation of mast cells could also explain protection against unrelated allergens.

2 | MATERIALS AND METHODS

2.1 | Mice

Female 8–16 weeks old BALB/c (National Cancer Institute, Frederick, MD) and IL-10^{-/-} (Jackson Laboratories, Bar Harbor, ME) mice were housed in micro-isolator cages under a standard 12 hour light/dark cycle with *ad libitum* access to chow and water. All studies were approved by the Uniformed Services University for the Health Sciences (USUHS) Institutional Animal Care and Use Committee.

2.2 | Sensitization

Mice were injected intraperitoneally with 50 µg of ovalbumin from chicken egg white (OVA) (A5503; Sigma, St. Louis, MO) and/or 50 µg of hemocyanin from the keyhole limpet *Megathura crenulata* (KLH; H7017; Sigma) adsorbed to Imject Alum (Thermo Scientific, Rockford, IL) adjuvant for 30 minutes in a final volume of 100 µL (aluminum hydroxide and magnesium hydroxide dose of 2 mg each). OVA and KLH were prepared in phosphate buffered saline (PBS), pH 7.4 (Quality Biological, Gaithersburg, MD). Mice were injected once per week for 3 weeks.

2.3 | Desensitization and challenge

Two weeks following the final sensitization, mice were injected intraperitoneally with 50 µg OVA in PBS, 50 µg KLH in PBS, 50 µg of bovine serum albumin (BSA) (A4503; Sigma) in PBS, or PBS once every hour for 6 hours. Injection volume was 100 µL. Mice were challenged intradermally in the ear with 10 µL of PBS (left) or 50 µg of OVA or KLH (right) diluted in PBS in a volume of 10 µL one hour following desensitization.

2.4 | Local anaphylaxis assay

The local anaphylaxis assay (LAA), used to assess sensitivity to challenge antigen, was performed as previously described.¹⁰ Briefly, mice were anesthetized with isoflurane gas. Mice were removed from the anaesthesia chamber and the left ear was placed on a piece of transparent tape that had been affixed, sticky side outward, to a 15 mL conical tube (BD, Franklin Lakes, NJ). The ear was injected intradermally with 10 µL of PBS using a 31 gauge needle attached to a 0.5 mL insulin syringe (Terumo, Somerset, NJ). The right ear was then placed on the tape and injected intradermally with 10 µL of volume containing 50 µg OVA or KLH. Three minutes after ear injections, 200 µL of a 0.5% Evans Blue dye (E2129; Sigma) in PBS solution was injected into the tail vein using a 27 gauge needle attached

to a 1 mL syringe (BD). Ten minutes after dye injection, mice were killed by CO₂ asphyxiation. Ears were surgically removed and placed into individual microcentrifuge tubes containing 700 μ L of spectrophotometric grade formamide (295876; Sigma-Aldrich, St. Louis, MO) at 63°C for 18–22 hours. Optical density (620 nm) of 200 μ L formamide after dye extraction was measured using a Victor-3V plate reader (Perkin Elmer, Waltham, MA).

2.5 | Systemic anaphylaxis challenge and scoring

Mice sensitized to OVA were challenged by intraperitoneal injection with either 0, 12.5, 25, 50, 100, or 200 μ g of OVA. Clinical scoring and rectal temperature readings were obtained at baseline before challenge and every ten minutes for 90 minutes following challenge. A scale ranging from 0 to 5 was adapted from Ganeshan et al¹² and implemented. Scores were associated with symptoms as follows: 0 = no symptoms, 1 = scratching/rubbing face, 2 = facial oedema, piloerection, or decreased activity, 3 = laboured respiration or cyanosis, 4 = respiratory distress or tremors, 5 = death. Severity levels were assigned to these values by our laboratory: 0 = asymptomatic, 1 = mild, 2 = moderate, 3 = moderately severe, 4 = severe, and 5 = extremely severe.

2.6 | IgE ELISA

At the indicated time-points, tail vein blood was collected in Microtainer plasma separator lithium heparin tubes (BD). Plasma was separated from cells by centrifugation at 10 000 *g* for 2 minutes at room temperature (RT). Plasma was removed and stored in cryovials (Sarstedt, Newton, NC) at –20°C. Plasma was brought to RT, diluted 1:50 in assay diluent, and OVA-specific IgE concentrations were determined using a mouse OVA-IgE ELISA (MD Bioproducts, St. Paul, MN) according to manufacturer instructions.

2.7 | mMCP1 ELISA

Two weeks after the final sensitizing injection, mandibular blood was collected in Microtainer plasma separator lithium heparin tubes at 0, 2.5, and 6 hours during the rush DS schedule. Plasma was separated from cells by centrifugation at 10 000 *g* for 2 minutes at RT. Plasma was removed and stored in cryovials at –20°C. Plasma was brought to RT and diluted 1:100 in assay diluent. mMCP-1 levels were determined using a mouse MCPT-1 (mMCP-1) ELISA (Thermo Fisher Scientific, Waltham, MA) according to manufacturer instructions.

2.8 | Proliferation and cytokine analysis

One hour after completing the desensitization schedule, BALB/c mice were killed. Spleens were removed and placed into RPMI-1640 containing 3% FBS (Valley Biomedical, Winchester, VA), 1% Pen-Strep (Cellgro, Mediatech, Herndon, VA), 1% L-glutamine (Cellgro), and 20 mmol/L HEPES (Cellgro). Single-cell suspensions were made by pushing spleens through a 70 μ m cell strainer (BD Falcon, Franklin

Lakes, NJ) with the plunger of a 3 mL syringe (BD). Red blood cells were lysed with ACK lysis buffer (Quality Biological). CD4⁺ T cells and CD11c⁺ dendritic cells (DCs) were isolated from sensitized (CD4⁺) or naïve (CD11c⁺) mice using L3T4 and N418 magnetic bead isolation kits (Miltenyi, Auburn, CA), respectively. Cells were cultured at a concentration of 2×10^6 cells/mL in RPMI-1640 supplemented with 10% FBS, 1% Pen-Strep, 1% L-glutamine, and 20 mmol/L HEPES for 3 days at a ratio of 10:1 CD4⁺ T cells: CD11c⁺ DCs. Cells were stimulated with media, 20 μ g/mL OVA or KLH, or 5 μ g/mL anti-CD3 (clone 17A2; eBioscience, San Diego, CA) and 2 μ g/mL anti-CD28 (clone 37.51; eBioscience). Proliferation was assessed using the Cell Proliferation ELISA, BrdU chemiluminescent kit (Roche, Indianapolis, IN) according to manufacturer instructions on a Victor-3V plate reader. Cytokine production was assessed using the Mouse Th1/Th2 6-Plex Panel (Invitrogen, Camarillo, CA) according to manufacturer instructions on a Bio-Plex 200 system (Bio-Rad, Hercules, CA).

2.9 | Histology

Ears were excised from mice following completion of the rush DS schedule and placed in 10% formalin. Ear tissue samples were sent to Histoserv (Germantown, MD) for processing and staining with Toluidine blue for mast cells. Images were captured on a Zeiss Axio Imager.M2 using Stereo Investigator software at 63 $\times$ magnification under oil. The percentages of mast cells that were degranulated in each experimental group were determined by a pathologist blinded to study group allocation. A total of 50 mast cells from each mouse were assessed for evidence of degranulation. Representative images of PBS (sham)-desensitized or OVA-desensitized mice are shown.

2.10 | Statistical analysis

For proliferation and cytokine assays, comparisons of relevant groups were made using an unpaired, two-tailed Student's *t* test. For ear challenge assays, differences in OD were compared within groups (left vs right ear) using a paired, two-tailed Student's *t* test, or among groups (treatment conditions) using an unpaired, two-tailed Student's *t* test. For all experiments, a value of *P* < 0.05 was considered significant.

3 | RESULTS

3.1 | OVA sensitization results in production of OVA-specific IgE and a positive local anaphylaxis assay

Assessing the impact of rush DS on allergic responses requires the use of a reproducible allergic model system with measurable outcomes. Cutaneous type I hypersensitivity reactions after allergen sensitization and challenge can be reliably assessed by the LAA. Female BALB/c mice were sensitized with OVA/alum or sham-sensitized with

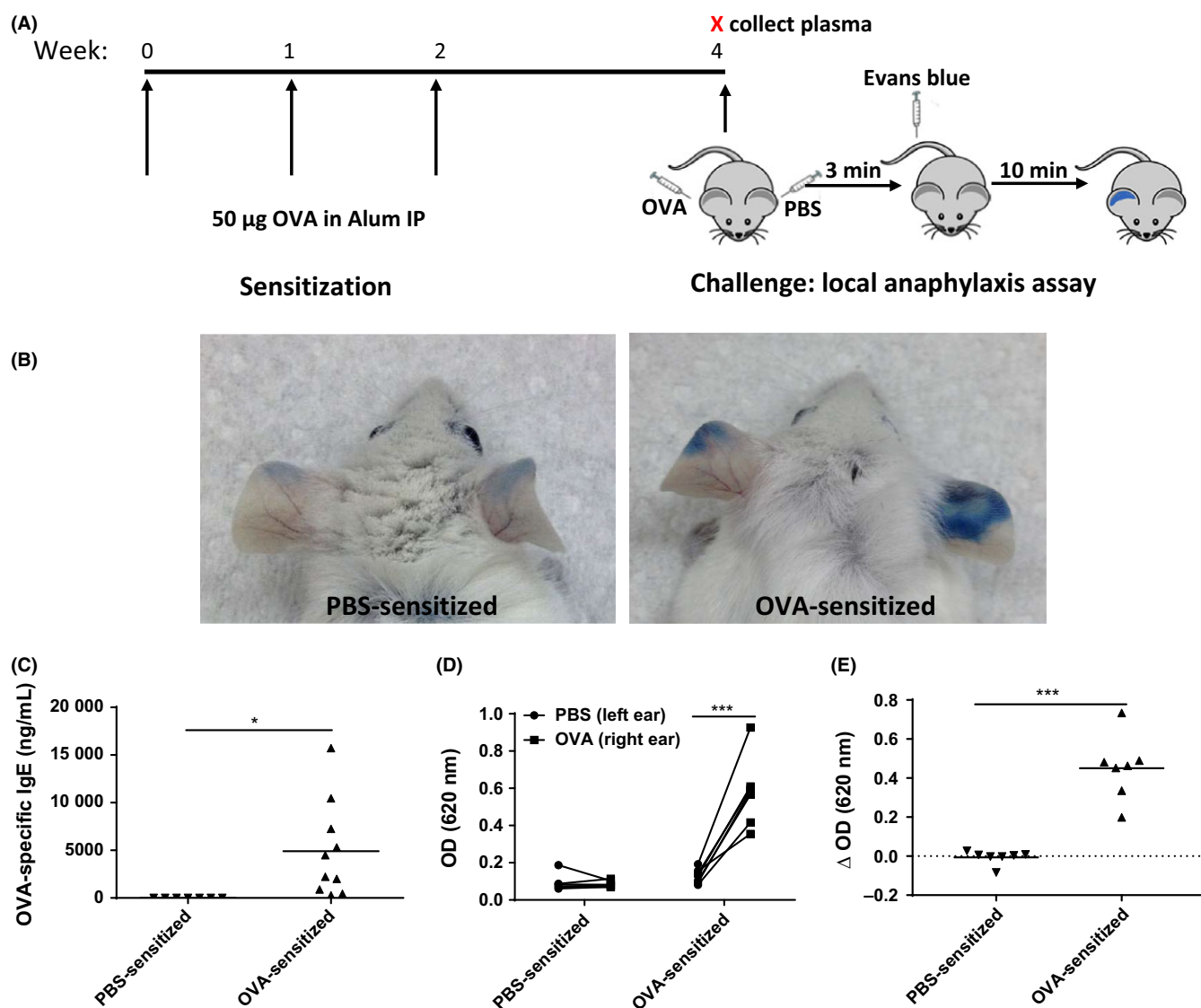


FIGURE 1 A, Mice were injected intraperitoneally once weekly for 3 wk with PBS/alum or OVA/alum, rested for 2 wk and then plasma was collected or a local anaphylaxis assay (LAA) was performed. Ten minutes after dye administration, ears were removed and Evans blue dye was extracted in formamide overnight at 63°C. B, Representative pictures of mice that were sensitized with either PBS or OVA and underwent the LAA prior to ear removal. Note the intense blue colour of the right ear in the OVA-sensitized mouse where intradermal challenge with OVA occurred. C, OVA-specific IgE levels were determined in mice sham-sensitized with PBS or sensitized with OVA. D, OD 620 nm was measured in ears of PBS (sham)-sensitized and OVA-sensitized mice after injection of PBS into the left ear (closed circles) and OVA into the right ear (closed squares). Lines connect the OD readings obtained for the left and right ears of individual animals. E, The change in OD (620 nm) was calculated for each mouse by subtracting the OD (620 nm) of PBS-challenged ears from the OD (620 nm) of OVA-challenged ears in matched animals. OD, Optical density; OVA, ovalbumin; PBS, phosphate buffered saline. * $P < 0.05$; *** $P < 0.001$

PBS/alum as illustrated in Figure 1A. OVA-sensitized but not PBS (sham)-sensitized mice exhibited blue right ears 13 minutes post-intradermal challenge with 50 µg of OVA and subsequent intravenous administration of Evans blue dye. Left ears, which were injected with PBS, showed little to no blue colouring in both groups. The baseline blue colouring that did occur in PBS-injected ears was likely due to the vascular permeability induced by the trauma of needle insertion and tissue blebbing from the injectate volume (Figure 1B). The development of an OVA-specific IgE response was observed in OVA-sensitized, but not PBS(sham)-sensitized mice

(Figure 1C). Following overnight extraction of dye from excised ears in formamide, there was a greater optical density (OD) resulting from dye extracted from the right ear than from the left, in OVA-sensitized but not PBS control mice (Figure 1D) after intradermal OVA challenge ($P < 0.0001$). The difference of OD between right and left ears of individual animals was significantly greater in OVA-sensitized than in PBS(sham)-sensitized mice (Figure 1E). The development of OVA-specific IgE and increased vascular permeability within 15 minutes of allergen injection demonstrate the utility of this hypersensitivity model in evaluating the impact of rush DS.

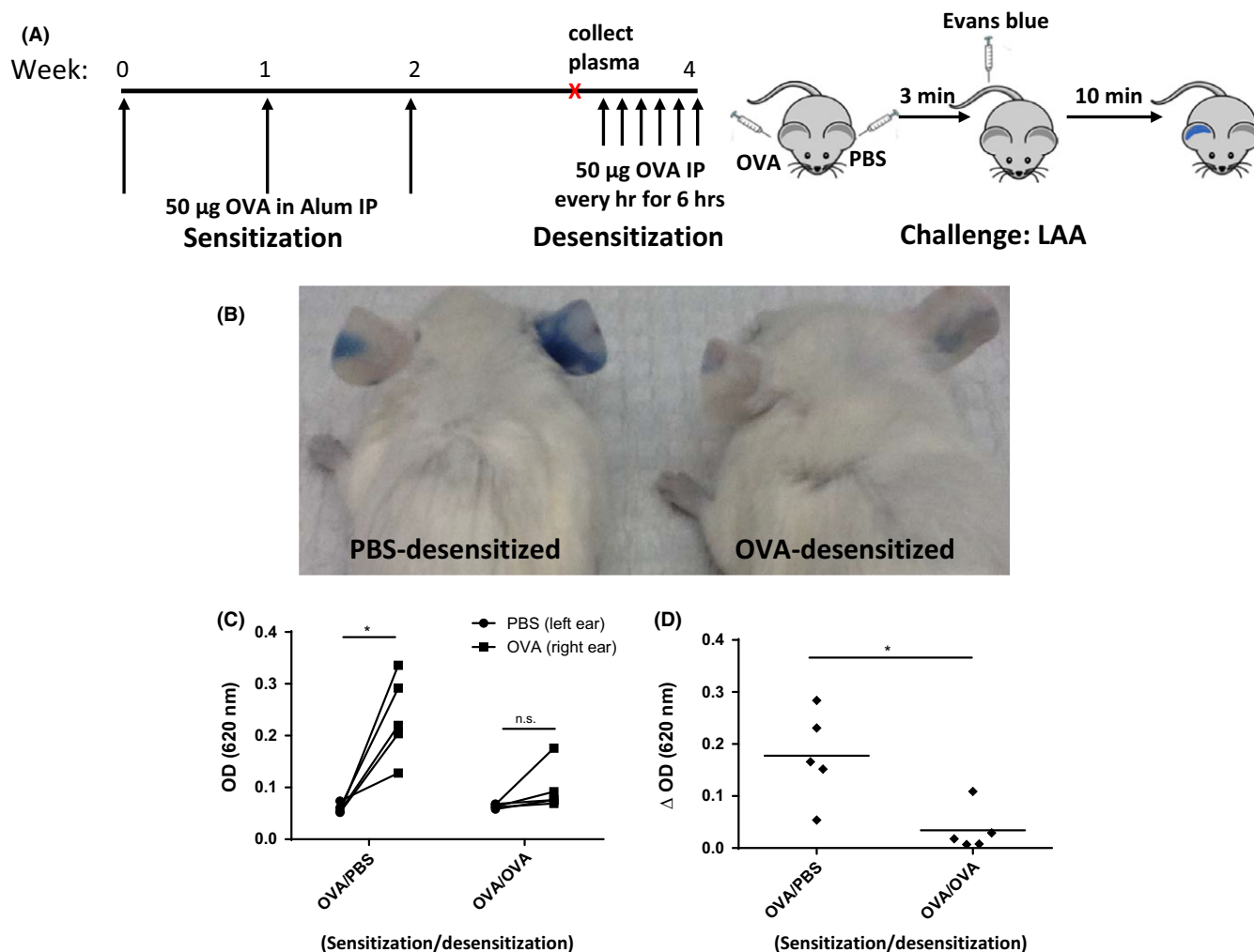


FIGURE 2 A, Mice were sensitized weekly for 3 wk with OVA/alum, rested for 2 wk, and then desensitized once per hour for 6 h by intraperitoneal injection of 50 µg of OVA or administered a sham desensitization schedule of PBS. One hour after desensitization, plasma was collected or the LAA was performed. B, Representative picture of PBS (sham)-desensitized and OVA-desensitized mice after PBS injection into the left ear and OVA injection into the right ear. Note the deep blue colour of the right ear of the PBS (sham)-desensitized mouse, which is absent in the right ear of the OVA-desensitized mouse. C, OD was measured in PBS-challenged (left, closed circles) and OVA-challenged (right, closed squares) ears in OVA-sensitized mice that were PBS (sham)-desensitized (OVA/PBS) or OVA-desensitized (OVA/OVA). Lines indicate ears from the same mouse. D, The change in OD was calculated for each mouse by subtracting the OD (620 nm) of PBS-challenged ears from the OD (620 nm) of OVA-challenged ears in desensitized mice. LAA, local anaphylaxis assay; OD, Optical density; OVA, ovalbumin; PBS, phosphate buffered saline. * $P < 0.05$

3.2 | Rush desensitization decreases immediate hypersensitivity measured by the local anaphylaxis assay

To begin investigating the mechanisms by which rush DS confers protection against allergic challenge, we tested whether mice that had been sensitized to OVA could be desensitized using a rush DS protocol in which 50 µg OVA was administered hourly by intraperitoneal injection for six hours (Figure 2A). We designed this protocol to achieve desensitization within a 24-hour timeframe. Following intradermal OVA challenge and subsequent dye administration, PBS (sham)-desensitized mice exhibited blue colouring in their right ears. In contrast, mice that were desensitized with OVA showed noticeably less blue colour in

their right ears (Figure 2B). The OD of dye extracted from the right ears was greater in PBS (sham)-desensitized mice than OVA-desensitized mice (Figure 2C). The difference in OD at 620 nm in PBS (sham)-desensitized mice was significantly greater than in OVA-desensitized mice (Figure 2D). This indicates that the rush DS protocol results in measurable and reproducible clinical desensitization.

3.3 | CD4⁺ T cell proliferation and CD4⁺ T cell cytokine production are unaltered by rush desensitization

To determine the immunological changes induced by rush DS, groups of OVA-sensitized mice that were PBS (sham)-desensitized

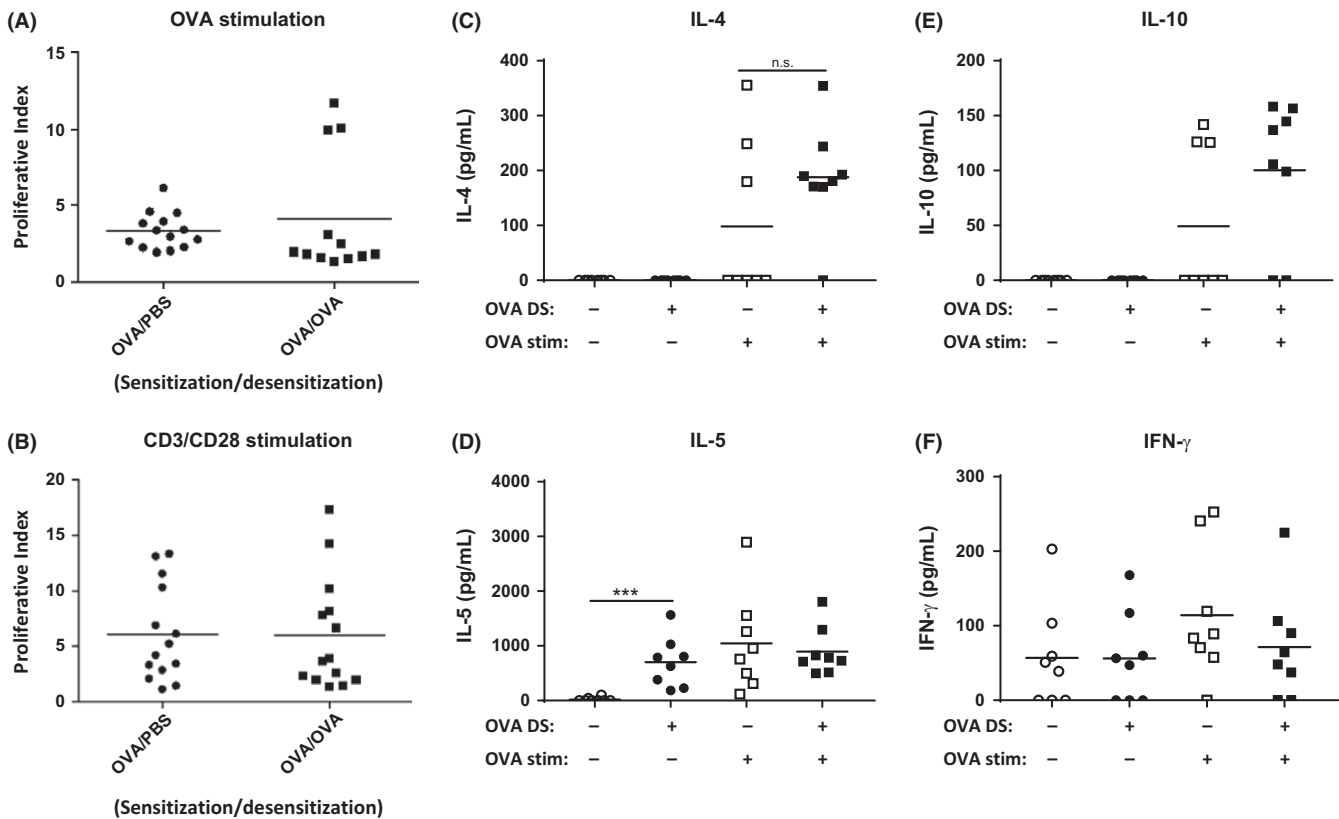


FIGURE 3 Proliferation of CD4⁺ T cells in co-culture with CD11c⁺ dendritic cells from PBS (sham)-desensitized and OVA-desensitized mice after stimulation for 3 d with either OVA (A) or anti-CD3/anti-CD28 (B). IL-4 (C), IL-5 (D), IL-10 (E), and IFN-γ (F) levels were assessed from CD4⁺ T cells from PBS (sham)-desensitized or OVA-desensitized mice after 3 d in culture with either media or OVA stimulation. OVA, ovalbumin; PBS, phosphate buffered saline. *** $P < 0.001$

(OVA/PBS) or OVA-desensitized (OVA/OVA) were killed one hour after the final rush DS injection. As seen in Figure S1, there was no reduction of OVA-specific IgE plasma levels in mice that were desensitized with OVA relative to those desensitized with PBS. To investigate the effects of rush DS on CD4⁺ T cell function, CD4⁺ T cell proliferation and cytokine production in response to OVA stimulation were measured one hour after the final PBS or OVA injection. Proliferation in response to OVA was not different between PBS (sham)-desensitized controls and OVA-desensitized mice (Figure 3A). Similarly CD4⁺ T cell proliferation in response to polyclonal stimulation with anti-CD3/anti-CD28 was not diminished by rush DS (Figure 3B). CD4⁺ T cell cytokine production was assessed after 3 days of ex vivo stimulation with OVA or media alone. While spontaneous release of IL-5 was greater from CD4⁺ T cells of OVA-desensitized mice, there were not any significant differences in IL-4, IL-5, IL-10, or IFN-γ production after OVA stimulation (Figure 3C-F).

3.4 | Rush desensitization with one allergen results in clinical desensitization to an unrelated allergen

To test whether mice with multiple sensitivities could be desensitized to one allergen using the other allergen as the desensitizing agent, mice were first sensitized to OVA and KLH simultaneously.

They were then either sham-desensitized with PBS or desensitized with OVA and challenged subsequently with KLH by intradermal injection. Mice that were sham-desensitized with PBS (OVA + KLH/PBS) responded to challenge with KLH as expected with high ODs of dye extracted from their right ears (Figure 4A,C). Mice desensitized with OVA (OVA + KLH/OVA), however, did not respond robustly to KLH challenge (Figure 4A). Instead, they appeared to be desensitized like a mouse sensitized to OVA alone would be by OVA desensitization. OVA and KLH-sensitized mice that were desensitized with OVA exhibited significantly reduced differences in OD after KLH challenge compared to OVA and KLH-sensitized mice that were PBS (sham)-desensitized (Figure 4B, $P = 0.0058$). This ability of one allergen to desensitize an animal to a second allergen required that the animal be previously sensitized to both allergens, as mice that were sensitized to both OVA and KLH and administered rush DS with the irrelevant antigen BSA responded to KLH challenge as if they had been desensitized with only PBS (Figure 4C,D). Additionally, desensitization did not occur due to cross-reactivity between KLH and OVA, as OVA desensitization did not result in clinical desensitization against KLH in mice that had only been sensitized with KLH (Figure 4E,F). These results demonstrate that desensitizing an individual with multiple hypersensitivities to a single allergen has the effect of desensitizing to multiple allergens. Thus, desensitizing a person with an allergen to which they are mildly allergic could

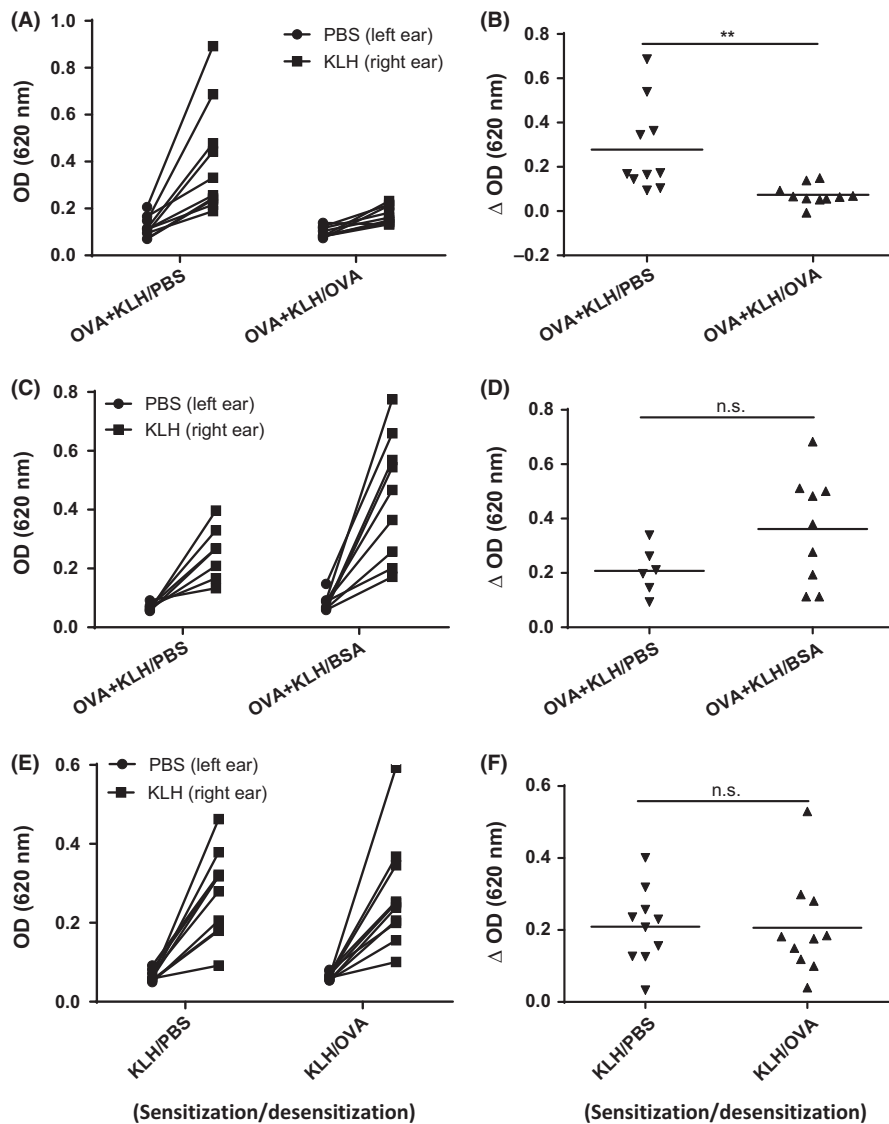


FIGURE 4 Optical density (OD) of ears from OVA + KLH-sensitized (A, C) or KLH-sensitized (E) BALB/c mice that were either sham-desensitized with PBS or desensitized with OVA (A, E), or sham-desensitized with PBS or control-desensitized with BSA (C) and then challenged with KLH (A, C, E). Left and right ears from individual mice are connected with diagonal lines. Panels B, D, and F represent the change in OD (OD 620 nm of right ear less OD 620 nm of left ear) from mice in panels A, C, and E, respectively. BSA, bovine serum albumin; KLH, Keyhole limpet hemocyanin; OVA, ovalbumin; PBS, phosphate buffered saline. ** $P < 0.01$

protect them against responding to other allergens to which they are highly allergic.

3.5 | Secondary allergen desensitization does not result in alterations to T cell proliferation or secreted cytokine profiles

To determine whether the desensitizing allergen, OVA, had any impact on the ability of T cells from OVA and KLH-sensitized mice to either proliferate or produce cytokines in response to KLH stimulation, spleens were removed one hour after desensitization and $CD4^+$ cells were isolated. $CD4^+$ T cells were co-cultured with $CD11c^+$ DCs and stimulated with either OVA or anti-CD3/anti-CD28 for 3 days. Proliferation in response to KLH and anti-CD3/anti-CD28 stimulation was the same for both PBS (sham)- and OVA-desensitized mice (Figure 5A,B). Similarly, there were no significant differences in cytokine production between $CD4^+$ T cells after incubation with media or OVA, with the exception of IL-5 and IFN- γ , which were significantly increased following media stimulation in OVA-

desensitized mice as compared to PBS (sham)-desensitized mice (Figure 5C-F).

3.6 | Rush desensitization with 50 μ g of OVA causes subclinical degranulation of mast cells

To determine the dose of OVA that causes subclinical activation of mast cells, we conducted a dose-response study of OVA in sensitized mice. OVA/alum sensitization resulted in detectable specific IgE in all sensitized animals (Figure S2A). After OVA/alum sensitization, groups of mice were challenged with single intraperitoneal injections of 0, 12.5, 25, 50, 100, or 200 μ g of OVA. Doses of 25 and 50 μ g were sufficient to induce mast cell degranulation as measured by circulating mMCP-1 levels one hour after challenge (Figure S2B). Hypothermia occurred only when mice were injected with 200 μ g of OVA (Figure S2C). We also evaluated clinical symptoms based on a scoring rubric that includes evaluation for symptoms such as scratching, facial oedema, and laboured breathing.¹² Moderate anaphylaxis symptoms (piloerection, decreased activity) were observed in all mice

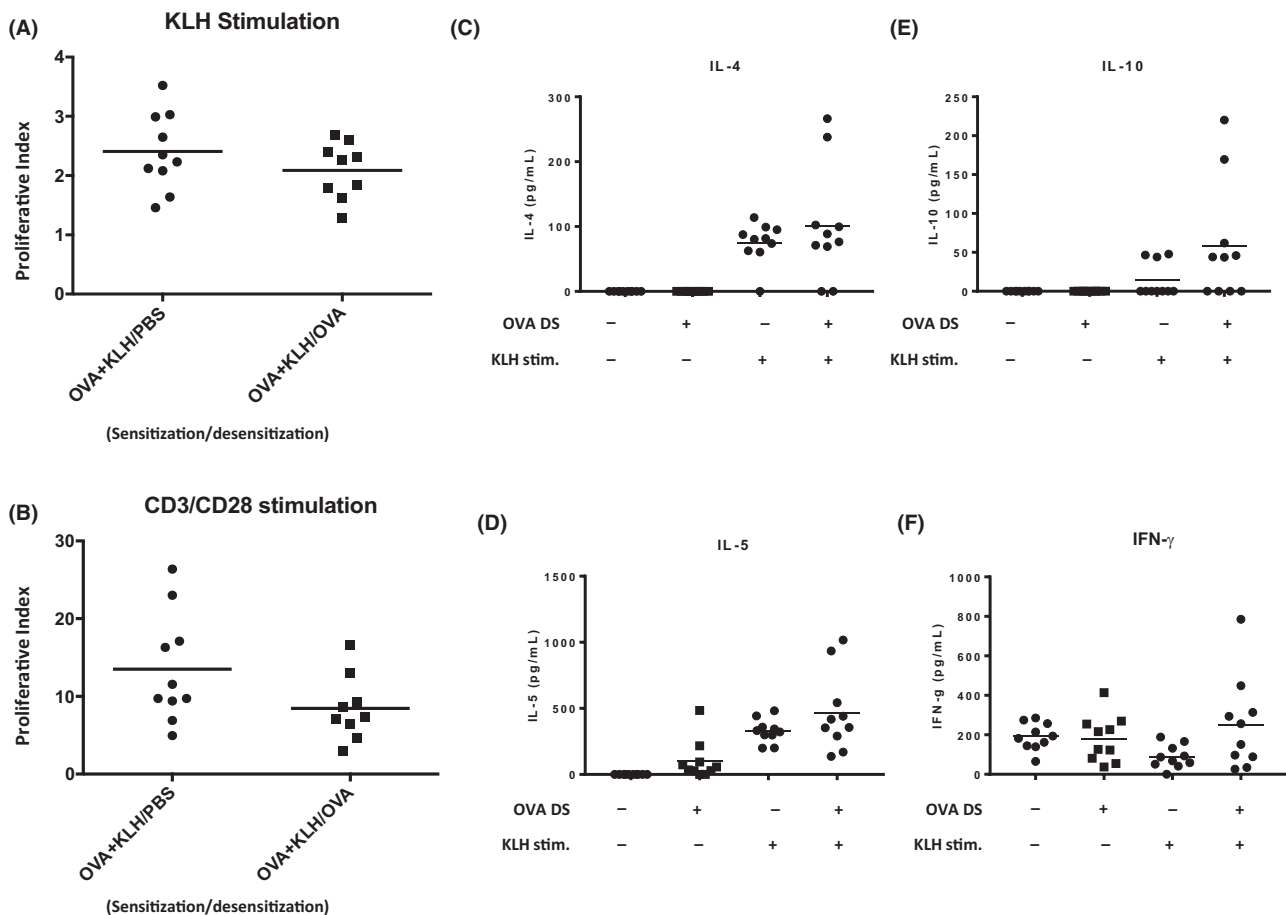


FIGURE 5 Proliferation of CD4⁺ T cells incubated with CD11c⁺ dendritic cells and stimulated for 3 d in vitro with KLH (A) or anti-CD3/anti-CD28 antibody (B). Cell supernatants were also collected and assessed for the production of IL-4 (C), IL-10 (D), IL-5 (E), and IFN- γ (F) following stimulation for 3 d with either media or KLH. All cells were isolated from the spleens of mice sensitized with OVA + KLH and desensitized with OVA or sham-desensitized with PBS as described in the materials and methods section. KLH, Keyhole limpet hemocyanin; OVA, ovalbumin; PBS, phosphate buffered saline

challenged with 200 μ g, while on average mice challenged with 50 or 100 μ g exhibited only mild symptoms (Figure S2D). Plasma levels of OVA-specific IgE did not correlate with the degree of mast cell activation for any concentration of allergen challenge, demonstrating that any level of detectable specific IgE was sufficient to cause anaphylaxis upon allergen challenge (Figure S2E). In contrast, a strong correlation was observed between plasma mMCP-1 concentration drawn 90 minutes after allergen challenge and maximal temperature drop ($r = 0.9166$, $P < 0.001$, Figure S2F).

The impact of rush DS on mast cell degranulation was evaluated by assessing mMCP-1 levels and tissue histology of mast cells after administration of the rapid desensitization regimen (50 μ g of OVA given by intraperitoneal injection every hour for 6 hours starting 2 weeks after sensitization). Although not statistically significant, compared to PBS (sham)-desensitized mice, serum levels of mMCP-1 were elevated in OVA-desensitized mice at 2.5 hours post-administration of the initial dose of desensitizing allergen (Figure 6A, $P = 0.091$). mMCP-1 was also elevated in OVA-desensitized mice at 6 hours post-administration of initial desensitizing dose of allergen (Figure 6A, $P = 0.026$).

To directly assess mast cell alterations in response to rush DS, histological assessment was performed on ears that were excised from mice desensitized with OVA or sham-desensitized with PBS ($n = 5$ per group). No evidence of mast cell degranulation was observed in 4 of the 5 mice that were sham-desensitized with PBS (Figure 6B). In contrast, all five of the mice that were OVA-desensitized demonstrated evidence of mast cell degranulation (Figure 6B). The average percentage of mast cells that exhibited a degranulated morphology was 7.2% in the PBS (sham)-desensitized group and 43% in the OVA-desensitized group. These results demonstrate that the desensitization regimen used in this experiment caused subclinical degranulation of mast cells.

3.7 | Rush desensitization does not require IL-10

Regulatory mechanisms in place in healthy individuals and those undergoing successful allergen-specific immunotherapy include production of interleukin 10 (IL-10) by both T and B cells.¹³ Thus, we investigated whether IL-10 played a role in our rush DS model. Female IL-10^{-/-} mice were sensitized with OVA and then mock-

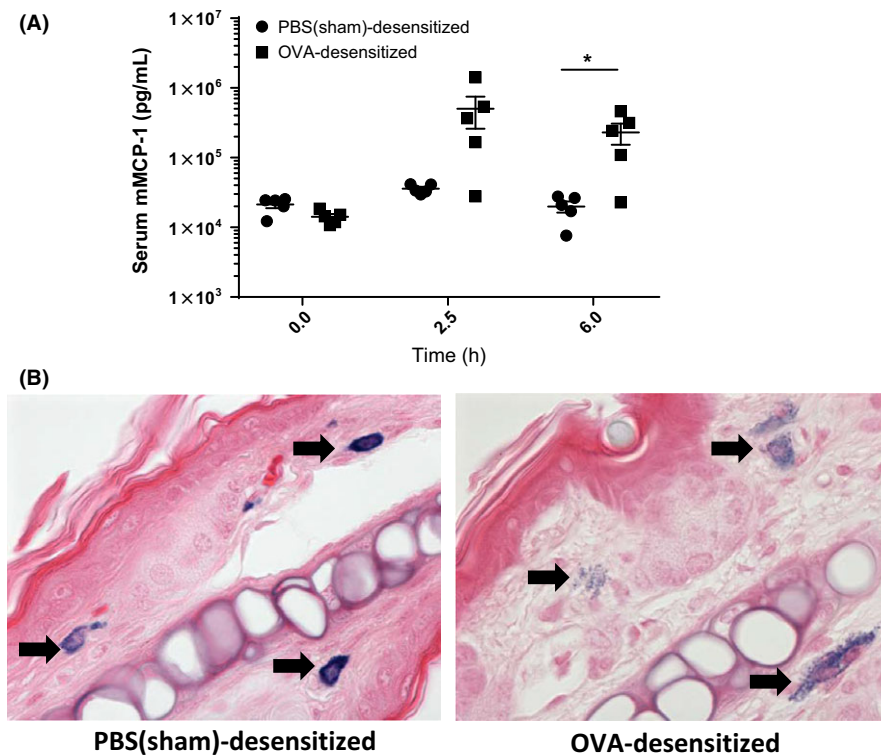


FIGURE 6 A, Serum levels of murine mast cell protease 1 (mMCP-1) in response to desensitization with ovalbumin (OVA) or phosphate buffered saline (PBS) (sham) at 0, 2.5 and 6 h following the beginning of the schedule. B, Toluidine blue stain for mast cells of ear tissue from mice undergoing desensitization with OVA or PBS (sham). Magnification at 630 $\times$, mast cells are indicated by arrows

desensitized with PBS or desensitized with OVA as above. Lack of IL-10 had no impact on the ability of mice to be desensitized. ODs at 620 nm of dye extracted from the right ears of both groups of mice were similar in magnitude (Figure S3A) and the change in OD was also the same in both groups (Figure S3B). This demonstrates that rush DS in our model does not require IL-10.

4 | DISCUSSION

Rush DS is an important clinical tool for overcoming medication allergies. However, because the underlying mechanism of rush DS is poorly understood, clinicians are limited in their ability to optimize schedules, particularly for polysensitized individuals. In this study, we employed a mouse model of rush DS that uses the model allergen OVA to address mechanistic questions regarding rush DS.

We developed a schedule whereby desensitization, as measured by the LAA, could be achieved in a short period of time of less than 1 day. Similar to another group who described a rush DS model that relied on increasing doses of intravenous antigen, we observed no adverse effects in our mice during our protocol.⁹ We found that repeated administration of low-dose OVA over six hours resulted in a significantly decreased response to high-dose allergen challenge in sensitized mice. Of note, our single-allergen desensitization protocol also resulted in protection against bystander allergens in dually sensitized mice. Our active sensitization model allowed us to explore several potential mechanisms for allergic protection that may not have been adequately addressed with a passive sensitization model, including allergen-induced T cell proliferation and cytokine secretion,

differential levels of circulating IgE, and a requirement for IL-10. Ultimately, we found and provide evidence that alterations to mast cells best explain the clinical protection conferred by rush DS observed in our model.

By nature, rush DS does not occur over as long of a timeframe as traditional allergen-specific immunotherapy (ASIT), and thus likely engages different immune mechanisms from ASIT. Unlike the long-lasting tolerance induced by ASIT, the tolerance induced in patients that undergo rush DS protocols is transient. In our model, we found that rush DS did not immediately reduce allergen-specific IgE levels in circulation (Figure S1). Additionally, we found that there were no changes in the ability of CD4⁺ T cells to proliferate in response to allergen. This result is in contrast to the decrease in proliferation that has been seen in antigen-specific T cells in humans repeatedly exposed to bee venom over several months.¹⁴ We also observed no difference in the cytokines produced by CD4⁺ T cells following desensitization. Effective rush DS in our model did not require IL-10, as we found that IL-10^{-/-} and wildtype mice were equally protected against allergic challenge in the LAA (Figure S3A,B). As neither allergen-specific IgE levels nor CD4⁺ T cell responses were altered in our rush DS model, these results indirectly support the idea that rush DS is altering the function of allergy effector cells such as mast cells.

Indeed, it is thought that the earliest mechanisms of desensitization occur through suppression of the function of mast cells and basophils.^{13,15} It has also been shown that rapid desensitization alters the magnitude of inflammatory mediators released following allergen challenge.¹⁶ We chose to look specifically at mast cells since they had been previously shown to be required for ear swelling in the LAA¹¹ and were found to be functionally suppressed in a murine

oral desensitization model.¹⁷ The results of our study suggest that the effector function of mast cells is reduced by rush DS through repeated activation and subclinical degranulation of these cells. This concept is supported by the ability of activating anti-IgE and anti-IgE receptor antibodies to induce polyclonal DS.¹⁸ In our model, we found that rush DS caused subclinical degranulation of mast cells dependent on the presence of IgE to the desensitizing antigen. Although varying levels of serum OVA-IgE were observed within the OVA-sensitized group of mice, mast cell degranulation did not correlate with serum OVA-IgE levels, which indicated to us that all mice with detectable OVA-specific IgE were clinically sensitized (Figure S2E). While we observed no adverse events in our animals during administration of the rapid desensitization protocol, we found that serum levels of mMCP-1 were significantly elevated at 6.0 hours in mice receiving OVA desensitization as compared to PBS controls. Furthermore, we found that repeated, systemic administration with 50 µg of OVA during the desensitization protocol resulted in subclinical degranulation of mast cells in the ears of OVA-sensitized mice. This is consistent with a long-standing hypothesis that rush DS works by causing piecemeal degranulation of mast cells at a level below that which causes clinical disease yet renders mast cells depleted of granule contents.^{19,20} While activation of basophils and mast cells during the time of rush DS has not been extensively studied in humans, a small study found that basophils become activated in children undergoing bee venom rush DS.²¹ Additionally, it is well recognized that allergic reactions are a common adverse effect of rush DS. For example, a recent study evaluating desensitization regimens for bee and wasp venom reported that allergic reactions occurred in 13.7% of patients on an ultra-rush regimen, 14.3% of patients on a rush regimen, and 26.5% of patients on a conventional immunotherapy regimen.²² This suggests that mast cell and basophil activation likely occur to some level in many patients undergoing desensitization.

A previous case report showed that desensitization to a single allergen can result in bystander desensitization, or allergen desensitization in a non-specific manner.²³ In vitro studies, however, have found that rush DS only protects against challenge with the desensitizing allergen.^{8,9,24} Thus, we tested whether or not rush DS results in bystander DS in our in vivo OVA/KLH model. We found that dually sensitized mice could be desensitized to both allergens by performing the desensitization with only one allergen. However, when the novel antigen BSA was administered in the desensitizing protocol, desensitization was not achieved against a sensitizing allergen. We also demonstrated that cross-reactivity of allergens was not the mechanism of bystander DS. These results demonstrate that desensitization to bystander allergen is dependent on the presence of allergen-specific IgE to the antigen used in the rush DS protocol.

The observation that desensitization against one allergen confers clinical protection to another allergen supports the concept that rapid DS works primarily by causing mast cells to become functionally non-responsive. When a host is sensitized to an allergen, repeated administration of that allergen can result in degranulated mast cells that are then unresponsive to all allergen challenges. As

we showed in our study, however, this process can only occur if one is sensitized to the allergen being used for the desensitization protocol for the generation of allergen-specific IgE. Additionally, these findings indicate that it could be possible to desensitize a patient to multiple allergens using fewer allergens in the desensitization cocktail than are being targeted for desensitization.

In summary, we have demonstrated that both allergen-specific and bystander rush DS can be achieved in a mouse model. Rush DS must occur using an allergen to which the individual was previously sensitized. Rush DS does not require IL-10, does not alter the ability of CD4⁺ T cells to proliferate, does not change the quantities of IL-4, IL-5, or IFN-γ produced by CD4⁺ T cells, and does not result in a decrease in allergen-specific IgE levels in plasma. We demonstrated, however, that rush DS results in subclinical degranulation of mast cells and release of the mast cell-specific mediator mMCP-1. Subclinical degranulation of mast cells in our model most likely results in depletion of their granule contents during rush DS, effectively decreasing the quantity of inflammatory mediators, such as histamine, that they have available to release in response to allergen challenge.

The effect we observed on bystander allergens suggests that individuals with multiple allergies could be desensitized to all of them through rush DS to a single allergen. We hypothesize that subclinical degranulation of effector cells is likely an important mechanism for bystander desensitization. In line with current understanding of rush DS, we theorize that the state of desensitization that results from subclinical degranulation lasts for a short period of time, eventually resulting in recurrent sensitization to the allergen once granule contents are restored.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest with this study.

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REFERENCES

1. Simpson CR, Newton J, Hippisley-Cox J, Sheikh A. Incidence and prevalence of multiple allergic disorders recorded in a national primary care database. *J R Soc Med*. 2008;101(11):558-563.
2. Land MH, Kim EH, Burks AW. Oral desensitization for food hypersensitivity. *Immunol Allergy Clin North Am*. 2011;31(2):367-376, xi.
3. Calabria CW. Accelerated immunotherapy schedules. *Curr Allergy Asthma Rep*. 2013;13(4):389-398.

4. Castells M. Rapid desensitization of hypersensitivity reactions to chemotherapy agents. *Curr Drug Saf*. 2006;1(3):243-251.
5. Lee CW, Matulonis UA, Castells MC. Rapid inpatient/outpatient desensitization for chemotherapy hypersensitivity: standard protocol effective in 57 patients for 255 courses. *Gynecol Oncol*. 2005;99(2):393-399.
6. Castells M. Desensitization for drug allergy. *Curr Opin Allergy Clin Immunol*. 2006;6(6):476-481.
7. Akdis CA. Therapies for allergic inflammation: refining strategies to induce tolerance. *Nat Med*. 2012;18(5):736-749.
8. Sancho-Serra Mdel C, Simarro M, Castells M. Rapid IgE desensitization is antigen specific and impairs early and late mast cell responses targeting FcεRI internalization. *Eur J Immunol*. 2011;41(4):1004-1013.
9. Oka T, Rios EJ, Tsai M, Kalesnikoff J, Galli SJ. Rapid desensitization induces internalization of antigen-specific IgE on mouse mast cells. *J Allergy Clin Immunol*. 2013;132(4):922-932.e16.
10. Evans H, Killoran KE, Mitre E. Measuring local anaphylaxis in mice. *J Vis Exp*. 2014;92:e52005.
11. Fox EM, Torrero MN, Evans H, Mitre E. Immunologic characterization of 3 murine regimens of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2015;135(5):1341-1351.e1-7.
12. Ganeshan K, Neilsen CV, Hadsaitong A, Schleimer RP, Luo X, Bryce PJ. Impairing oral tolerance promotes allergy and anaphylaxis: a new murine food allergy model. *J Allergy Clin Immunol*. 2009;123(1):231-238.e4.
13. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*. 2014;133(3):621-631.
14. Meiler F, Zumkehr J, Klunker S, Rückert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med*. 2008;205(12):2887-2898.
15. Plewako H, Wosińska K, Arvidsson M, et al. Basophil interleukin 4 and interleukin 13 production is suppressed during the early phase of rush immunotherapy. *Int Arch Allergy Immunol*. 2006;141(4):346-353.
16. Eberlein-König B, Ullmann S, Thomas P, Przybilla B. Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitization. *Clin Exp Allergy*. 1995;25(8):704-712.
17. Woo HY, Kim YS, Kang NI, et al. Mechanism for acute oral desensitization to antibiotics. *Allergy*. 2006;61(8):954-958.
18. Khodoun MV, Kucuk ZY, Strait RT, et al. Rapid polyclonal desensitization with antibodies to IgE and FcεRIα. *J Allergy Clin Immunol*. 2013;131(6):1555-1564.
19. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2011;127(1):18-27; quiz 28-9.
20. Giavina-Bianchi P, Aun MV, Motta AA, Kalil J, Castells M. Classification of angioedema by endotypes. *Clin Exp Allergy*. 2015;45(6):1142-1143.
21. Cichocka-Jarosz E, Dorynska A, Pietrzyk JJ, Spiewak R. Laboratory markers of mast cell and basophil activation in monitoring rush immunotherapy in bee venom-allergic children. *Immunotherapy*. 2011;3:1013-1017.
22. Bozek A, Kolodziejczyk K. Safety of specific immunotherapy using an ultra-rush induction regimen in bee and wasp allergy. *Hum Vaccin Immunother*. 2018;14(2):288-291.
23. Ciprandi G. Clinical bystander effect exerted by allergen immunotherapy: a hypothesis. *Eur Ann Allergy Clin Immunol*. 2015;47(2):62-63.
24. Ang WX, Church AM, Kulis M, Choi HW, Burks AW, Abraham SN. Mast cell desensitization inhibits calcium flux and aberrantly remodels actin. *J Clin Invest*. 2016;126(11):4103-4118.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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