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Inhibiting phosphatase SHIP-1 enhances suboptimal IgE-mediated activation of human blood basophils but inhibits IgE-mediated activation of cultured human mast cells

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ABSTRACT

IgE-mediated activation of basophil granulocytes and mast cells follows a bell-shaped dose-response curve. The decreased activation at supraoptimal allergen stimulation is thought to be associated with SH2-containing inositol-5′-phosphatase 1 (SHIP-1). SHIP-1 phosphorylation is inversely related to IgE-mediated releasability of basophils. This study sought to clarify the regulatory role of SHIP-1 in degranulation of basophil granulocytes and mast cells by selective inhibition of the phosphatase function of SHIP-1with 3-α-aminocholestane (3-α-AC).

Six grass pollen allergic patients, six non-responder patients and six cultured human primary mast cell lines were included. The effect of 3-α-AC (1–60 μM, 30 min, 37 °C) was analyzed at individual suboptimal, optimal and supra-optimal allergen concentrations. The activity, upregulation of CD63, measured at different conditions was compared to evaluate the maximal effect of selective SHIP-1 inhibition. Basophils of five non-responder patients were treated with 3-α-AC (10 μM, 30 min, 37 °C).

At high concentrations (> 60 μM) of 3-α-AC, cells appeared to enter apoptosis. The median reactivity increased from 27.1% to 44.9% $CD63^+$ basophils at 10 μM of 3-α-AC and suboptimal allergen stimulation ($p = 0.0153$). There was no effect on blood basophils of 3-α-AC at optimal or supra-optimal allergen concentrations. In contrast, treatment with more than 6 μM 3-α-AC significantly inhibited mast cell reactivity. 10 μM 3-α-AC reduced median reactivity from 32.85% to 16.5% CD63 + mast cells ($p = 0.0465$). Treatment with 3-α-AC did not increase response of basophils of non-responder patients.

Modulating blood basophils with 3-α-AC enhanced reactivity only at suboptimal allergen concentration, and basophils from non-responders did not regain responsiveness to IgE stimulation. 3-α-AC inhibited the IgE response of mast cells in a dose dependent manner.

1. Introduction

The degranulation process of basophil granulocytes and mast cells is, as any other cellular function, tightly controlled by several regulatory mechanisms. The bell-shaped dose-response curve of IgE-mediated activation emphasizes this. The bell-shaped curve could be explained by a local optimum of ratios of allergen and IgE, as known from titration of antigen against a constant amount of antibody [1]. Decreased activation at supraoptimal allergen stimulation, however, is now thought to be associated with SH2-containing inositol-5′-phosphatase 1 (SHIP-1) [2]. SHIP-1 dephosphorylates phosphatidylinositol-3,4,5-trisphosphate (PIP3), a secondary messenger involved in degranulation of basophil granulocytes and mast cells but may also have adapter functions.

This study sought to clarify the regulatory role of the phosphatase function of SHIP-1 in basophil granulocyte and mast cell degranulation by examining the functional consequences of selective inhibition of the phosphatase function of SHIP-1 with 3-α-aminocholestane (3-α-AC) [3]. Earlier studies have reported that SHIP-1 is highly phosphorylated at supra-optimal allergen stimulation [4] and that murine SHIP-1-knockout mast cells failed to express a dose-response curve with a descending part at high allergen concentrations [2]. Therefore, it was expected that a selective SHIP-1 inhibitor would increase the degranulation of both basophil granulocytes and mast cells, especially at

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supraoptimal allergen stimulation.

SHIP-1 is a 145 kD molecule with an N-terminal Src homology 2 (SH2) domain and a central phosphatase domain [5] and in the C-terminal part several PxxP sequences that may bind to SH3 domains and two NPXY sequences that, when phosphorylated, may bind SH2 or PTB domains as known from other adapter proteins [6]. As adapter proteins tend to not have enzymatic function and it is not known how SHIP-1 regulates IgE-mediated signal transduction [7], we investigated the significance of the phosphatase of SHIP-1 in this context.

It is suggested that SHIP-1 phosphorylation is inversely related to IgE-mediated releasability of basophils [4]. The inhibitor, 3-α-AC, was used to explore whether it was possible to rescue blood basophils of patients classified as non-responders in basophil activation testing (BAT), whose blood basophils are not activated through FcεRI even though their skin mast cells are reactive.

2. Materials and methods

2.1. Antibodies and reagents

3-α-aminocholestane was purchased from Echelon Biosciences Inc. (Salt Lake City, Utah). Grass pollen extract (Aquagen® Phleum pratense) was obtained from ALK-Abello (Hørsholm, Denmark) and natural Der p 2 allergen was obtained from Indoor Biotechnologies (Charlottesville, Virginia). Purified mouse anti-human IgE (clone G7-18) from BD Biosciences (San Jose, California) and anti-human FcεRIa (clone AER-37, CRA1) from Biolegend (San Diego, California) were positive controls. Basophils were identified as SS-low cells binding Alexa Fluor® 647 anti-human CD193 (clone 5E8, CCR3) from Biolegend and activation was assessed with FITC anti-human CD63 (clone H5C6).

Ficoll-Paque media solution was from Amersham Pharmacia Biotech (Uppsala, Sweden). AC133 cell isolation kit and LS^+ columns were purchased from Miltenyi Biotech (Bergish-Gladbach, Germany). Stem Span medium was from Stem Cell Technologies (Vancouver, Canada), IL-3, SCF and IL-6 were from R&D Systems (Abingdon, United Kingdom) and IL-4 was from PeproTech (London, England). Trypan blue 0.5% w/w was purchased from Ampliqon (Odense, Denmark). Mast cells were sensitized with 80 kU_A/L recombinant human IgE consisting of truncated IgE [8] and equimolar concentrations (7% each) of specific rIgE clones (H12, H10 and P4E [9]). BD FACS™ Lysing Solution was from BD Biosciences.

2.2. Inhibition of SHIP-1

The selective SHIP-1 inhibitor, 3-α-AC, was used according to the manufacturer's instruction. Whole blood containing basophils or cultured human mast cells were incubated with inhibitor at final concentrations of 0–60 μM for 30 min at 37 °C. The effect of SHIP-1 inhibition was analyzed by the relative increase in CD63 upregulation compared to cells treated with vehicle (PBS).

2.2.1. Grass pollen allergic patients

Grass allergic patients (n = 10) seen in our clinic gave informed consent. The project had been approved by the local ethics committee (Nr 1-10-72-104-15). Inclusion criteria were a positive skin prick test for grass, a history of seasonal rhino conjunctivitis and no ongoing immunotherapy. Included patients completed a standardized allergy questionnaire from the allergy clinic at Aarhus University Hospital (Table 1).

2.2.2. Non-responder individuals

Six non-responder individuals had expressed < 20% CD63+ basophil granulocytes to FcεRI crosslinking in a clinical diagnostic BAT [10] (median age 45 years, 4 women, one individual suspected of honey allergy and five individuals suspected of one or more drug allergies). They were retested in the present series with FcεRI crosslinking within one year of the date on which the clinical BAT was performed.

2.2.3. Basophil activation test (BAT)

Heparinized whole blood (4 ml) from ten grass allergic patients (Table 1) was drawn outside the grass pollen season and aliquots of 100 μl were incubated for 30 min at 37° with 10 μl of increasing allergen concentration ($1{,}6 \times 10^{-6}$ ng/ml to $1{,}6 \times 10^{2}$ ng/ml) and 10 μl of an antibody master-mix containing titrated volumes of FITC-conjugated anti-CD63 and Alexa Fluor 647-conjugated anti-CD193. A final concentration of 50 μg/ml anti-IgE antibody was used as positive control and PBS was used as negative control. After allergen stimulation, the blood was hemolysed with 2 ml of lysing solution for 15 min in dark at room temperature. Afterwards the samples were centrifuged (400*g*, 4 °C, 8 min) and the supernatant was removed. Pellet from all samples were analyzed on BD FACS CANTO IITM flow cytometer, where 180.000 events were recorded to ensure appropriate basophil counts. All data was analyzed in FlowJo, LLC, version 10.2 for Windows (Ashland, Oregon). Suboptimal, optimal and supraoptimal allergen concentrations were determined, and the effect of 3-a-AC was explored only at these allergen concentrations.

For six grass pollen allergic patients the suboptimal, optimal and supraoptimal allergen concentrations were determined. Aliquots of blood pretreated with concentrations of 3-α-AC ranging from 1 u M to 60 u M for 30 min were incubated with these allergen concentrations and processed as described above. Samples were also incubated with 10 u M 3-α-AC for 15 and 45 min, with similar results as for 30 min, The BAT used to retest the non-responder individuals described in Section 2.3.2 was identical with the positive control described above. Aliquots of blood of non-responders were treated with 1–30 u M 3-α-AC for 30 min before activation by FcεRI crosslinking.

2.2.4. Mast cell preparation

Functional human mast cells were derived from peripheral progenitors of anonymous blood bank donors as described [11]. Mononuclear cells from buffy coats were isolated on a Ficoll-Paque density gradient. A $CD133^+$ cell isolation kit and a LS^+ separation column were used according to manufacturer's instructions (Miltenyi Biotec, 130-097 − 049 and 130-042 − 401) to enrich hematopoietic stem cells (median cell recovery : 1×10^6 stem cells per buffy coat).

2.2.5. Mast cell culturing

Mast cells were prepared as described earlier [11]. Purified $CD133^+$ stem cells were resuspended in Stem Span medium containing IL-6 (50 ng/ml), stem cell factor (100 ng/ml) and penicillin/streptomycin (100 μg/ml). Stem cells were cultured for 8 weeks in a humidified CO_2 incubator (5%) at 37 °C to become mature functional mast cells [11]. The culture medium was supplemented with IL-3 (1 ng/ml) in weeks 0, 1 and 2 and from week 6 IL-4 (10 ng/ml) and IgE /see Section 2.6) were added to the medium.

Culture medium was renewed once a week by centrifugation (400 G, 4 °C, 5 min), removal of supernatant and resuspension in fresh medium. Cells were counted by Trypan blue staining and the cell-density was adjusted to 5×10^5 cells/ml. Mast cell lines were routinely quality ensured by metachromatic staining, IgE mediated histamine release and CD63 activity measurements (Table 2).

2.2.6. Mast cell sensitization and activation

Mast cells were sensitized against house dust mites during the last two weeks of culture (week 6 and 7) with 80 kU/L total IgE [12] consisting of 21% specific IgE clones (H12, H10 and P4E, 1:1:1) and 79% non-specific Fc-fragments of IgE antibodies [13]. After IgE sensitization, 90 μl of cell suspension was exposed to 10 μl of recombinant Der p 2 allergen (10 μg/ml to 10×10^{-7} μg/ml) for 30 min at 37 °C. 50 μg/ml of anti-IgE antibody was used as positive control and PBS was used as negative control. The cells were then incubated with 4 μl of a titrated volume of FITC-conjugated anti-CD63 for 20 min at 4 °C. 2 ml of

Table 1
Clinical characteristics of included patients. Symptom scores from 0 to 3 refer to symptom severity each month. 0 corresponds to no symptoms, 1 to mild symptoms, 2 to moderate symptoms and 3 corresponds to severe symptoms. The questionnaire was completed at inclusion and outside the grass pollen season.

Clinical characteristic	
Gender, female/male	5/5
Age in years, average (range)	32 (19–56)
Positive skin test for grass	10/10
Duration of allergy in years, average (range)	20,3 (6–41)
Asthma at inclusion	1/10
Asthma in childhood	2/10
Atopy in family	10/10
Other sensitizations, average (range)	3 (1–10)

Symptom score, average (range)

Jan	Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
0 (-)	0 (-)	0.3 (0–1)	1.2 (0–2)	2.3 (1–3)	2.7 (1–3)	2.6 (1–3)	2.4 (1–3)	1.2 (0–2)	0.1 (0–1)	0 (-)	0 (-)

Table 2
Mast cell line quality assurance. Metachromatic staining with alcian blue and staining for serine proteases, tryptase and chymase, evaluate cell maturation. CD63 upregulation is measured as the response to stimulation with 50 µg/ml anti-IgE antibodies for 30 min at 37 °C to represent the degranulation and thereby activation of the cultured mast cells. All measurements were performed in week 8 and all numbers are given in percent.

	Alcian blue	Tryptase	Chymase	Histamine Release	CD63 expression
Median [%]	94	99	7	30	57
Range [%]	85–100	95–100	2–18	16–46	49–73

PBS was added to every sample to wash off excess antibodies. Samples were centrifuged (400*g*, 4 °C, 10 min), the supernatant was removed, and the pellet was analyzed on BD FACS CANTO IITM flow cytometer. 10.000 events were recorded. All data was analyzed in FlowJo, LLC, version 10.2 for Windows (Ashland, Oregon).

2.3. Statistics

Nonparametric tests were used as data was not normally distributed according to D'Agostino-Pearson normality test (Graphpad Prism V7.0 (La Jolla, CA). Statistical significance between the effect mediated by 3-α-AC in basophils and mast cells was evaluated by unpaired Mann-Whitney tests. Friedman ANOVA tests were performed on mast cell data and basophil data to evaluate the significance of dose-dependent changes in IgE-mediated reactivity; $p < 0.05$ was considered significant.

3. Results

3.1. Toxicity of 3-α-AC

At concentrations above 60 µM, 3-α-AC induced mast cell death (Fig. 1) corresponding well with previous findings [3]. Most mast cells lost their functional size and granularity when treated with 100 µM of 3-α-AC (Fig. 1B, C).

3.2. Natural variation in allergic responses

As SHIP-1 activity depends on stimulation level [4,14], a dose-response curve of each included basophil and mast cell sample (Table 2) was analyzed to define suboptimal, optimal and supra-optimal stimulation levels (Fig. 2, Tables 3 and 4). Unlike the basophil response (Fig. 2A), the dose-response curve of mast cells did not show a supra-optimal response (Fig. 2B).

3.3. Titration of 3-α-AC in basophil granulocytes and mast cells

The measured CD63 upregulation on cells treated with 3-α-AC was compared with CD63 upregulation on cells treated with vehicle to assess the effect of 3-α-AC (Fig. 3A). 3-α-AC did not affect the activity of blood basophils at optimal or supraoptimal allergen concentration. We selected 10 u M 3-α-AC as the most likely concentration to have an effect on blood basophil granulocytes. At suboptimal allergen concentrations, 3-α-AC increased the fraction of activated basophils from 26.5% $CD63^+$ basophils with vehicle to 49.6% at 10 µM 3-α-AC (IQR: 34.7–68.2; $p = 0.0153$) at 10 µM of 3-α-AC (Fig. 4A).

At concentrations > 3 µM, 3-α-AC decreased the activity of mast cells at both suboptimal and optimal allergen stimulation (Fig. 3B). At suboptimal stimulation, the median activity decreased from 32.85% (IQR: 18.1–50.88) $CD63^+$ mast cells to 16.5% (IQR: 7.08–20.03) at 10 µM of 3- α-AC. At optimal allergen stimulation, the median activity decreased from 59.35% (IQR: 47.14–68.93) $CD63^+$ mast cells to 33.85% (IQR: 5.42–48.4) at 10 µM of 3-α-AC (Fig. 4B).

3.4. Different effects of 3-α-AC

The optimal concentration of 10 µM 3-α-AC upregulated basophils CD63 at suboptimal allergen concentration (Fig. 4A). The inhibitor decreased the activity of mast cells at both suboptimal and optimal allergen concentrations (Fig. 4B). The maximal reactivity decreased from 56.7% $CD63^+$ mast cells to 38.3% ($p = 0.0313$) and the sensitivity (EC50) decreased from 0.015 µg/ml Der p 2 for untreated cells to 0.031 µg/ml Der p 2 (ns) for mast cells treated with 10 µM of 3-α-AC. A comparison of the two titration curves at optimal allergen stimulation emphasized that 3-α-AC has effect on blood basophils at suboptimal allergen concentration, but seems to inhibit degranulation of cultured human mast cells (Fig. 5). At concentrations > 6 µM 3-α-AC, the mast cell response decreased significantly, suggesting differential regulation by SHIP-1 of degranulation in blood basophils and cultured human mast cells.

3.5. 3-α-AC does not offer relief for non-responder basophil granulocytes

Due to a low but reproducible effect of 3-α-AC (Fig. 3A) on suboptimal basophil activation, it was explored whether 3-α-AC could rescue blood basophils of patients classified as non-responders to respond to FcεRI crosslinking by IgE and allergen. One of six patients with earlier clinical non-responder results, had spontaneously reverted into a responder one year later (not shown). Blood basophils of the five remaining non-responder patients were treated with the inhibitor, 3-α-AC, but remained unresponsive to FcεRI stimulation (Fig. 6).

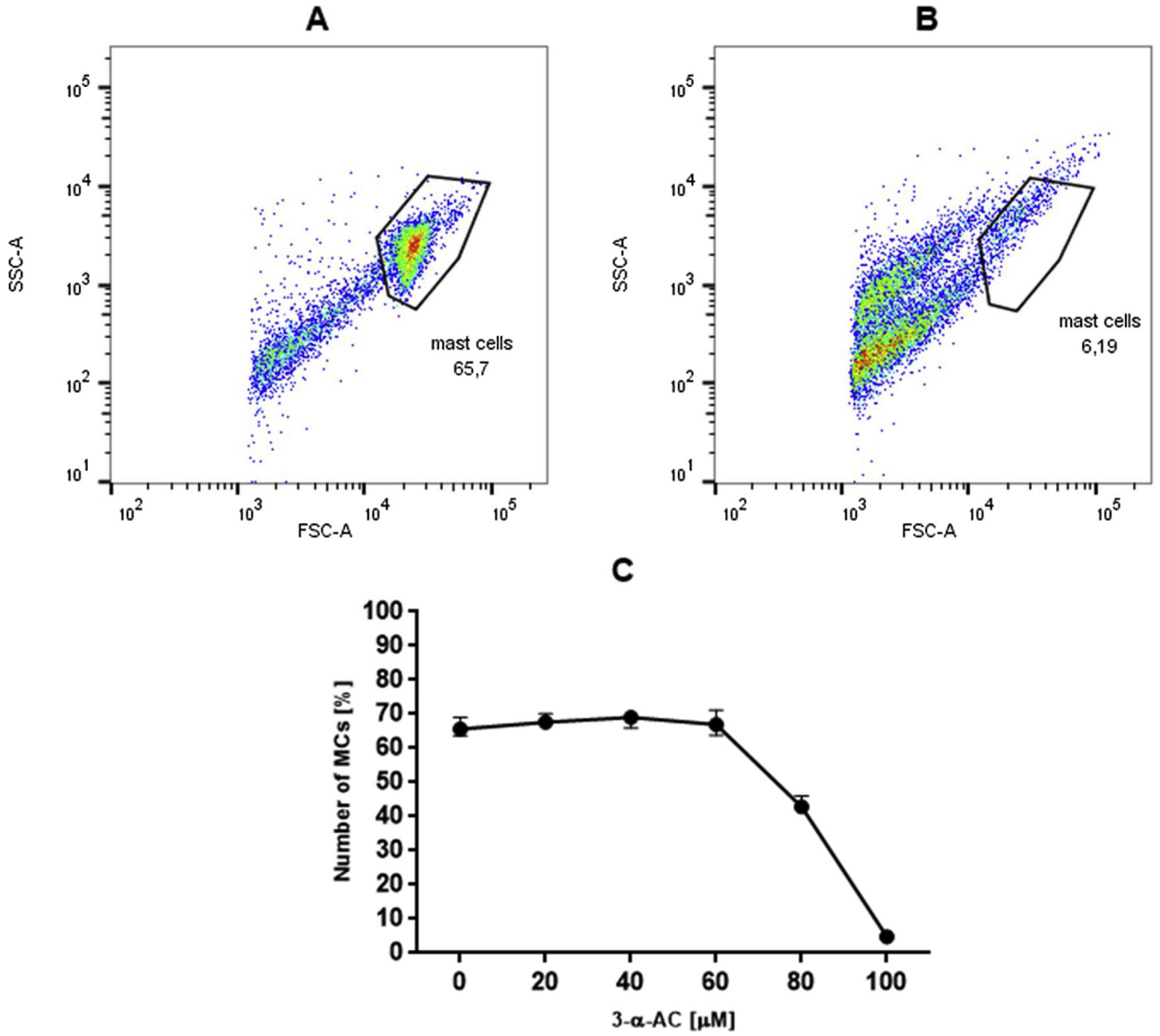

Fig. 1. Determination of the toxicity of 3-α-AC. **A**, Measured events for mast cells pre-incubated with vehicle. The marked region shows a typical mast cell population defined by size and granularity. In this example (cell line 3416 A), 65.7% of the total measured events is considered as normal functioning mast cells. B, Treatment with 100 μM 3-α-AC for 30 min depletes mast cells and leaves a tail of debris (6.19%). C, Determination of toxicity of increasing concentrations of 3-α-AC on cultured mast cells (n = 3, Median ± IQR) for 30 min at 37 °C. At concentrations beyond 60 μM, 3-α-AC appears to be toxic for mast cells.

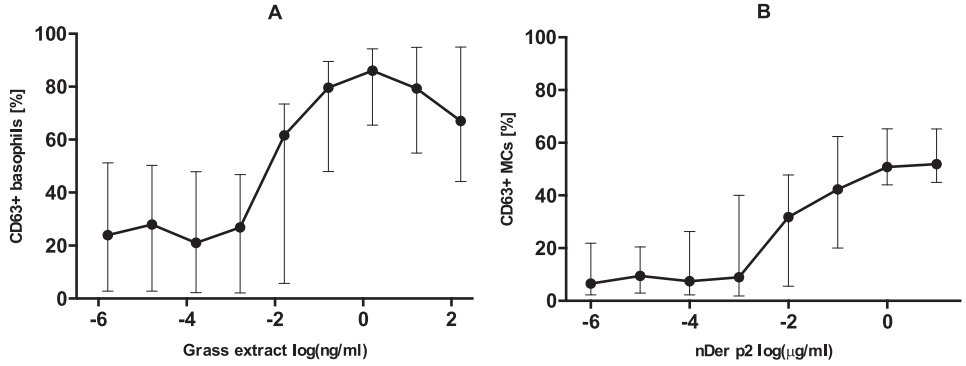

Fig. 2. Dose-response curves of untreated basophils and mast cells A) CD63 BAT (n = 10 grass allergic patients, ± IQR) with grass extract B) CD63 activation of primary human mast cells (n = 6, ± IQR) sensitized with IgE specific for Der p 2 and challenged with rDer p 2.

Table 3
Individual allergen concentrations used for titration of 3-α-AC in basophils. For three donors (D1, D3 and D4), the dose-response curve did not decrease remarkably at high concentrations of grass pollen extract.

Donor	Sub-optimal concentration [ng/ml]	Optimal concentration [ng/ml]	Supra-optimal concentration [ng/ml]
D1	1.6	16	*160*
D2	0.16	1.6	160
D3	0.016	1.6	*160*
D4	0.016	1.6	*160*
D5	0.0016	0.16	16
D6	*0.00016*	0.16	160

Table 4
Individual allergen concentrations used for titration of 3-α-AC in mast cells. No supraoptimal concentration of the mast cell lines could be determined as none showed a decreased response at the highest concentrations of allergen. Lines 4116 A, 0517 A, 0617 A and 0817 A had similar dose-response curves.

Cell line	Sub-optimal concentration [μg/ml]	Optimal concentration [μg/ml]
4116A	0.01	1
0217A	0.01	10
0316A	0.1	1
0516A	0.01	1
0616A	0.01	1
0816A	0.01	1

4. Discussion

The phosphatase activity of SHIP-1 is suppressed by 3-α-AC, a selective inhibitor developed in 2010 [3]. Since the development, the inhibitor has been used in mice strains to compare the effect of selective SHIP-1 inhibition with complete ablation of the enzyme. SHIP-1 knockout mice experienced several hematopoietic abnormalities and struggled with a massive myeloid cell infiltration of the lungs. In general, this uncontrolled growth of hematopoietic cells caused a remarkably reduced life span compared to mice expressing SHIP-1 [15]. Selective SHIP-1 inhibition by 3-α-AC increased the number of myeloid immunoregulatory cells and granulocytes without triggering the myeloid-associated lung infiltration observed in SHIP-deficient mice [3]. These findings support the fundamental role of SHIP-1 in production and growth of hematopoietic immune cells and that complete absence of the enzymatic aspect of SHIP-1 disturbs the balance of blood cells. This was in favor of chemical inhibition of SHIP-1, where the enzyme and its catalytic function is not completely removed from the cells.

in vitro studies of 3-α-AC have until now primarily focused on leukemia cells, where selective chemical SHIP-1 inhibition has been found to trigger apoptosis (12.5 μM) [3,16]. This is consistent with our finding that mast cells loose viability at 3-α-AC concentrations above 60 μM. As the cell biology of basophil granulocytes and mast cells may differ from that of cancer cells, the parameters of the study must be adjusted according to cell type and primary end point. The optimal parameters in the present study were found in preliminary experiments (data not shown) testing solvent, incubation time and optimal concentrations inspired by a study from 2013 of AQX-1125, a chemical activator of SHIP-1 [17]. Chemical SHIP-1 activation with AQX-1125 showed that it was possible to affect the regulatory role of SHIP-1 in the degranulation process in murine mast cells without affecting the survival of the cells negatively and the findings support the established theory of a stimulation dependent activity of SHIP-1. However, the decrease in absolute numbers was low (14% to 8%) compared with the degranulation in mast cells from which SHIP-1 had been deleted (60%). A study from 2014 evaluated the effect of AQX-1125 on airway responses to allergen stimulation in mild- to-moderate asthmatic patients and concluded that AQX-1125 had no effect on the early response after an allergen challenge [18].

The present experiments with blood basophils concur with the above; there was no negative effect on cell survival and a reproducible enhancement of degranulation at suboptimal allergen concentration at 10 μM of 3-α-AC, but no effect at optimal or supraoptimal concentrations of allergen. As with AQX-1125 [17,18], results obtained with basophil granulocytes indicate that selective chemical modulation of SHIP-1 only results in minor changes in actual degranulation. This is consistent with the notion that SHIP has several domains with related functions, and that the phosphatase function is not important for degranulation of blood basophils.

We found a different response to 3-α-AC in blood basophils and cultured mast cells. This may be due to the difference in IgE sensitization between blood basophils sensitized in vivo and mast cells sensitized in vitro. However, both responses are similar to our findings in other studies were the patients were more carefully characterised; in a different study, Grass pollen allergen Phl p 5 specific IgE contributed a median of 10.2% (IQR 8.7%–16.2%, max 21%) of total IgE of 24 patients [19]. In other published work (on which our selection of the 21% Der p 2 specific recombinant human IgE originally was based), this figure is similar (maximal 21%, Table 1, [20]. In basophil granulocytes, 3-α-AC enhanced IgE mediated activation at suboptimal allergen concentration, whereas 3-α-AC inhibited mast cell activation at 10 μM. These findings suggest that mast cells may have a different use of PIP3 than basophil granulocytes. Alternatively, the mast cells may not have been sufficiently differentiated as we did not attain supraoptimal activation with the mast cells used in this series of experiments. However, the mast cells used in this study were metachromatic, expressed tryptase and were activated through IgE and relevant allergen.

SHIP-1 down regulates PI3K signaling by converting PIP3 to PI-3,4-P2 and acts as an adapter protein that may bind SH2 and SH3 domains [21]. The literature describing AQX-1125 and 3-α-AC focuses on the catalytic function of the enzyme [3,17]. If only the catalytic function of SHIP-1 is inhibited by 3-α-AC binding, the protein could still mediate specific protein-protein interactions, amongst others with proteins harboring SH2 and SH3 domains. This means that SHIP-1 function in quenching the allergic response may be recruiting relevant proteins into the signalosome rather than the conversion of PIP3 to PI-3,4-P2, which

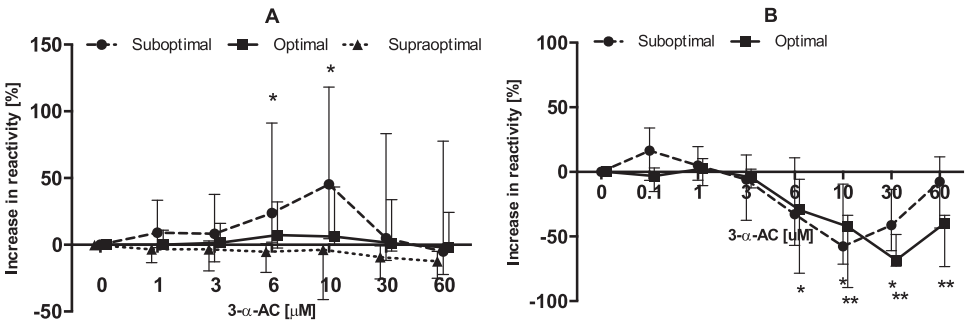

Fig. 3. The effect of SHIP-1 phosphatase inhibition by 3-α-AC.
Titrating 3-α-AC in a) basophils (n = 6, median ± IQR) at suboptimal, optimal and supraoptimal stimulation and b) mast cells (n = 6, median ± IQR) at suboptimal and optimal stimulation. The relative increase is calculated as $100*((R_{+3\text{-}\alpha\text{-}AC} - R_{control})/R_{control})$. $p < 0.05$ = *, $p < 0.005$ = **.

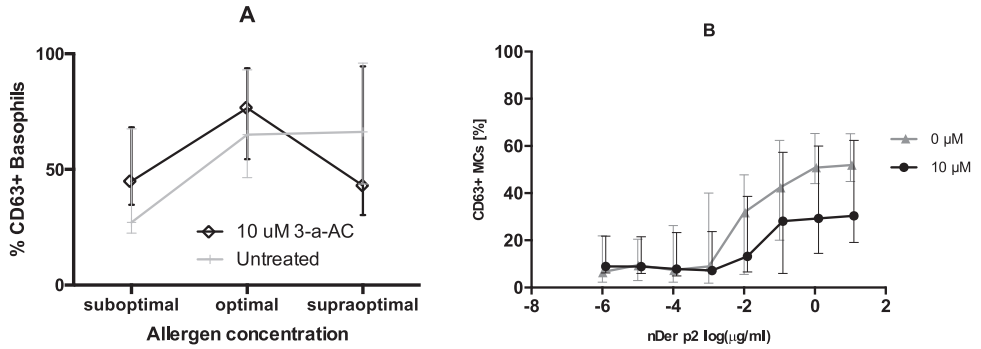

Fig. 4. Effect of 10 µM 3-α-AC (black) or vehicle (PBS, grey) on A) Basophils reactivity at suboptimal, optimal and supraoptimal allergen concentrations, ns (n = 6, median ± IQR) or B) Mast cells, p = 0,0313 for reactivity, ns for sensitivity (n = 6, median ± IQR).

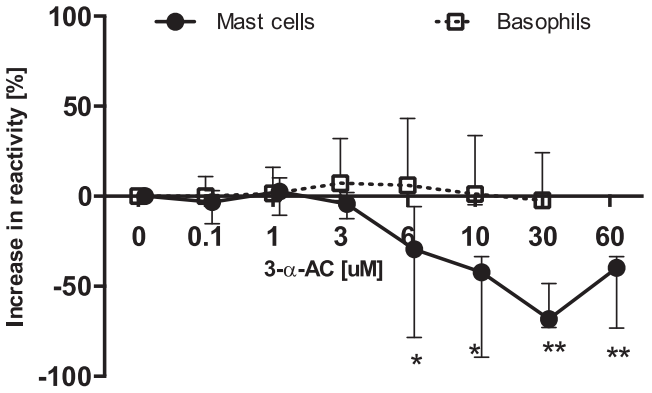

Fig. 5. Comparison of effect of 3-α-AC on mast cells (solid) and basophils (dotted) at optimal allergen concentrations. (n = 6, median ± IQR). Friedmanns test, p = 0.0016 for mast cells. p < 0.05 = *, p < 0.005 = **.

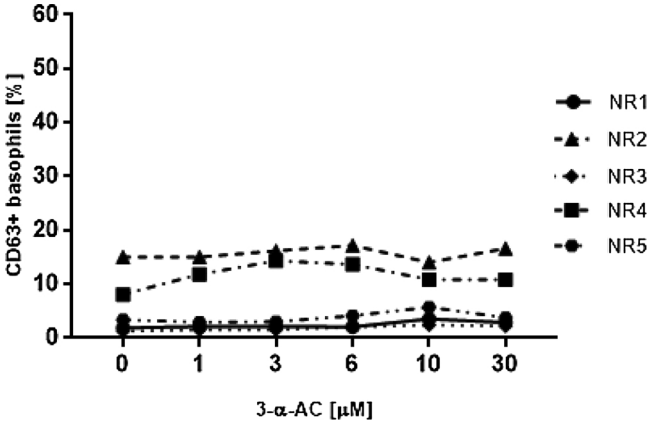

Fig. 6. 3-α-AC does not reinstate activation of basophils from non-responders. Basophils from five non-responders (< 20% CD63 in positive IgE control in a clinical BAT) incubated with increasing concentrations of 3-α-AC for 30 min at 37 °C before stimulation with anti-IgE (0.1 ng/ml). The curves show the absolute upregulation of CD63 based on their negative controls.

could explain why the effect of chemical modulation is not as expected. In a recent review, a possible mechanism for SHIP-1 as the central adapter protein has been proposed in the negative signalosome associated with IgE-mediated activation of mast cells [7]. SHIP-1 may associate with phosphorylated ITAM domain of FcεRI??. SHIP-1, in turn, recruits numerous other proteins like Lyn, Shc and p62dok into the signalosome. P62doc is an interaction partner of RasGAP, that inhibits p21RAS, and hence the MAPK ERK1/2. ERK1/2 may act through a feedforward loop to phosphorylate Syk [22] and phosphorylate the Calcium sensor STIM-1 [23]. These adapter functions gathered from several organisms and cell types summarized in [7] may be central in the pathway regulating degranulation of mast cells and basophil granulocytes through the IgE-allergen interaction. These regulatory mechanisms are superimposed on a possible stoichiometric optimal effect of interaction of multiple epitopes of each allergen molecule and multiple clones of IgE binding these epitopes, presented by FcεRI [1].

As there was a possible low effect of 3-α-AC on basophil activation at suboptimal allergen concentration, we explored whether it was possible to rescue blood basophils of patients classified as non-responders in diagnosing basophil activation tests. We found, surprisingly, that one non-responder reverted spontaneously into responder confirming that the non-responder phenotype may not be stable [24]. Treatment with 3-α-AC could not rescue non-responders suggesting that other mechanisms may be responsible for non-responsiveness of basophil granulocytes of these patients.

5. Conclusion

In contrast to other adaptor proteins, SHIP-1 also has phosphatase function, and it was hitherto not known how SHIP-1 controls signal transduction through IgE and FcεRI [7]. We could not assign a central role to the lipid phosphatase aspect of SHIP-1 in blood basophil or mast cell activation by inhibiting the enzyme with 3-α-AC, and 3-α-AC could not rescue basophil activation of non-responders.

Declaration of interest

None.

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References

[1] M. Heidelberger, F.E. Kendall, A quantitative theory of the Precipitin reaction: III. The reaction between crystalline egg albumin and its homologous antibody, J. Exp. Med. 62 (1935) 697–720.
[2] K. Gimborn, E. Lessmann, S. Kuppig, G. Krystal, M. Huber, SHIP down-regulates FcepsilonR1-induced degranulation at supraoptimal IgE or antigen levels, J. Immunol. 174 (1950 2005) 507–516.
[3] R. Brooks, G.M. Fuhler, S. Iyer, M.J. Smith, M.-Y. Park, K.H.T. Paraiso, et al., SHIP1 inhibition increases immunoregulatory capacity and triggers apoptosis of hematopoietic cancer cells, J. Immunol. 2010 (184) (1950) 3582–3589.
[4] B.F. Gibbs, A. Räthling, D. Zillikens, M. Huber, H. Haas, Initial signal strength controls basophil FcεRI-mediated signaling which is down-regulated by SH2 domain-containing inositol 5-phosphatase, J. Allergy Clin. Immunol. 118 (2006) 1060–1067.
[5] A. Ming-Lum, S. Shojania, E. So, E. McCarrell, E. Shaw, D. Vu, et al., A pleckstrin homology-related domain in SHIP1 mediates membrane localization during Fcγ receptor-induced phagocytosis, FASEB J. 26 (2012) 3163–3177.
[6] L.R. Rohrschneider, J.F. Fuller, I. Wolf, Y. Liu, D.M. Lucas, Structure, function, and biology of SHIP proteins, Genes Dev. 14 (2000) 505–520.

[7] M. Huber, Activation/Inhibition of mast cells by supra-optimal antigen concentrations, Cell Commun. Signal 11 (2013) 7.
[8] R.K. Jensen, M. Plum, L. Tjerrild, T. Jakob, E. Spillner, G.R. Andersen, Structure of the omalizumab fab, Acta Crystallogr. Sect. F Struct. Biol. Commun. 71 (2015) 419–426.
[9] L.H. Christensen, J. Holm, G. Lund, E. Riise, K. Lund, Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge, J. Allergy Clin. Immunol. 122 (2008) 298–304.
[10] H.J. Hoffmann, E.F. Knol, M. Ferrer, L. Mayorga, V. Sabato, A.F. Santos, et al., Pros and cons of clinical basophil testing (BAT), Curr. Allergy Asthma Rep. 16 (2016), https://doi.org/10.1007/s11882-016-0633-6.
[11] M. Holm, H.B. Andersen, T.E. Hetland, C. Dahl, H.J. Hoffmann, S. Junker, et al., Seven week culture of functional human mast cells from buffy coat preparations, J. Immunol. Methods 336 (2008) 213–221.
[12] P.M. Frandsen, I.J.M.K. Krohn, H.J. Hoffmann, P.O. Schiøtz, The influence of IgE on cultured human mast cells, Allergy Asthma Immunol. Res. 5 (2013) 409–414.
[13] C. Hjort, P.O. Schiøtz, M. Ohlin, P.A. Würtzen, L.H. Christensen, H.J. Hoffmann, The number and affinity of productive IgE pairs determine allergen activation of mast cells, J. Allergy Clin. Immunol. 140 (2017) 1167–1170 e2.
[14] M. Huber, C.D. Helgason, J.E. Damen, L. Liu, R.K. Humphries, G. Krystal, The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 11330–11335.
[15] C.D. Helgason, J.E. Damen, P. Rosten, R. Grewal, P. Sorensen, S.M. Chappel, et al., Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span, Genes Dev. 12 (1998) 1610–1620.
[16] G.M. Fuhler, R. Brooks, B. Toms, S. Iyer, E.A. Gengo, M.-Y. Park, et al., Therapeutic potential of SH2 domain-containing inositol-5'-phosphatase 1 (SHIP1) and SHIP2 inhibition in cancer, Mol. Med. Camb. Mass 18 (2012) 65–75.
[17] G.R. Stenton, L.F. Mackenzie, P. Tam, J.L. Cross, C. Harwig, J. Raymond, et al., Characterization of AQX-1125, a small-molecule SHIP1 activator: part 1. Effects on inflammatory cell activation and chemotaxis in vitro and pharmacokinetic characterization in vivo, Br. J. Pharmacol. 168 (2013) 1506–1518.
[18] B.R. Leaker, P.J. Barnes, B.J. O'Connor, F.Y. Ali, P. Tam, J. Neville, et al., The effects of the novel SHIP1 activator AQX-1125 on allergen-induced responses in mild-to-moderate asthma, Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol. 44 (2014) 1146–1153.
[19] J.M. Schmid, P.A. Würtzen, R. Dahl, H.J. Hoffmann, Pretreatment IgE sensitization patterns determine the molecular profile of the IgG4 response during updosing of subcutaneous immunotherapy with timothy grass pollen extract, J. Allergy Clin. Immunol. 137 (2016) 562–570.
[20] S.G.O. Johansson, A. Nopp, H. Oman, J. Ankerst, L.O. Cardell, R. Grönneberg, et al., The size of the disease relevant IgE antibody fraction in relation to 'total-IgE' predicts the efficacy of anti-IgE (Xolair) treatment, Allergy 64 (2009) 1472–1477.
[21] K. Roget, M. Malissen, O. Malbec, B. Malissen, M. Daëron, Non-T cell activation linker promotes mast cell survival by dampening the recruitment of SHIP1 by linker for activation of T cells, J. Immunol. 2008 (180) (1950) 3689–3698.
[22] R. Xu, R. Seger, I. Pecht, Cutting edge: extracellular signal-regulated kinase activates syk: a new potential feedback regulation of fcε receptor signaling, J. Immunol. 163 (1999) 1110–1114.
[23] E. Pozo-Guisado, D.G. Campbell, M. Deak, A. Alvarez-Barrientos, N.A. Morrice, I.S. Alvarez, et al., Phosphorylation of STIM1 at ERK1/2 target sites modulates store-operated calcium entry, J. Cell. Sci. 123 (2010) 3084–3093.
[24] K.J. Puan, A.K. Andiappan, B. Lee, D. Kumar, T.S. Lai, G. Yeo, et al., Systematic characterization of basophil anergy, Allergy 72 (2017) 373–384.