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Recovery from desensitization of IgE-dependent responses in human lung mast cells

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running head: mast cell recovery from desensitization

Abstract

Background Clinical desensitization and oral food immunotherapy are therapeutic interventions that allow individuals who react adversely to an allergen (drug or food) to be made tolerant to the allergen. However, tolerance is brief and allergen hypersensitivity can recur within days following allergen withdrawal.

Objective We hypothesize that the reason these treatments are temporary reflects rapid recovery of mast cells from a desensitized state. We sought to test this.

Methods Desensitization of IgE-mediated histamine release from human lung mast cells was explored by methods that partially replicate the pattern of treatment during clinical desensitization. Specific and nonspecific desensitization and changes in surface IgE were examined following desensitization. Recovery from desensitization was also studied.

Results Desensitization of mast cell responses was readily induced with concentrations of antigen or anti-IgE that were suboptimal for secretion. There was little or no nonspecific desensitization when lung mast cells were exposed to antigens. There was no loss of cell surface IgE following desensitization. Removing the desensitizing stimulus from the media following desensitization allowed the cells to recover with half-point of recovery of ~1.5 days and complete recovery after 5 days. Both the functional response and histamine content recovered within this timeframe. The recovery appeared possible because both antigens and anti-IgE dissociated rapidly from cells after washing to remove excess stimulus.

Conclusion and Clinical Relevance Human lung mast cells readily recover from a desensitized state following removal of desensitizing antigen. This finding provides a potential explanation for the ephemeral nature of clinical desensitization.

Keywords

human lung mast cell, IgE, anti-IgE, antigen, histamine, desensitization

Introduction

Clinical desensitization is a therapeutic intervention that allows individuals who would normally react adversely to a drug to be made tolerant to the drug [1]. This approach may be useful when therapeutic alternatives are unavailable so facilitating administration of the drug would be of obvious benefit to the patient. For example, clinical desensitization has been applied successfully in chemotherapy to allow use of certain agents that would otherwise promote adverse drug events in some people [2]. A benefit of the process is that desensitization can be induced rapidly by exposing the hypersensitive individual to low concentrations of the drug and then escalating the concentrations over a short timeframe [1-3]. The downside is that tolerance to the drug is brief and hypersensitivity to the drug can recur within days once the drug is withdrawn [3].

A second therapeutic arena that is receiving intense interest is the use of foods to induce a desensitized state in food allergic individuals [4,5]. In recent years it has become increasingly evident that several months of careful escalation of a food exposure in an allergic subject does not result in much or any obvious immune deviation [6]. While it would be desirable to induce long-lasting tolerance to a particular food, the subjects instead experience only a transient tolerance [6]. Because the tolerance is transient, it is now being called ‘desensitization.’ It is not known whether this ‘desensitization’ is the result of cellular desensitization. The time courses of recovery from either

clinical drug desensitization or food desensitization are somewhat different but still fall within a range of days to weeks. However, the broad range of recovery periods may reflect differential clearance of the desensitizing allergen from the body.

At the cellular level there has been a number of studies of desensitization using both basophil and mast cell models [7-14]. Emphasis has been placed on determining whether subthreshold dosing of the cells in vitro mimics the process observed in vivo. With respect to subthreshold dosing, it is clear that mast cells and basophils can be desensitized with concentrations of an IgE-mediated stimulus that otherwise do not induce much or any measurable secretion [15-17].

What has not been studied in any detail is whether primary human mast cells recover from an IgE-mediated desensitized state. Since tissue mast cells can survive for many months and IgE receptor is constantly being synthesized, it is likely that desensitization of the mast cell is neither a terminal condition nor one that permanently alters the IgE-dependent response. However, from the standpoint of understanding recovery from drug or food desensitization, a useful starting point would be to determine whether these cells recover from desensitization in vitro and, if so, how rapidly recovery occurs.

In this study we have investigated the effects of IgE-dependent desensitization on human lung mast cells. Moreover, we have investigated how human lung mast cells recover from desensitization and the mechanisms that this might entail.

Materials and Methods

Materials

The following were purchased from the sources indicated; collagenase, human serum albumin (HSA), Percoll, mouse-IgG₁-FITC (Sigma, Poole, UK); gentamicin, penicillin/streptomycin, fungizone, RPMI 1640 (Invitrogen, Paisley, UK); JW8-IgE (BioServ UK Ltd, Sheffield, UK); anti-c-kit-PE (Miltenyi Biotec, Woking, UK); monoclonal (IgG₁) anti-c-kit-PE (Miltenyi Biotec, Surrey, UK); mouse IgG₁-PE, goat IgG-FITC (Caltag, Invitrogen, Paisley, UK); goat anti-mouse λ -LC-FITC (AbD

Serotec, Oxford, UK); anti-IgE-FITC (Abcam, Cambridge, UK); anti-human IgE, clone HP6061 (Strattech Scientific Ltd, Newmarket, UK); FBS (PromoCell, Heidelberg, Germany). All other compounds and reagents were from Sigma (Poole, UK).

5-iodo-4-hydroxy-3-nitrophenacetyl (NIP)₂₀-HSA was a gift from Dr Birgit Helm (Sheffield University). Benzyl penicilloate (BPO)-IgE, BPO₃₂-HSA and BPO-epsilon-amino-caproic acid (BPO-EACA) were prepared as described [18].

Buffers

Phosphate buffered saline (PBS), pH 7.3, contained (mM): NaCl 137; Na₂HPO₄.12H₂O 8; KCl 2.7; KH₂PO₄ 1.5. +PBS was PBS additionally supplemented with: CaCl₂.2H₂O 1 mM; MgCl₂.6H₂O 1 mM; glucose 5.6 mM; HSA 30 µg/mL.

Lung tissue

Human lung tissue was obtained from surgical resections with the approval of the National Research Ethics Service (REC reference: 15/NW/0657). Written, informed consent was obtained from participants.

Cell isolation

Mast cells were isolated from human lung tissue using methods described in detail elsewhere [19]. In brief, macroscopically normal tissue from lung resections was disrupted physically and enzymatically (collagenase Ia) to generate a mixed cell suspension of which 3 to 13% of the cells were mast cells. Mast cells were visualized by microscopy using an alcian blue stain. This method generated approximately 6 x 10⁵ mast cells per g of tissue. Mast cells of enhanced purity (15 to 71% purity; median, 38%) were used in this study and were prepared by flotation of preparations over discontinuous Percoll gradients [20]. We investigated the profile of cell types in four Percoll

preparations by performing cytopins and differential cell counts based on morphology. The cell profile was; mast cells $59\pm7\%$ (mean $\pm$ SEM), eosinophils $5\pm2\%$, neutrophils $<1\%$, lymphocytes $26\pm6\%$, macrophages $10\pm4\%$.

Mediator release

Mediator release experiments were performed essentially as described elsewhere [19]. Experiments were performed in +PBS buffer. Histamine released into supernatants was measured using an automated fluorometric method [21]. In most experiments, mediator release was initiated with a mouse anti-human IgE, 6061P (Hybridoma Farms, MD). Alternatively, mast cells were activated with antigen. Cells were passively sensitized (20 h, 37 °C) with a chimeric IgE (human heavy chain, murine lambda light chain), JW8-IgE (~200 ng/mL) that recognizes the hapten, NIP [22]. After sensitization, cells were washed twice and activated with the antigen, NIP₂₀-HSA. Mast cells were also passively sensitized (20 h, 37 °C) with BPO-specific IgE (~400 ng/mL), the cells washed and then challenged with BPO₃₂-HSA.

Desensitization protocol

In desensitization experiments, mast cells were incubated without or with anti-IgE or antigen for discrete periods of time (1 to 48 h). Mast cells were typically incubated at a density of 0.1×10^6 mast cells per ml in RPMI-1640 buffer supplemented with penicillin (10 Units/mL), streptomycin (10 µg/mL), gentamicin (50 µg/mL), fungizone (1 µg/mL), HSA (2.5 mg/mL) and supplemented with additional calcium to a final concentration of 1 mM. After the desensitization treatment the cells were washed three times. Cells were then used in mediator release experiments or processed for flow cytometry.

In some experiments, recovery from desensitization was also explored. Desensitization induced by anti-IgE or antigen was allowed to proceed typically for 4 h the cells were then washed, resuspended in supplemented RPMI buffer and allowed to recover for 1, 3 or 5 days. After the desensitization treatment the cells were washed three times and used in mediator release experiments.

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In all desensitization and recovery experiments, the design was such that all treatment conditions were harvested at the same time. So for example in experiments investigating the time-dependence of desensitization where incubation times for desensitization were 1, 8 and 24 h, the control set involved incubating mast cells for 24 h in buffer, the 1 h desensitization set involved incubating mast cells for 23 h in RPMI buffer and then for 1 h with desensitizing treatment, the 8 h desensitization set involved incubating cells for 16 h in buffer and then 8 h in desensitizing treatment along with a 24 h desensitization set. All treatment sets were thus harvested at the same time and studied experimentally at the same time. In recovery experiments, a similar procedure was followed. If cells were desensitized for 4 h but not allowed to recover, they actually experienced 24 h of culture in RPMI buffer, washed and 4 h of desensitization before washing again and challenge. In parallel, cells experiencing recovery were desensitized for 4 h, washed and cultured for 24 h to recover before challenge. Control cells experienced 4 h of buffer alone, washing and 24 h of RPMI buffer alone before challenge.

Flow cytometry

Flow cytometry experiments were performed on mast cells using methods that have been described in detail elsewhere [19]. Mast cells were identified as the c-kit (CD117) positive population using a monoclonal (IgG₁) anti-c-kit-PE (A3C6E2).

Mast cells were first fixed (Medium A, Fix & Perm kit, Invitrogen, Carlsbad, CA, USA) before incubation with fluorochrome-conjugated antibodies (1:5 to 1:20). In order to determine JW8-IgE density in the CD117+ population, a goat anti-mouse λ -light chain-FITC was used alongside relevant controls. In order to determine IgE density in the CD117+ population, a mouse (IgG₁) anti-IgE-FITC (BE5) was used alongside relevant controls.

Samples were run on an LSR II flow cytometer (BD Biosciences, Oxford, UK) and data captured using DIVA software. Analysis of data was performed using FlowJo software (Tree Star, Oregon, USA). The amount of fluorescence in the gated population was quantified as net Mean

Fluorescence Index (MFI) which was calculated as the geometric mean of the specific antibody signal subtracted by the geometric mean of the relevant control signal.

Data analysis

Data were analysed using GraphPad Prism (version 6, La Jolla, CA, USA). Statistical significance was assessed utilising either Student's t test or repeated measures ANOVA as appropriate.

Results

Characteristics of desensitization of mast cells with anti-IgE Ab

Some characteristics of desensitization in human lung mast cells have been examined previously [23,24]. To provide the boundary conditions for the current studies, desensitization with a pan-specific crosslinking agent was examined. The anti-IgE Ab (6061P) used was a monomeric monoclonal murine IgM antibody. Preliminary studies established that it was possible to induce a partial or complete level of desensitization with subthreshold or suboptimal concentrations of anti-IgE. Results presented in the Supporting Information repository (Fig. S1) demonstrate that 50% desensitization occurred with 2 ng/mL anti-IgE and nearly complete desensitization with 20 ng/mL (the optimum for secretion being 2 µg/mL). Desensitization was time-dependent. At these lower concentrations of stimulus, a half-maximal effect occurred between 1 and 8 h (Fig. S1, Supporting Information). Preliminary studies demonstrated that sequential escalation of desensitizing concentrations could establish a state of desensitization without significant secretion (Fig. S2, Supporting Information). Sequential addition of 0.2, 0.6, 1.2, 2, 3, 5 and 8 ng/mL of anti-IgE made at 30 min intervals up to 3 h resulted in 4% released histamine and 80% reduction in subsequent histamine release at optimal concentrations for stimulation.

Specificity of desensitization

We investigated what effects an overnight treatment with antigen or anti-IgE would have on non-crossreacting stimuli (so-called nonspecific desensitization). Following passive sensitization with NIP-specific IgE (JW8-IgE) mast cells were washed and then incubated for a further 24 h with or without anti-IgE (2 μ g/mL) or NIP-HSA (10 ng/mL). The overnight treatment with anti-IgE abolished the response to both anti-IgE and NIP-HSA whereas the overnight antigen treatment selectively abrogated the response of mast cells to NIP-HSA but not to anti-IgE (Fig. 1A).

In addition, mast cells were passively co-sensitized with both JW8-IgE and a BPO-specific IgE. After sensitization overnight, the cells were washed and incubated (24 h) with or without an optimal releasing concentration (10 ng/mL) of either NIP-HSA or BPO-HSA. The overnight treatment with BPO-HSA abolished the subsequent response of mast cells to BPO-HSA but had no effect on the NIP-HSA response (Fig. 1B). Conversely, overnight treatment with NIP-HSA abolished the mast cell response to NIP-HSA but not to BPO-HSA.

Desensitization was not accompanied by loss of IgE

In preliminary experiments, we investigated the expression of IgE by mast cells. Mast cells were incubated overnight with or without JW8-IgE (~200 ng/mL). The cells were then washed and fixed and the density of either JW8-IgE or total IgE was assessed by flow cytometry. JW8-IgE, a chimeric IgE consisting of human heavy chain and mouse λ -light chain [22], was detected by using an antibody raised against the mouse λ -light chain. The data show that JW8-IgE was very clearly expressed by mast cells following passive sensitization (Fig. 2A). Moreover, there was an increase in the expression of total IgE following passive sensitization (Fig. 2B). Overall, there was a $34 \pm 9\%$ increase (n=6) in total IgE levels following passive sensitization with JW8-IgE (Fig. 2C).

Having established conditions for detection of IgE on mast cells, the effects of desensitizing treatments on IgE expression were determined. Cells were first incubated overnight with JW8-IgE. After washing, the cells were incubated with or without anti-IgE (0.02, 0.2 or 2 μ g/mL) for 4 h. After

these desensitizing treatments, the cells were washed and the density of JW8-IgE detected by flow cytometry. Desensitizing concentrations of anti-IgE had little effect on JW8-IgE expression on mast cells (Fig. 3A). Overall, there was essentially no change in JW8-IgE density with treatments of 20 and 200 ng/mL of anti-IgE and ~9% reduction in JW8-IgE after treatment with 2000 ng/mL. Functional experiments performed in parallel indicated that desensitizing treatments led to substantial loss of functional responses to either anti-IgE (Fig. 3B) or NIP-HSA (Fig. 3C) without loss of IgE expression.

Mast cells recover from desensitization

Mast cells were incubated for 4 h with anti-IgE (20 ng/mL) to induce desensitization. The cells were then washed to remove excess unbound anti-IgE and incubated for an additional 24 h in buffer to establish whether the cells would recover from the desensitization treatment. Recovery was observed and there were two principal aspects to this. First, a period of recovery allowed mast cells to replenish histamine lost during the desensitization phase (Fig. S3, Supporting Information). Second, mast cells recovered from a desensitized state responded effectively to anti-IgE. Cells allowed to recover for 24 h from desensitization showed ~40% recovery in the response to anti-IgE compared to cells that had been desensitized and not allowed to recover (Fig. 4A). The effects of longer recovery periods of 3 (Fig. 4B) or 5 days (Fig. 4C) from desensitization were investigated. The data show that greater recovery (~70% of the control anti-IgE response) was observed when the cells were allowed to recover for 3 days and complete recovery was observed if the cells were allowed to recover over 5 days (Fig. 4D).

Recovery was not restricted to desensitization by anti-IgE Ab since similar results were obtained with antigen. Mast cells that had been passively sensitized with JW8-IgE were exposed to a desensitizing concentration of NIP-HSA (0.1 ng/mL) for 4 h, washed extensively to remove excess unbound antigen and then allowed to recover for 24 h. The data show that the response of mast cells to NIP-HSA (0.1 – 10 ng/mL) was substantially improved in those cells allowed to recover compared to the desensitized set (Fig. S4, Supporting Information).

The ability to recover from desensitization was strongly linked to the desensitizing concentration of stimulus (Fig. 5). Mast cells that had been exposed to a very high desensitizing concentration (2 $\mu\text{g/mL}$) of anti-IgE were less able to respond to either anti-IgE or NIP-HSA after a recovery period (24 h) compared to cells that had been exposed to lower desensitizing concentrations (0.02 or 0.2 $\mu\text{g/mL}$) of anti-IgE.

Recovery could be accelerated somewhat by the addition of IgE during the recovery period. Mast cells were desensitized with anti-IgE (0.2 $\mu\text{g/mL}$) for 4 h and then washed extensively and allowed to recover (20 h) in the absence or presence of JW8-IgE (~ 200 ng/mL). The data show that desensitized mast cells that then recovered in the presence of JW8-IgE responded effectively to NIP-HSA but that this response was reduced compared to that seen with control cells. Similarly, although the addition of extra IgE during the recovery period improved the response to anti-IgE, this response was still diminished somewhat compared to the control response (Fig. S5, Supporting Information).

Disaggregating IgE cross-links has no effect on recovery

Although the cells were washed free of excess stimulus prior to incubation for recovery, this approach may not lead to loss of IgE-bound stimulus because aggregates may be stable for considerable periods of time [2]. To test whether dis-aggregation would enhance recovery, mast cells were passively sensitized with BPO specific-IgE and histamine release induced by BPO-HSA was assessed in the absence and presence of increasing concentrations of the hapten, BPO-EACA (0.001 – 0.1 mM). As a monovalent hapten, BPO-EACA is known to interfere with BPO-HSA induced cross-links [18]. These preliminary experiments indicated that (a) 0.01 mM BPO-EACA completely abolished the response to BPO-HSA and (b) that the washing procedures employed, effectively removed BPO-EACA from the subsequent reactions (Fig. S6, Supporting Information).

In subsequent experiments, desensitization was induced by incubating mast cells with BPO-HSA (10 ng/mL for 4 h), the cells were washed extensively and allowed to recover (24 h) in the absence or presence of BPO-EACA (0.01 mM). Subsequent responses to both BPO-HSA (100

ng/mL) and anti-IgE (2 μ g/mL; for control purposes) were investigated. The data show that recovery from desensitization of the response to BPO-HSA was unaffected by the presence of BPO-EACA (Fig. 6). Consistent with the absence of nonspecific desensitization noted above, desensitization to BPO-HSA did not affect the response to anti-IgE Ab.

Antigen and anti-IgE Ab readily dissociate from IgE

The failure of BPO-EACA to accelerate recovery from desensitization induced by BPO-HSA implied that there were no sustained cross-links to disrupt suggesting that washing of the cells might remove bound antigen from surface IgE. Using a method first used in the 1980s [26], the relative durability of a pre-established cell surface aggregate (established with either anti-IgE Ab or antigen) was examined by a rapid dilution test. A time-course experiment was performed in which mast cells were stimulated in a small volume (50 μ L) with either antigen or anti-IgE at concentrations ten-fold lower than maximal (therefore, on the ascending limb of the dose-response curve, where a 20-fold dilution would produce much less release). At discrete intervals (10 sec to 10 min) the reaction was altered by the addition of PBS-EDTA (5 mM) or diluted 20-fold by the addition of 1 ml of +PBS buffer (containing all the supplements required for histamine release) and secretion allowed to proceed for a further 10 min. The experiment showed that diluting the reaction volume with +PBS led to a blunted response (relative to a full 10 minute secretion). This was true whether anti-IgE (Fig. 7A), NIP-HSA (Fig. 7B) or BPO-HSA (Fig. 7C) was used to induce release. These results suggested that each of these stimuli dissociate relatively rapidly.

Discussion

The principal purpose of this study was to evaluate whether human lung mast cells recover from desensitization. In agreement with a large body of work in alternative mast cell and basophil systems, we could readily demonstrate that incubation with either anti-IgE or antigen attenuated the subsequent

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response of mast cells to either anti-IgE or antigen. The surprising observation emerging from this study was that mast cells could readily recover from a desensitized state.

In the context of desensitization, studies in human basophils have shown that there are several operating mechanisms that are likely to influence the subsequent response of the cells following desensitization. These include negative feedback loops like the activation of SHIP (SH2 domain-containing inositol 5' phosphatase) or longer-term effects like the loss of cell surface IgE [27,28]. With respect to the latter, even in basophils, this is a very slow and incomplete process [25]. In the current study, no evidence was found for the loss of cell surface IgE. This does not imply that the retained surface IgE continues to signal, most evidence from a variety of models (although with different mechanisms), suggests that it does not [29-31]. For human basophils, it appears that there is a multi-step process that begins with the cessation of signalling [30,31]. Although immobilization of the receptor occurs, whether immobilized receptor is ultimately internalized [32] is not entirely clear [11]. Although there was no evidence for internalization of IgE in human lung mast cells within the timeframe of desensitization in these studies, future studies may find a very slow process of partial internalization in the continued presence of antigen.

The surprising observation was that mast cells could readily recover from a desensitized state even without explicitly inducing dissociation of pre-established aggregates. Recovery was most readily observed when the cells were desensitized with submaximal concentrations of stimulus. There was limited recovery if the cells were stimulated at concentrations that induce maximal release. This result may suggest something about the underlying mechanism of desensitization. For example, a process that results from the accumulation of an activation inhibitor (e.g, progressive activation of SHIP), might display a large upper boundary leading to excessive accumulation of inhibitor while a depletion-based process like loss of functional IgE or an activation intermediate can at best reach the lower boundary of zero. Since a low concentration (20 ng/mL) and a high concentration (2000 ng/mL) of anti-IgE both lead to significant desensitization (although not quite equal under the timeframes typically used) and aggregates established at either concentration are readily disaggregated (see Fig. 7), the mechanism that appears to explain more readily the lack of recovery

following desensitization induced by a strong stimulus would be a process involving the accumulation of an inhibitor. However, as discussed below, other results might be explained by depletion of activation elements (including loss of functional IgE).

Speculation about mechanisms aside, the results do suggest that recovery is a function of the magnitude of stimulation. Longer times might improve recovery of strongly desensitized cells but since the typical process of clinical desensitization is to escalate doses gradually, it seems likely that the mast cell experiences only a progressively weak signal from which recovery is more readily achievable.

One issue regarding recovery that we considered was whether maximal histamine release that was considerably less than 100% represented secretion of all functional granules. In such a scenario, 30% release might be the best an IgE-mediated signal could induce, not because the signalling steps were weak but because only 30% of the granules were ready for secretion. If this occurred, then stimulating with a maximal releasing concentration (2000 ng/mL) of anti-IgE Ab would secrete all the releasable granules and even if the cell recovered from its state of desensitization, there would be no 'secretable granules' left to secrete. The cell would appear not to recover. For two reasons, this explanation does not have strong support. First, as shown in Fig. S3 (Supporting Information), one aspect of the mast cell recovery is the re-accumulation of histamine. This is unlike the experience with human basophils which fail to restore histamine over this timeframe [33]. While it is possible that these newly-synthesized granules are immature and not readily releasable, the starting point for testing release after recovery appears similar to the starting point with freshly isolated cells. Second, there is no correlation between recovery and the extent of secretion.

It was of interest that when mast cells were exposed to additional antigen-specific IgE during the recovery period that the response to anti-IgE Ab improved, although the response was still reduced by about a third compared to the control response with anti-IgE. In contrast to the speculation above that the desensitization process may involve accumulation of an inhibitor of activation, these results suggest that replacement of functional IgE can partially overcome the state of desensitization.

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As noted above, one model of desensitization that finds support both in studies of human basophils and rodent mast cells suggests that aggregated IgE and its receptor are segregated into regions of the membrane that do not allow access to proximal activation elements leading to a non-functional [30-32] but potentially reversible IgE/receptor once aggregates dis-aggregate. Supplying new IgE in distinct uninvolved receptors would be expected to bypass the non-functional IgE and give the appearance of better recovery. Our data showing that desensitization was not accompanied by a loss of cell surface IgE is consistent with this model of non-functional IgE/receptor.

Another unexpected observation was the rapidity that crosslinks could be dis-aggregated. The wash kinetics suggest that anti-IgE Ab and antigens lead to aggregates that are easily dissociated (see Fig. 7). This is also in contrast to observations in human basophils where aggregates were relatively stable over time [25]. This behaviour is probably important in the recovery observed. It also explains why the addition of monovalent hapten (BPO-EACA) during the recovery period after cells had been desensitized with BPO-HSA failed to improve recovery. These findings are somewhat difficult to reconcile as an expectation would be that the affinity of BPO-HSA for BPO-specific IgE on the mast cell would be quite high and that, even with washings to remove excess unbound antigen, BPO-HSA would be bound in multiply branched aggregates and persist after washing away unbound antigen [25]. One caveat is that the method used in this study to assess the transience of formed aggregates necessarily worked with 'young' aggregates. Other studies have suggested that aggregate size evolves to large sizes over time and also suggest that these aggregates become more difficult to dis-aggregate [25,34]. In addition, the mast cells that are typically isolated from lung tissue express 10-fold lower densities of FcεRI compared to human basophils [35,36] and higher densities of sensitized receptors might be expected to form larger branched aggregates. This possibility may also serve to explain why desensitization is largely specific for the desensitizing antigen. In basophils, the extent of nonspecific desensitization is a function of antigen-specific IgE density [37] but there is no *a priori* expectation for what density of IgE would represent a transition to nonspecific desensitization in mast cells. Indeed, the precise mechanisms that distinguish specific and nonspecific desensitization are unknown [30] so that it would be difficult to formulate a precise expectation for such a transition.

Nonspecific desensitization does occur in mast cells and studies by Zhao and colleagues with cultured skin mast cells demonstrated complete nonspecific desensitization [9]. Whether the densities of FcεRI or the biochemical pathways are different in the cultured cells is not clear. Notably, clinical desensitization appears to be specific for the desensitizing allergen [38].

The disparity in the nature of the response when comparing skin mast cells and lung mast cells might be related to the phenotype of mast cells. Human lung mast cells are mostly (~90%) of the MC_T phenotype, cells containing tryptase, whereas human skin mast cells are mostly of the MC_{TC} phenotype containing both tryptase and chymase [39]. Reports indicate that MC_T and MC_{TC} mast cells can be functionally heterogeneous [39]. By extension, it is possible that the nature of desensitization and recovery described in this paper using mostly MC_T mast cells may not necessarily be representative of all subsets of mast cells. This has relevant implications since, in the context of clinical desensitizations, different routes of administration (oral v intravenous) are likely to target different mast cell subsets.

There are few expectations regarding the rate of recovery from desensitization because the precise mechanisms are not understood. In human basophils, recovery from nonspecific desensitization required hours and there was no recovery from specific desensitization [25]. It may be that the recovery observed in these mast cell experiments reflected a re-establishment of a normal state for the signal transduction apparatus. These mechanistic issues aside, it appears that a formerly 'desensitized' receptor can recover its activation functionality and that this process requires several hours with complete recovery by 5 days.

In summary, in this study we demonstrated that normal tissue lung mast cells can be readily desensitized by sub-threshold concentrations of anti-IgE and antigen and there is little or no nonspecific desensitization. Moreover, we showed that mast cells can recover quite rapidly from desensitization following removal of excess unbound anti-IgE or antigen. These findings may be of relevance to characteristics seen in clinical desensitization, in particular the temporary nature of clinical desensitization. These results may also have relevance to observations that oral

immunotherapy becomes ineffective within days to weeks. If oral immunotherapy does not result in immune deviation but only induces mast cell desensitization, then recovery would be expected to be fast. The time-limiting process may be the time required to 'wash' the system of antigen rather than the time required for mast cells to recover from a desensitized state. The same arguments hold for clinical desensitization for drug allergies.

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Conflicts of interest

None

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Figure Legends

Figure 1 Specificity of the desensitization. (A) Mast cells were passively sensitized with JW8-IgE for 20 h. The cells were then washed twice and incubated for 24 h without or with NIP-HSA (10 ng/mL) or anti-IgE (2 µg/mL). After these incubations, the cells were washed three times and histamine release induced by NIP-HSA (10 ng/mL, shaded columns) or anti-IgE (2 µg/mL, open columns) assessed. (B) Mast cells were passively sensitized with JW8-IgE and BPO-IgE for 20 h. The cells were then washed twice and incubated for 24 h without or with NIP-HSA (10 ng/mL) or BPO-HSA (10 ng/mL). After these incubations, the cells were washed three times and histamine release induced by NIP-HSA (10 ng/mL, shaded columns) or BPO-HSA (10 ng/mL, open columns) assessed. Asterisks denote statistically significant ($P < 0.05$ at least) levels of desensitization compared to control (buffer). Values are means±SEM, n=4 for (A) and for (B).

Figure 2 Expression of IgE after passive sensitization. Mast cells were incubated with (+ passive) or without (no passive) JW8-IgE (~200 ng/mL) for 20 h after which the cells were fixed and specific expression of either JW8-IgE or total IgE was determined by flow cytometry. (A) Representative data are shown of JW8-IgE expression in the CD117+ population determined by incubating fixed cells with a goat anti-mouse λ-light chain Ab or corresponding isotype control. (B) In the same experiment, the same preparation was evaluated for total IgE expression using a mouse anti-human IgE mAb. (C) A total of 6 preparations, represented by different symbols in the graph, were evaluated for the expression of total IgE following passive sensitization. The data highlighted in red represent data calculated from values generated in (B).

Figure 3 Effect of desensitization on IgE density. Mast cells were passively sensitized with JW8-IgE for 20 h after which the cells were washed. The cells were then challenged with or without anti-IgE (0.02, 0.2 or 2 μ g/mL) for 4 h after which the cells were fixed. (A) The density of JW8-IgE after treatments was determined by detecting mouse λ -LC expression in the CD117+ population by flow cytometry. Effects of desensitizing treatments with anti-IgE (0.02, 0.2 or 2 μ g/mL for 4 h) on histamine release induced by (B) anti-IgE (2 μ g/mL) or (C) NIP-HSA (10 ng/mL) were followed in parallel. Values are means $\pm$ SEM, n=4. Asterisks denote statistically significant ($P < 0.05$ at least) reduction in histamine release following desensitizing treatments compared to control.

Figure 4 Recovery from desensitization. Mast cells were maintained in buffer throughout (control) or were incubated with anti-IgE (20 ng/mL) for 4 h (desensitized) or incubated with anti-IgE for 4 h, then washed extensively and allowed to recover in buffer (recovery) for either (A) 1 day, (B) 3 days or (C) 5 days. After these treatments, the cells were washed and the response to anti-IgE evaluated. (D) The data in (A), (B) and (C), for the highest concentration of anti-IgE (2 μ g/mL), were reworked to provide a relationship for the kinetics of recovery. Values are means $\pm$ SEM, n=4 (A), n=5 (B) and n=3 (C).

Figure 5 Concentration-dependence of recovery. Mast cells were passively sensitized with JW8-IgE for 20 h after which the cells were washed. Cells were incubated in buffer throughout (control) or were incubated with anti-IgE for 4 h (desensitized) or incubated with anti-IgE for 4 h, then washed extensively and allowed to recover in buffer for 24 h (recovery). Desensitizing treatments of anti-IgE were (A, D) 0.02, (B, E) 0.2 or (C, F) 2 μ g/mL. After these treatments mast cells were activated with either (A, B, C) anti-IgE (2 μ g/mL) or (D, E, F) NIP-HSA (10 ng/mL) for histamine release. Values are means $\pm$ SEM, n=6 (A and D), n=4 (B and E) and n=5 (C and F). Statistical annotations: * statistically significant ($P < 0.05$ at least) desensitization compared to control; # denotes statistically significant ($P < 0.05$ at least) recovery from desensitization.

Figure 6 Effect of BPO-EACA on recovery from desensitization induced by BPO-HSA. Mast cells were passively sensitized with BPO-specific IgE for 20 h after which the cells were washed. Cells were incubated in buffer throughout (control) or were incubated with BPO-HSA (10 ng/mL) for 4 h (desensitized) or incubated with BPO-HSA for 4 h, then washed extensively and allowed to recover in buffer for 24 h (recovery). In parallel, the effect of the hapten, BPO-EACA (0.01 mM) on recovery from desensitization (recovery + EACA) was investigated. Cells were also incubated in buffer for 4 h and then for a further 24 h with BPO-EACA (EACA). After these treatments the cells were washed extensively and then challenged with either (A) BPO-HSA (100 ng/mL) or (B) anti-IgE (2 µg/mL) for 30 min for histamine release. Values are means±SEM, n=3. Asterisks denote statistically significant ($P < 0.05$ at least) reductions in histamine release compared to control.

Figure 7 Time course of histamine release. Mast cells were activated with either (A) anti-IgE (0.2 µg/mL), (B) NIP-HSA (1 ng/mL) or (C) BPO-HSA (10 ng/mL) for discrete time intervals (10, 20, 30, 40, 60, 120, 300 and 600 sec) after which the reaction was terminated by the addition of PBS-EDTA or diluted 20-fold by the addition of +PBS and histamine release allowed to proceed for a further 10 min. Values are means±SEM, n=4 for (A), (B) and (C).

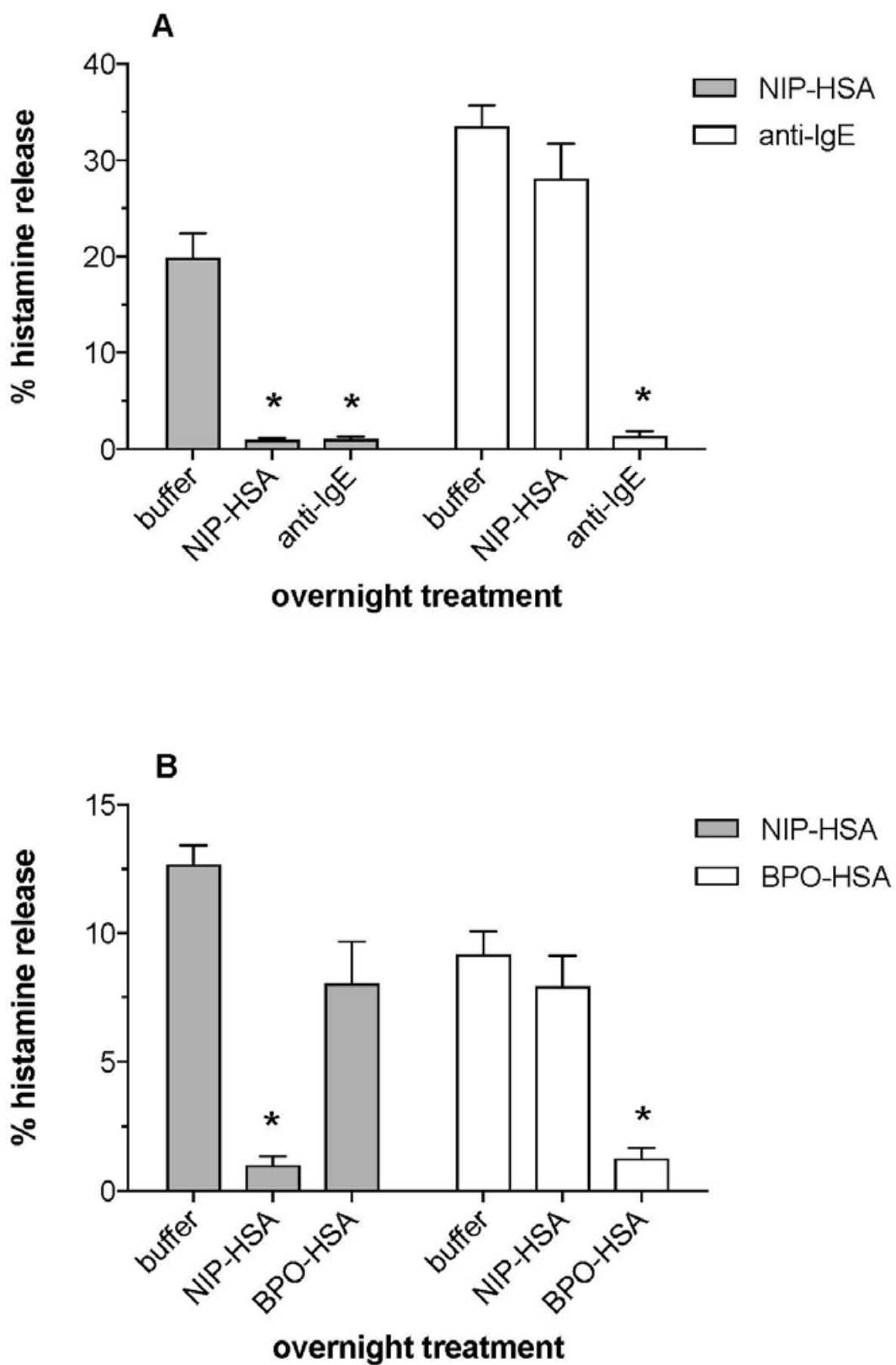


figure 1

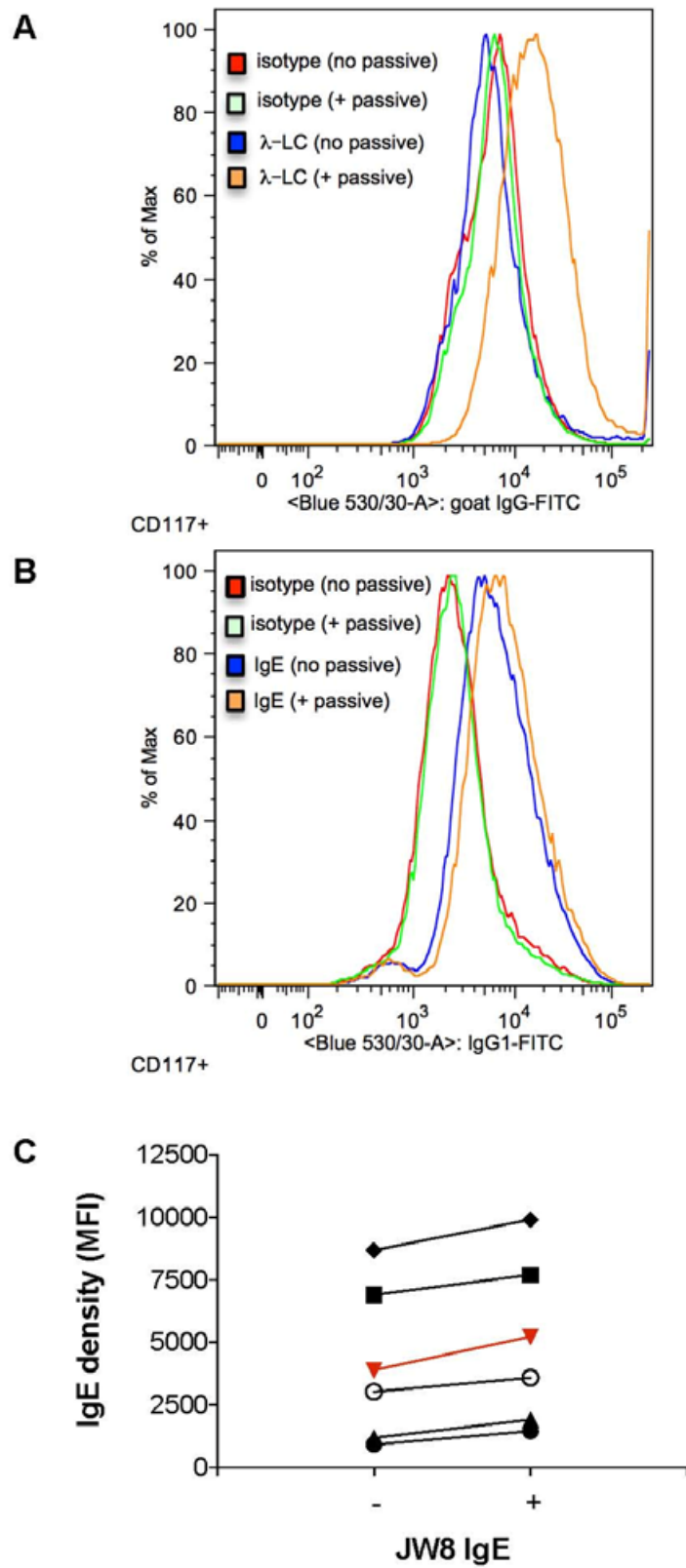


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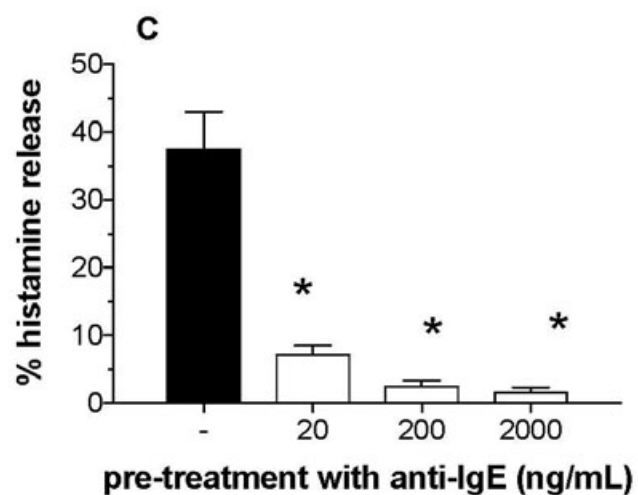
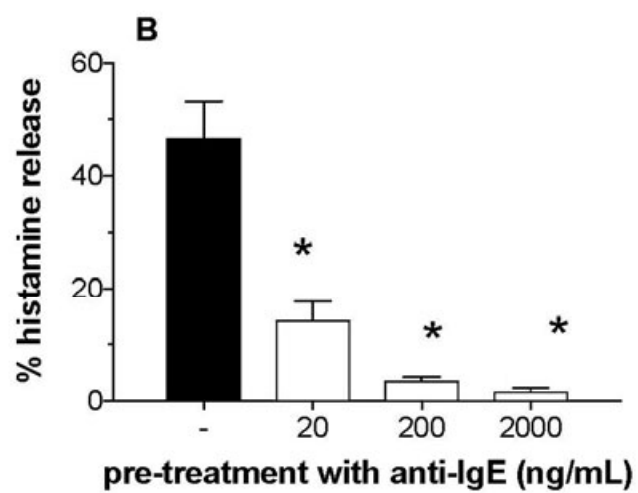
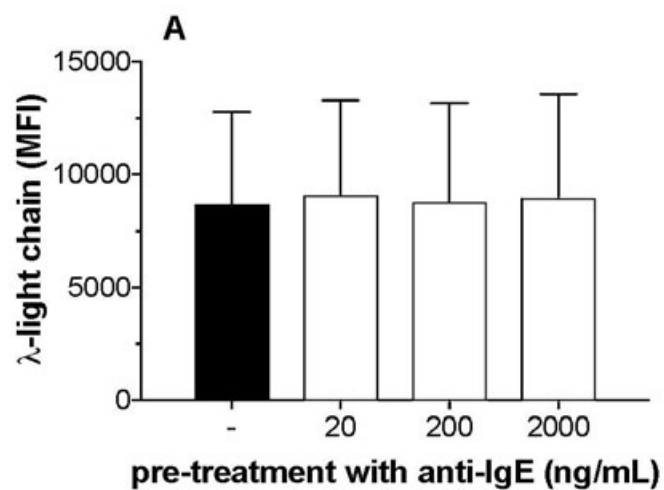


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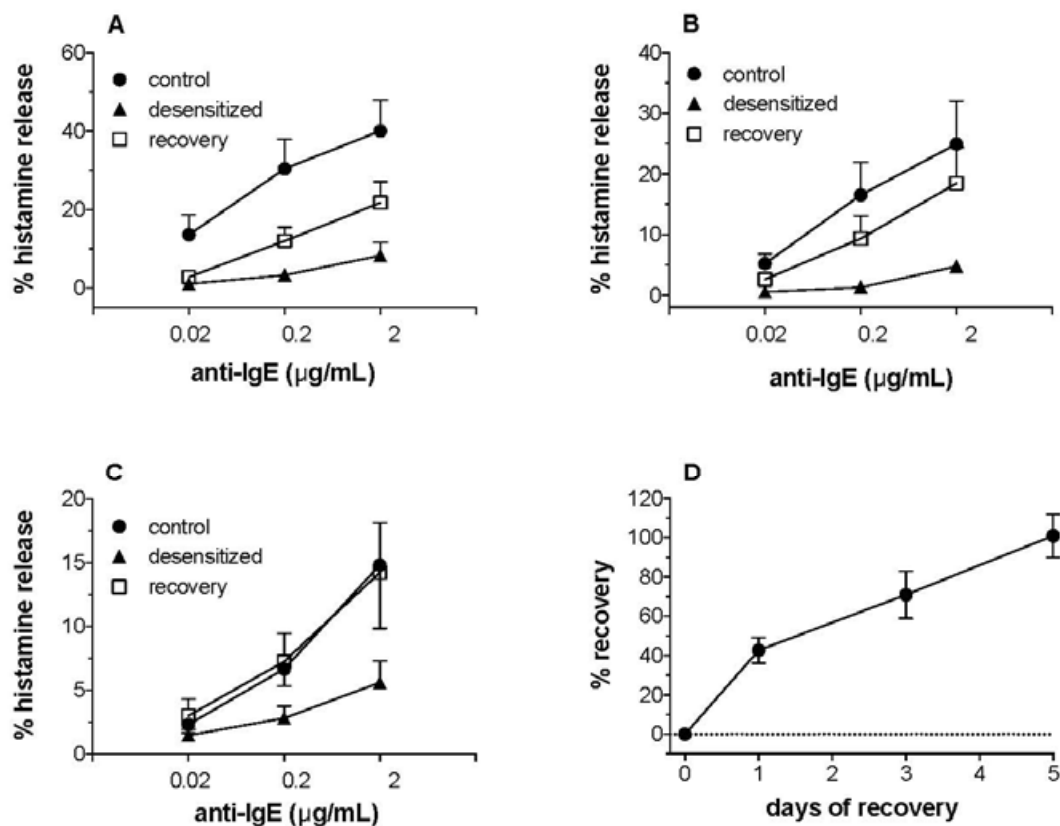


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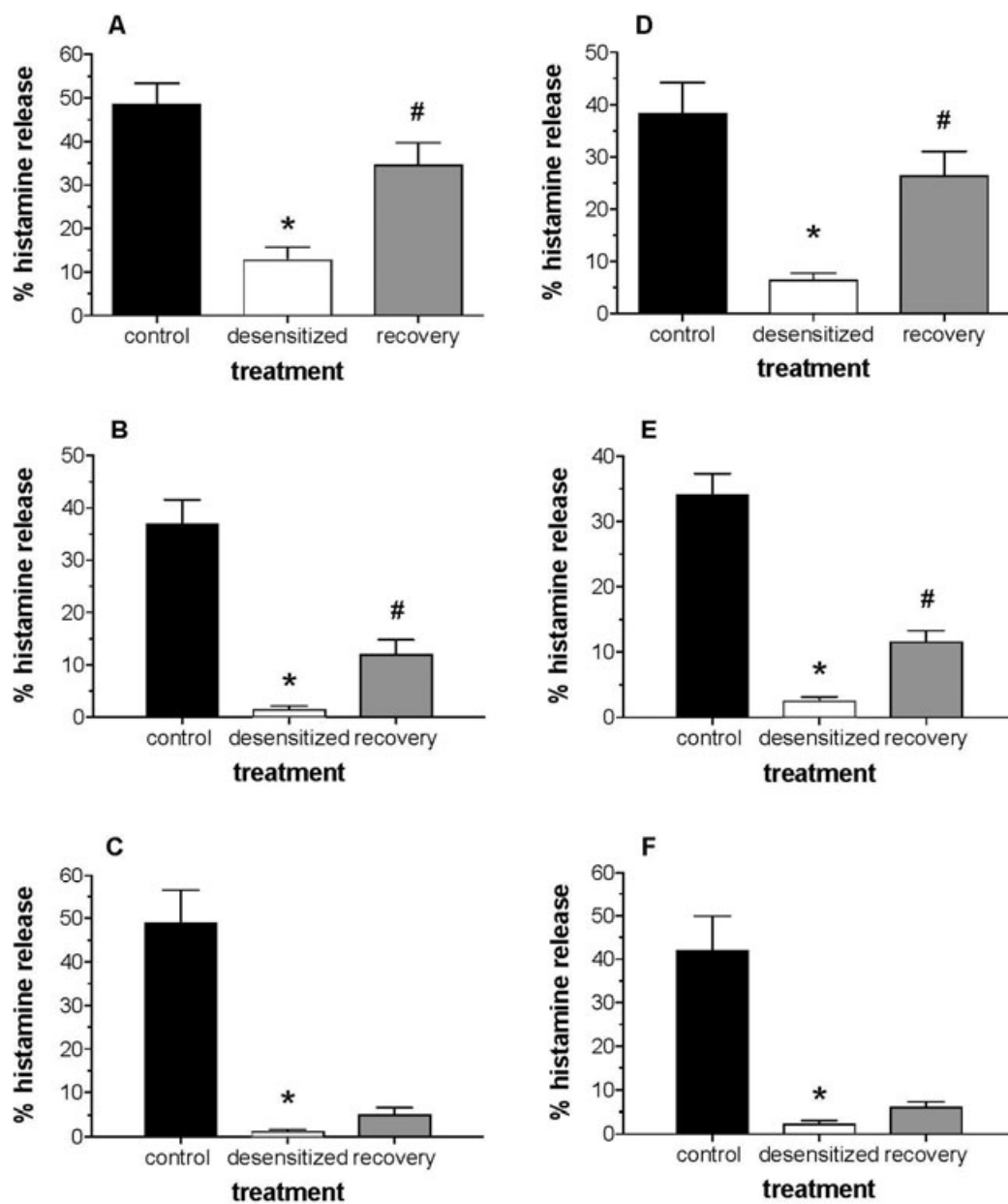


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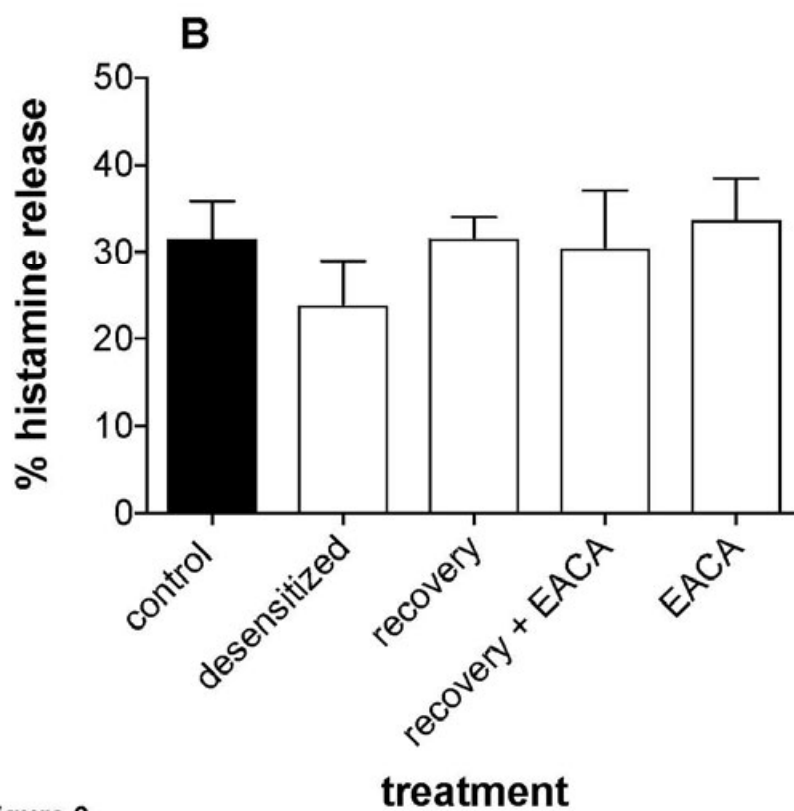
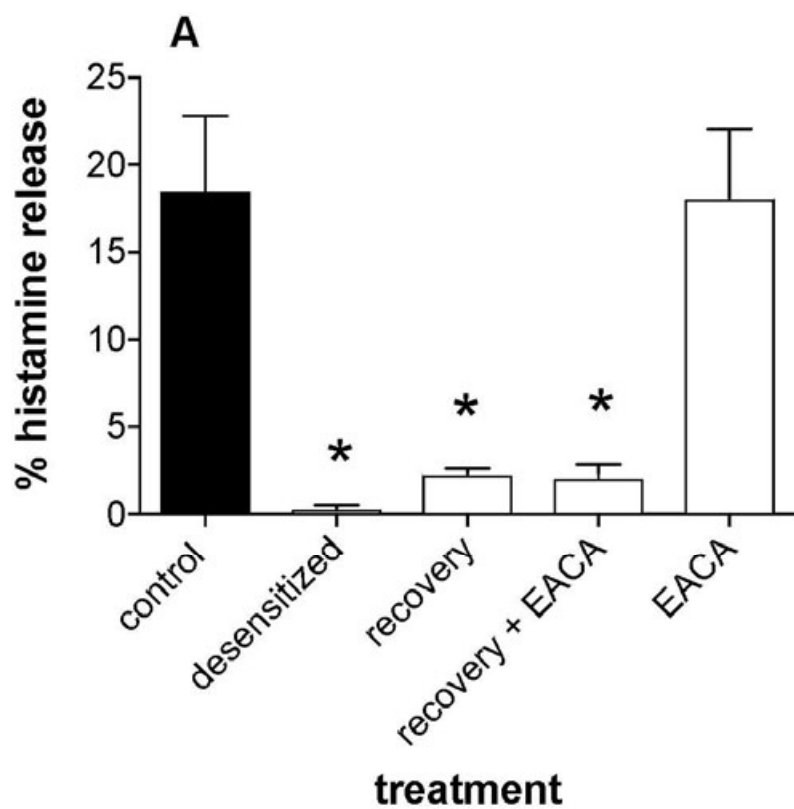


figure 6

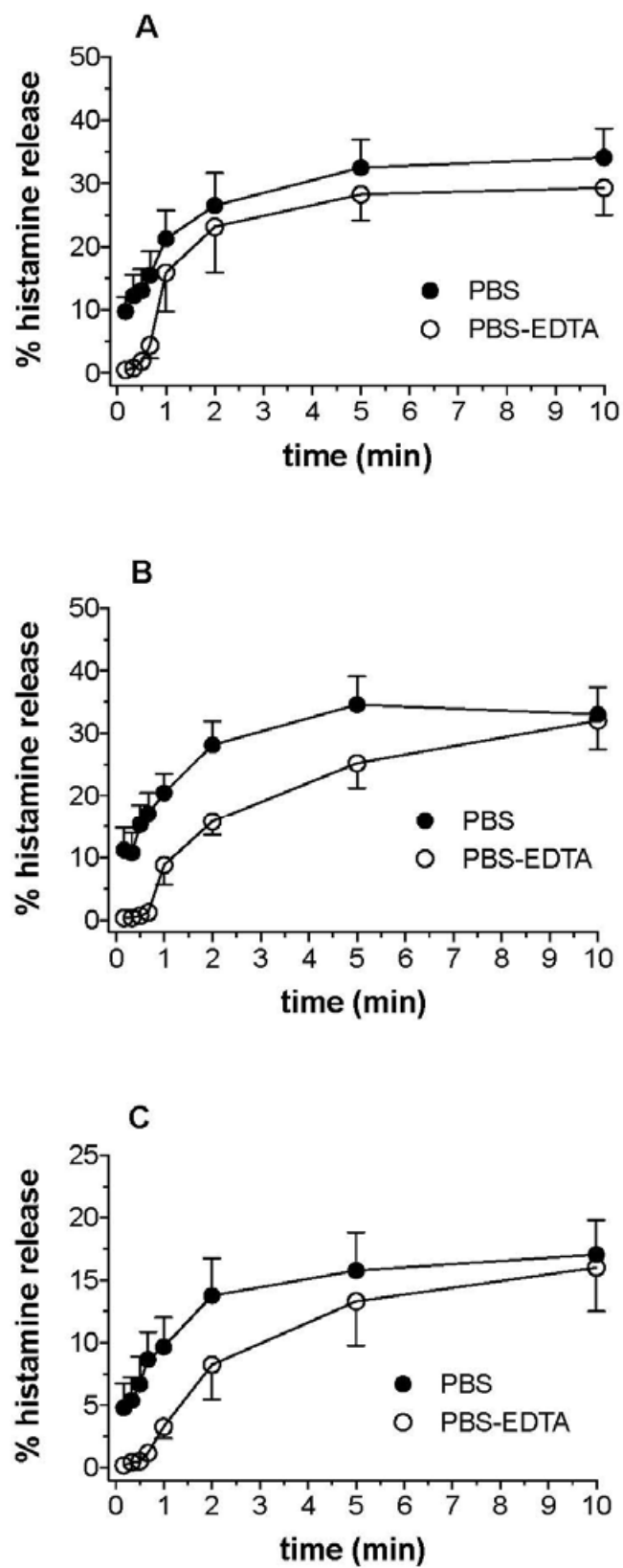


figure 7