

Histamine-releasing factor/translationally controlled tumor protein (HRF/TCTP)-induced histamine release is enhanced with SHIP-1 knockdown in cultured human mast cell and basophil models

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Abstract: Previously, we demonstrated a negative correlation between histamine release to histamine-releasing factor/translationally controlled tumor protein (HRF/TCTP) and protein levels of SHIP-1 in human basophils. The present study was conducted to investigate whether suppressing SHIP-1 using small interfering (si)RNA technology would alter the releasability of culture-derived mast cells and basophils, as determined by HRF/TCTP histamine release. Frozen CD34+ cells were obtained from the Fred Hutchinson Cancer Research Center (Seattle, WA, USA). Cells were grown in StemPro-34 medium containing cytokines: mast cells with IL-6 and stem cell factor (100 ng/ml each) for 6–8 weeks and basophils with IL-3 (6.7 ng/ml) for 2–3 weeks. siRNA transfections were performed during Week 6 for mast cells and Week 2 for basophils with siRNA for SHIP-1 or a negative control siRNA. Changes in SHIP-1 expression were determined by Western blot. The functional knockdown was measured by HRF/TCTP-induced histamine release. siRNA knockdown of SHIP-1 in mast cells ranged from 31% to 82%, mean $65 \pm 12\%$, compared with control ($n=4$). Histamine release to HRF/TCTP was increased only slightly in two experiments. SHIP-1 knockdown in basophils ranged from 34% to 69%, mean $51.8 \pm 7\%$ ($n=4$). Histamine release to HRF/TCTP in these basophils was dependent on the amount of SHIP knockdown. Mast cells and basophils derived from CD34+ precursor cells represent suitable models for transfection studies. Reducing SHIP-1 protein in cultured mast cells and in cultured basophils increases releasability of the cells. *J. Leukoc. Biol.* 84: 000–000; 2008.

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tumor-associated protein with no known function. In unrelated studies, we had identified a histamine-releasing activity that was found in late-phase fluids from nasal lavages, bronchoalveolar lavage fluids, and skin blister fluids that directly induced histamine release from basophils isolated from a subpopulation of allergic donors termed histamine-releasing factor (HRF) responders (HRF-R) [3]. After purification and cloning, this protein, now referred to as HRF, was found to be identical to TCTP and was also known as p23 [4].

Our group has focused on the extracellular functions of HRF/TCTP. It was originally described as a complete secretagogue for histamine and IL-4 secretion from basophils of allergic donor responders [5]. We have also shown that HRF/TCTP activates human eosinophils and inhibits T cells [6, 7]. During studies investigating the biology of HRF/TCTP, a hyper-releasable phenotype was identified using basophils from HRF/TCTP-R donors. The hyper-releasable basophils from these donors are also responsive to IL-3 and D₂O [8, 9]. Basophils showing hyper-releasability to HRF/TCTP were found to have lower levels of SHIP-1 compared with nonresponder basophils. There was a negative correlation between the levels of SHIP-1 protein in basophils and the histamine released by these cells when challenged with HRF/TCTP ($n=11$) [10]. These studies suggest that SHIP-1 may modulate releasability in human basophils.

The concept of releasability is not new in the field of stimulation of human basophils. In 1976, this term was first used by Lichtenstein and Conroy [11] to describe an event that applied to the in vitro study of release of chemical mediators from human basophils. It is accepted that the term releasability is the control of release of mediators from basophils in response to different stimuli and involves several biochemical events in addition to the surface density of IgE molecules. There have been reports of certain signaling molecule deficiencies in non-releasing basophils [12, 13] and publications that establish the importance of signaling events in basophil secretion [14, 15].

INTRODUCTION

Translationally controlled tumor protein (TCTP) was originally identified in the 1980s by Brawerman's groups [1, 2] as a

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However, to date, we are the first group to show the negative association of the phosphatase SHIP-1 with histamine release to HRF/TCTP in hyper-releasing basophils [10].

Other groups have also demonstrated the importance of the phosphatase SHIP-1 in human basophil secretion. Gibbs et al. [16] showed that SHIP-1 was highly phosphorylated when cells were stimulated with supraoptimal concentrations of anti-IgE. This study demonstrated an inverse relationship between SHIP-1 and IgE-mediated releasability. MacGlashan [17] has demonstrated that levels of spleen tyrosine kinase and to a lesser degree, SHIP-1 determine the variance in a population to maximum responsiveness to IgE-mediated activation of human basophils. The above studies coupled with our own published data all support a critical role for SHIP-1 in signal transduction events in these cells.

More recently, we have identified signal transduction events in human basophils that are associated with HRF stimulation [18]. In particular, we have demonstrated recently that basophils of HRF/TCTP donors had a sustained phosphorylation of ERK in response to anti-IgE [18]. We, therefore, undertook a study to determine if mast cells grown from CD34+ precursor cells retained the same hyper-releasable phenotype as that seen in basophils isolated from these HRF/TCTP-R donors [19]. Cultures of CD34+ cells from four HRF/TCTP-R and four HRF/TCTP-nonresponders (HRF/TCTP-NR) showed similar numbers of mast cells after 6 weeks of culture and had a similar amount of histamine content with a range of 0.6–2.4 ng/1000 mast cells. Additionally, IgE receptor expression on these cells and histamine release responses to multiple doses of anti-IgE antibody did not differ between the two groups. Contrary to the basophil response, the mast cells from HRF/TCTP-R did not demonstrate histamine release when stimulated with HRF/TCTP or to D₂O, a reagent long known to amplify releasability in basophils. Unlike the basophils from these subjects, mast cell levels of SHIP-1 were similar between the groups as were the kinetics of ERK phosphorylation, which peaked at 5 min for both groups. We concluded that cultured mast cells did not maintain the hyper-releasable phenotype that we see in basophils from HRF/TCTP-R. We hypothesized that this discrepancy between basophils and culture-derived mast cells might have resulted from the cytokine-rich milieu necessary to obtain the latter cell type, in that it potentially masked any inhibitory effects mediated by SHIP-1 [19].

To address this issue more directly, we considered altering SHIP-1 levels in basophils and mast cells using transfection. However, transfection of human basophils and mast cells has proved extremely difficult, likely as a result of the fact that these cells are at an end-stage in their development. To circumvent this technical difficulty, we used culture-derived cells, and although many groups have described this for mast cells [20–24], only a few investigators report the successful culture of basophils from precursor cells [25–29]. In this study, we show that sufficient numbers of basophils can be expanded from CD34+ cells that are suitable for transfection and signal transduction studies. As a result, we demonstrate a relationship between decreases in SHIP-1 protein levels and histamine release to HRF/TCTP.

MATERIALS AND METHODS

Buffers

PIPES buffer consisted of 25 mM PIPES, 110 mM sodium chloride, 5 mM KCl, PIPES-albumin glucose (PAG) as PIPES, 0.1% glucose, and 0.003% human serum albumin. For histamine release assays, PAG was supplemented with 5 mM calcium chloride. Lysis buffer (1×) was 20 mM Tris, pH 7.5, containing 1% Nonidet P-40, 5 mM EDTA, 1 mM Na₃PO₄, 5 mM Na₄PTO₇, 5 mM DDT, 5 mM NaF, and 1× protease inhibitor cocktail. Buffered 4% paraformaldehyde was made with 0.1 M Na₂HPO₄·7H₂O and 0.1 M NaH₂PO₄·H₂O, pH 7.4. Electrophoresis sample buffer was Invitrogen buffer (Invitrogen, Carlsbad, CA, USA) containing 5% 2-β-ME. Stripping buffer (1×) consisted of 2.3 M SDS, 2.5 M Trizma-base, pH 6.7, with 0.1 M β-ME.

Cell culture preparations

Human mast cells and basophils were cultured from purified CD34+ stem cells obtained from the Fred Hutchinson Cancer Research Center (Seattle, WA, USA). To derive mast cells, these precursor cells were cultured in StemPro-34 serum-free substitution complete medium (Invitrogen), supplemented with stem cell factor (SCF) and IL-6 (Invitrogen), both at 100 ng/ml [30]. Cells were harvested between 6 and 8 weeks. For human basophils, these CD34+ stem cells were also cultured in StemPro-34 medium but with IL-3 (Invitrogen/Biosource) at 6.7 ng/ml and harvested between 2 and 3 weeks. Mast cells were identified with Wright Giemsa stain, and basophils were stained with Alcian blue.

Small interfering (si)RNA transfection

The siRNA transfection was performed using Lipofectamine 2000 (Invitrogen) and two siRNA pools: one, a SHIP-1-targeted SMART pool and the other, a negative control pool from Dharmacon (Chicago, IL, USA). The mast cell transfections were performed at Week 6, whereas basophils were transfected at Week 2. The transfection procedure was repeated 48 h after the initial transfection based on a published method of Tkaczky et al. [31], and the cells were harvested 24 h later to assay protein levels and changes in function. Rhodamine-tagged siRNA (Qiagen, Valencia, CA, USA) was used to check transfection efficiency.

Basophil purification

For flow cytometry studies using primary basophils (see Fig. 2), the cells were purified from peripheral blood drawn from volunteers after informed consent was obtained in accordance with the Declaration of Helsinki. All blood-drawing was conducted under protocols approved by the Western Institutional Review Board (Seattle, WA, USA) or by Johns Hopkins University Institutional Review Board (Baltimore, MD, USA). Basophil-enriched cell suspensions were prepared on double Percoll gradients as described previously and then purified further by using negative selection [32, 33]. Primary basophil purity was between 95% and 99.9%, as determined by Alcian blue staining.

The histamine release (see Fig. 3A) in primary basophils was performed using enriched cell suspensions prepared on double Percoll gradients [32].

The representative, passive sensitization experiment (see Fig. 3B) was performed with primary dextran-sedimented basophils isolated as described previously [4].

Mediator release

Cultured mast cells and basophils were assayed for function after passively sensitizing with serum containing IgE (final concentration, 500 ng/ml) for 18–24 h or 48 h, respectively. After sensitization, the cells were stimulated with buffer, HRF/TCTP, anti-IgE, or anti-FcεRIα at the concentrations indicated for 45 min for histamine release or 4 h for IL-4. The supernatants were collected, and histamine release was measured by automated fluorimetry [34]. IL-4 was measured by ELISA (eBioscience, San Diego, CA, USA).

SHIP-1 quantitation

Knockdown was determined by comparing SHIP-1 protein levels of control and SHIP-1-specific, siRNA-treated cells using Western blots, as described previously in detail [10]. Briefly, cell pellets were washed with 1× PIPES,

resuspended in the presence of protease inhibitors, lysed in boiling SDS sample buffer with β -ME, and subjected to electrophoresis with protein transfer to nitrocellulose. Blots were probed with anti-SHIP-1 antibody and then stripped and reprobed with anti-PI-3K (anti-p85; Santa Cruz Biotechnology, Santa Cruz, CA, USA) as a control for protein loading. Additionally, the EZ-Q protein assay (Invitrogen) was used to insure equal protein loading in each experimental sample. This method of equal loading and comparing levels of intracellular signaling molecules is the standard method when using human basophils [10, 18]. Linearity of staining on Western blots was confirmed by loading increasing amounts of a standard T cell lysate and scanning to confirm relative increases of specific band intensity.

Flow cytometry

Basophils were fixed in 300 μ l-buffered 4% paraformaldehyde at room temperature for 5 min. Cells were flooded with 1 ml-filtered ice-cold PBS, 0.1% BSA, and pelleted at 400 g for 5 min at 4°C. The supernatant was removed, and the cells were resuspended in 250 μ l 10% DMSO in PBS. Cells were frozen at -80°C until further use. For the flow analysis, the cells were thawed and washed twice with PBS and then blocked (60 min, 0°) in the presence of 1 mg/ml human IgG to minimize nonspecific binding to Fc γ R as described previously [35]. Cells were divided into 100,000 cells per condition. Each condition was then incubated with anti-CD117 (c-kit; Immunotech, Marseille, France), anti-Fc ϵ R1 α /PE (eBioscience), or anti-CD123 (IL-3R) PE-Cy5 (BD PharMingen, San Diego, CA, USA) for 30 min on ice, followed by three washes in PBS. Samples were resuspended in PBS, 0.1% BSA, and analyzed using a FACSCalibur flow cytometer. At least 5000 events per experimental condition were analyzed. Data were expressed as mean fluorescence intensity (MFI) after subtracting values obtained using isotype control antibodies.

Recombinant HRF/TCTP production

HRF/TCTP preparations used in these studies include several GST-HRF/TCTP fusion protein productions or His-HRF/TCTP. GST-HRF/TCTP was produced in the baculovirus system and purified as described previously [18], and the His fusion protein was produced in *Escherichia coli*. Purified HRF/TCTP was judged to be greater than 95% by Coomassie blue staining of a SDS-PAGE gel. The protein concentration was determined by the bicinchoninic acid protein assay (Pierce, Rockford, IL, USA) and calculated based on the molecular mass of the HRF/TCTP (23 kD) as a portion of the total fusion protein.

Statistical analysis

Data are expressed as mean $\pm$ SE. Statistical significance was determined using the Student's *t*-test.

The histamine release concentration-dependence curves for primary (peripheral) blood and cultured basophils (see Fig. 3A) were fit to Gaussian curves, and the data for mast cells were fit to a sigmoid curve. The parameters derived from these fits and their error estimates were used to test for similarity among the curves.

RESULTS

SHIP-1 knockdown in human culture-derived mast cells

We first investigated the effect of SHIP-1 knockdown in culture-derived mast cells. A 92% transfection efficiency was achieved when using rhodamine siRNA in an initial experiment. Cells viewed under fluorescent light showed a characteristic punctate staining when compared with cells visualized under white light (data not shown).

Figure 1 shows that SHIP-1 siRNA experiments yielded an average of $65 \pm 12\%$ knockdown as determined by densitometric analysis of four experiments comparing SHIP-1 protein levels in the SHIP-1 siRNA knockdown cells versus the negative control siRNA pool ($P=0.014$). A representative Western

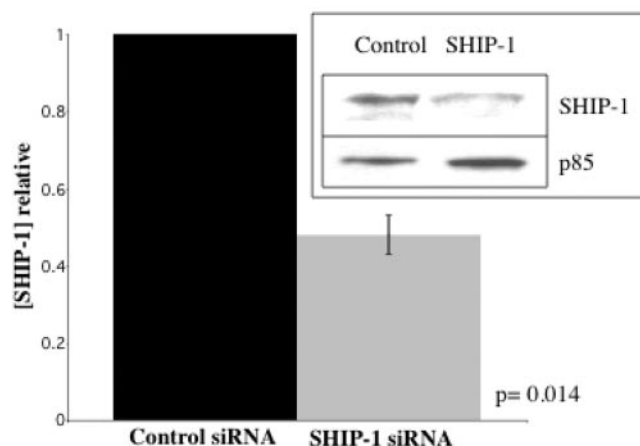


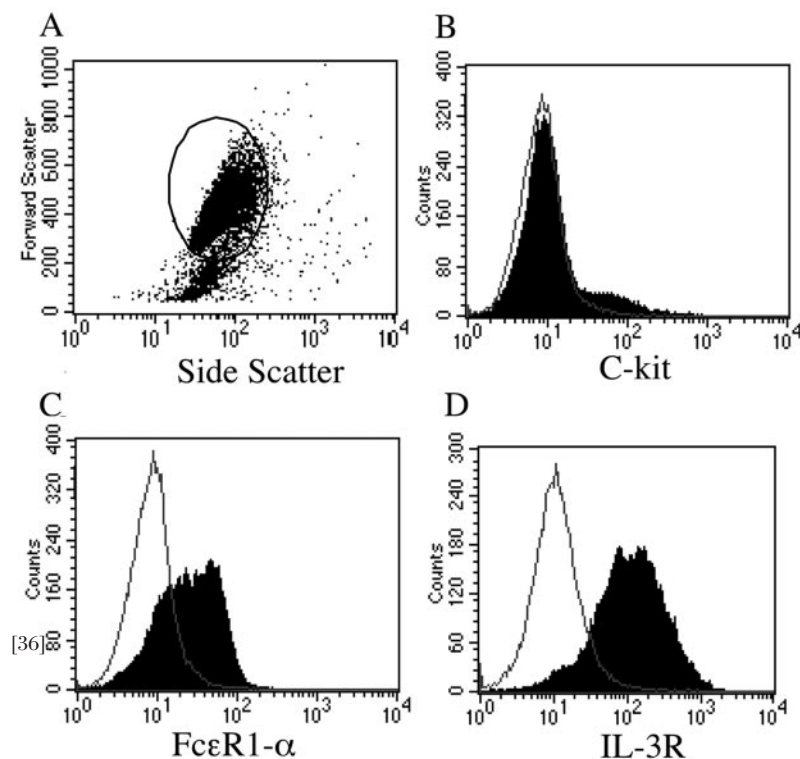
Fig. 1. SHIP-1 knockdown in human culture-derived mast cells. SHIP-1 siRNA experiments yielded an average of $65 \pm 12\%$ knockdown as determined by densitometric analysis comparing SHIP-1 protein levels in the SHIP-1 siRNA knockdown cells versus the negative control siRNA pool-treated cells ($n=4$). The inset depicts a representative Western blot of one of these knockdown experiments and the corresponding reprobe for expression of the p85 subunit of PI-3K.

blot of one of these knockdown experiments and the corresponding reprobe for expression of the p85 subunit of PI-3K (i.e., control for protein loading) are also shown. There are several ways to evaluate the effects of siRNA silencing. The three most common are evaluation of protein levels by Western blots, phenotype changes, and quantitative RT-PCR. We chose to use a combination of the first two methods for these experiments.

We demonstrated in two experiments that there were minimal changes in histamine release when the cells were stimulated with 4 μ M HRF/TCTP or 50 μ M HRF/TCTP (0–8%, 0–10%; data not shown). Parenthetically, the usual dose of HRF/TCTP used to stimulate basophils is 1.6–5 μ M.

Human culture-derived basophils

As SHIP-1 knockdown in mast cells yielded minimal histamine release responses to HRF/TCTP (even using a prohibitively high dose of HRF/TCTP), we explored the use of cultured-derived human basophils as an alternative for our siRNA transfection experiments. We first evaluated several phenotypic and functional parameters to assess whether cells derived in this manner resemble basophils isolated from blood. First, Alcian blue staining was variable between cultures ($n=6$), with a range of 3–51%, mean $20 \pm 5\%$ cells staining positive between Weeks 2 and 3 of culture, which is when the experiments were performed. These results are not unexpected, as all of the cells are not at maturity. However, **Figure 2** shows flow cytometric analyses of cultured human basophils at 3 weeks of culture, which indicate characteristics similar to those commonly seen in primary human basophils. A representative experiment of three basophil cultures is shown. First, the forward- and side-scatter properties indicated considerable heterogeneity among the cell population (A). However, the



	MFI	
	Cultured	Primary
c-kit (CD117)	6.5 ± 0.5	4 ± 1*
FcεRIα	15 ± 1.5	94 ± 24
IL-3R(CD123)	281 ± 55	788 ± 407

Fig. 2. Flow cytometric analysis of cultured human basophils. (A) The forward- and side-scattered properties. c-kit expression (B) was relatively low (MFI=6.5) and comparable with those levels published previously for primary human basophils (MFI=4±1). FcεRIα (C) and IL-3R (D) are depicted. The table lists the average MFIs of these three markers on culture-derived basophils at 3 weeks (21.3±0.3 days; n=3) versus those isolated from blood (n=3–4). *, Published data from ref.

markers examined were found as expected for blood basophils and appear to be distributed on the entire population of cells, as indicated by the unimodal staining distribution. This suggests a population of cells all expressing characteristics of human basophils, despite the heterogeneity in scatter and Alcian blue staining. Specifically, c-kit expression (B) was relatively low (MFI=6.5) and comparable with those levels published previously for primary human basophils (MFI=4±1) [36]. However, there is a small second peak of c-kit + staining cells in these cultures, accounting for 8.2 ± 1.2% of the entire cell population. As expected, FcεRIα was present (C) as was IL-3R (D), although levels for both markers

were generally lower than those typically detected on blood basophils. The table lists the average MFIs of these three markers on culture-derived basophils (n=3) versus those isolated from blood (n=3–4).

Assessment of functional characteristics also indicated that culture-derived basophils resemble blood basophils. **Figure 3A** demonstrates that the sensitivity of culture-derived basophils to anti-IgE stimulation was similar to that for primary basophils isolated from blood yet substantially more sensitive than what was observed for culture-derived mast cells. Curve-fitting the data derived from primary blood basophils, cultured basophils, and cultured mast cells shows that the optimums

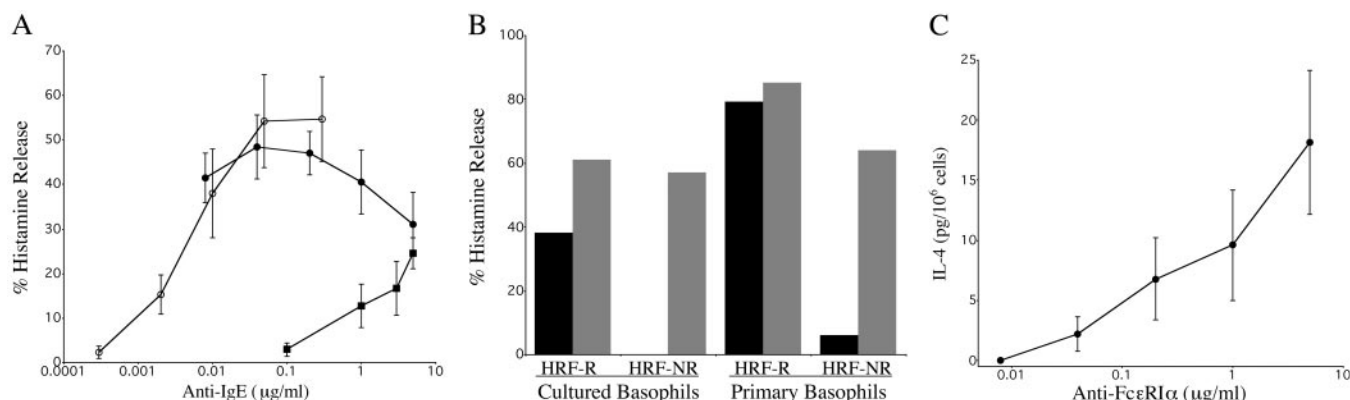


Fig. 3. Histamine release of cultured mast cells and cultured basophils as compared with primary basophils. (A) Cultured basophils (●; n=4) show a similar dose-response curve to primary basophils (○; n=6). This sensitivity is remarkably increased compared with cultured mast cells (■; n=3) at optimum and 50% of maximum release points (P=0.001). (B) Passive sensitization of cultured basophils. Response of HRF/TCTP (4 μM, black bars) demonstrates histamine release when the cultured basophils or the primary basophils are sensitized with HRF/TCTP-R. Anti-IgE (0.1 μg/ml, gray bars) does not discriminate between responder or nonresponder serum in cultured or primary basophils. The cultured basophil experiment is representative of two. The primary basophil experiment was done with the exact same serum used for the cultured basophils (n=1). (C) Cultured basophils (●) produce IL-4 (n=5) when stimulated with antibody to FcεRIα.

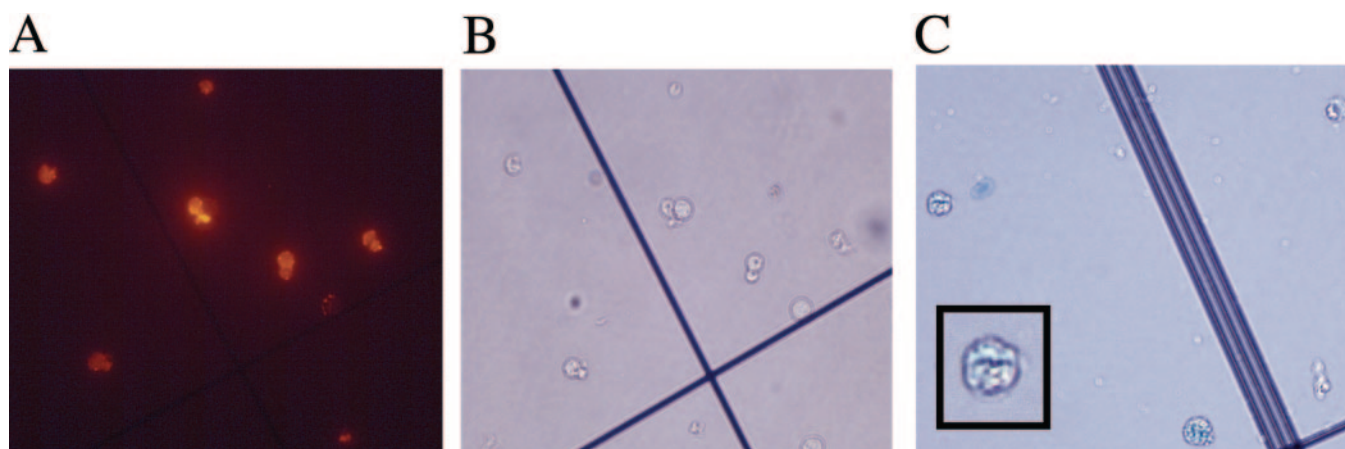


Fig. 4. Transfection of human culture-derived basophils. The fluorescent light exposure indicating rhodamine-positive cells (A) is compared with cells identified in the same field using white light exposure (B). The average efficiency was $43 \pm 17\%$, with a range of 24–77% ($n=3$). This is representative of three experiments. (C) The typical morphology of a culture-derived basophil stained with Alcian blue, and the inset details a higher magnification.

were statistically similar between primary blood basophils and cultured basophils but not between cultured basophils and cultured mast cells ($P=0.001$). The 50% of maximum release points were also statistically different between cultured basophils and cultured mast cells ($P=0.001$). For example, optimal histamine release occurred at concentrations of anti-IgE antibody in the range of 100 ng/ml for blood and culture-derived basophils. In contrast, histamine release by culture-derived mast cells was in the range of 1–5 $\mu\text{g/ml}$ anti-IgE, which is identical to what is reported for tissue-derived mast cells using this stimulus [36]. Figure 3B shows that cultured basophils and primary basophils released histamine to HRF/TCTP when sensitized with HRF/TCTP-R serum. The sensitivity of cultured and primary basophils to HRF/TCTP histamine release was increased compared with the histamine release from cultured mast cells. There was no histamine released to HRF/TCTP when any of these cells were sensitized with HRF/TCTP-NR serum. As expected, anti-IgE-induced histamine release did not discriminate between responder or nonresponder serum in cultured or primary basophils. This passive sensitization experiment was performed twice with cultured basophils using different HRF/TCTP-R and HRF/TCTP-NR sera, and a representative experiment is shown. The experiment using primary basophils was performed only once using the exact serum as shown for the cultured cells. This is actually representative of our standard passive sensitization protocol that has been performed numerous times over the years in our laboratory [4]. Furthermore, there is an Fc ϵ RI α concentration-dependent production of IL-4 in the cultured basophil model (Fig. 3C). This is consistent with published reports of cultured basophils [29]. In the same cultures, in the absence of any IL-4 release from the mast cells, these cells did release histamine (data not shown). Additionally, similar to the primary basophils, the cultured basophils released IL-4 (45 ± 12 pg/ 10^6 cells) when stimulated with 500 ng/ml ionomycin (data not shown).

We next explored the capacity to transfect culture-derived basophils by using rhodamine-labeled siRNA to determine the relative transformation efficiency in these cells. **Figure 4**

shows results from a representative of three experiments. Shown is the fluorescent light exposure indicating rhodamine-positive cells (A), which are compared with cells identified in the same field using white light exposure (B). (C) The typical morphology of a culture-derived basophil with an enlarged cell in the inset. The average transfection efficiency was $43 \pm 17\%$ (range 24–77%), which was considerably less than what was observed for culture-derived mast cells. The recovery was 48% and 45%, and the viability was 32–36% in two experiments. This viability was no different from the control without Lipofectamine. Therefore, this viability is not a result of the transfection process. There was considerable variation of viability between cultures (range 10–72%, average $36 \pm 7\%$). Other cytokines might increase viability, but this will take empirical testing. The increased histamine release sensitivity in these cells, as compared with the mast cells, led us to further explore SHIP-1 knockdown in these cultured basophils.

In four experiments, an average decrease of $51.8 \pm 7\%$ (range 34–69%, $P=0.016$) in SHIP-1 protein levels was observed compared with cells transfected with control siRNA (**Fig. 5A**). A representative Western blot shows decreased SHIP-1 protein relative to p85, which was examined to control for protein loading (inset). All knockdown experiments were done between Weeks 2 and 3 of culture (16.25 ± 5 days). Of note, the viability was not significantly different between control and SHIP-1 siRNA-treated cells ($35 \pm 14\%$ vs. $37 \pm 15\%$, respectively; $n=4$).

Table 1 contains data for all four knockdown experiments and corresponding changes to HRF/TCTP-induced histamine release using 1.6–5 μM . No change in histamine release is seen in Experiment 4, where only a 34% reduction in SHIP-1 is noted. As shown in Figure 5B, knockdown experiments with 49% or better show a significant difference in HRF/TCTP-induced histamine release ($P=0.005$; $n=3$). Anti-IgE-induced histamine release is not significantly different in these same experiments (data not shown). (C and D) Data from Experiment 2 in the table, where there was a 69% knockdown. An increased responsiveness to HRF/TCTP was observed over a wide range of concentrations (Fig. 5C). Additionally, Figure 5D

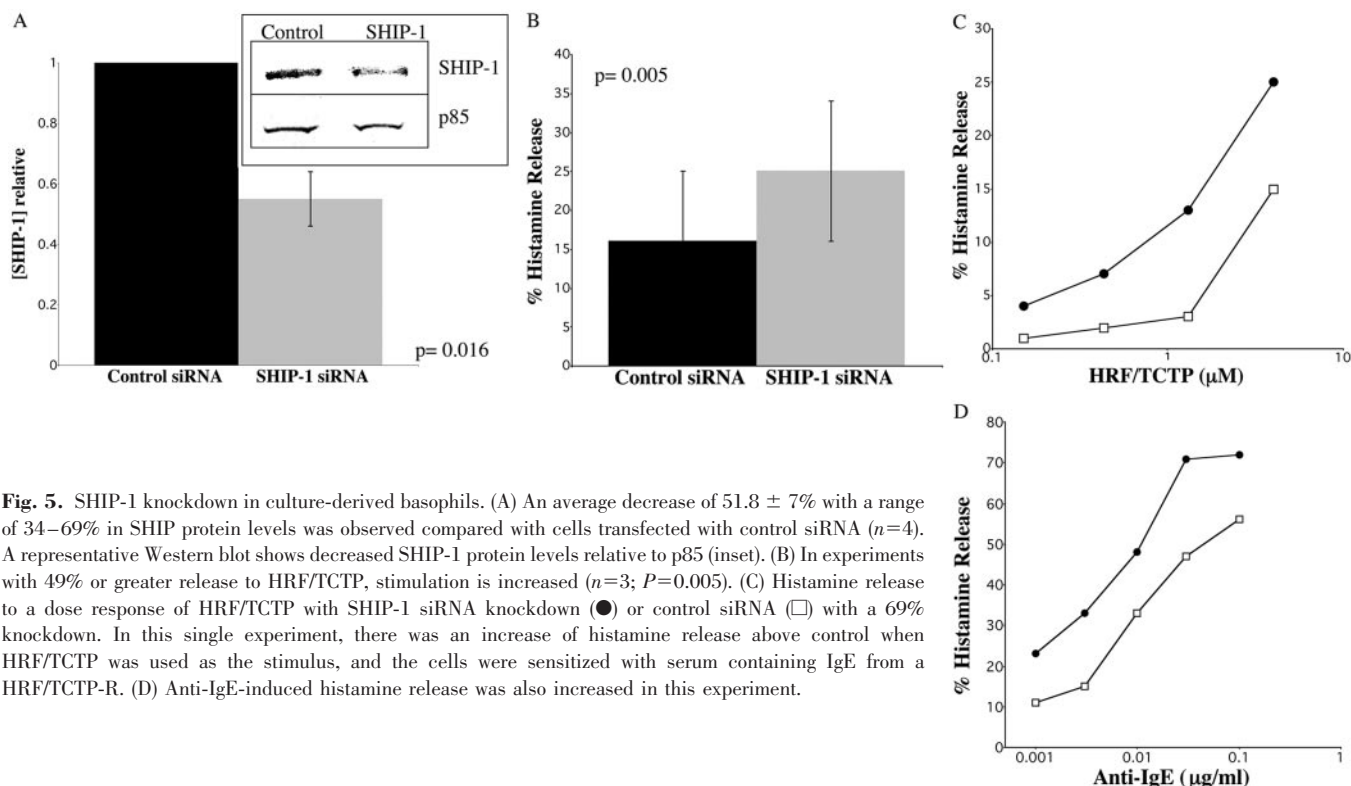


Fig. 5. SHIP-1 knockdown in culture-derived basophils. (A) An average decrease of $51.8 \pm 7\%$ with a range of 34–69% in SHIP protein levels was observed compared with cells transfected with control siRNA ($n=4$). A representative Western blot shows decreased SHIP-1 protein levels relative to p85 (inset). (B) In experiments with 49% or greater release to HRF/TCTP, stimulation is increased ($n=3$; $P=0.005$). (C) Histamine release to a dose response of HRF/TCTP with SHIP-1 siRNA knockdown (●) or control siRNA (□) with a 69% knockdown. In this single experiment, there was an increase of histamine release above control when HRF/TCTP was used as the stimulus, and the cells were sensitized with serum containing IgE from a HRF/TCTP-R. (D) Anti-IgE-induced histamine release was also increased in this experiment.

shows a similar trend in anti-IgE-mediated histamine release in the same experiment, suggesting that a more robust reduction in SHIP-1 can alter anti-IgE-induced release. In all of these experiments, HRF/TCTP-R serum was used to sensitize the cells as described in Materials and Methods.

Therefore, SHIP-1 knockdown was associated with increased histamine release to HRF/TCTP in cultured mast cells and cultured basophils; however, the sensitivity of the cultured basophil model is increased when compared with the cultured mast cell.

DISCUSSION

The data presented in this manuscript represent the first direct evidence linking histamine release to HRF/TCTP with decreased levels of SHIP-1 protein. In fact, until now, all of our previous reports had relied mostly on correlative data to support this relationship. For example, we first noticed that ba-

sophils from HRF/TCTP-R had reduced levels of SHIP-1 protein compared with basophils from subjects whose cells did not release histamine to HRF/TCTP [10]. In this study, a strong inverse correlation was observed between release to HRF/TCTP and reduced SHIP-1 protein levels. Although SHIP-1 levels appeared much higher in other cell types (monocytes and T cells) examined in these HRF/TCTP-R subjects, we could not fully exclude the possibility that some other signaling component might also contribute to the increased responsiveness seen in these basophils. Naturally, rodent models for which SHIP-1 is deficient had established more definitively the concept that the increased responsiveness of bone marrow-derived mast cells to FcεRI or SCF stimulation might be linked to reduced SHIP-1 [37].

These data show that CD34+ stem cells can be used to generate cultured cells that resemble human mast cells and human basophils and that both can be transfected with siRNA. SHIP-1 knockdown is associated with increases in histamine release to HRF/TCTP when sensitized with HRF/TCTP-R IgE in mast cells and basophils. The reasons that HRF/TCTP-induced histamine release was not observed when HRF/TCTP-NR IgE was used to passively sensitize the cells of SHIP-1 knockdown cells are unclear at this time. Perhaps, it could involve an additional serum component.

These data demonstrate for the first time that a cell culture model of human basophils exists to generate sufficient quantities of human basophils to perform transfection experiments. Vector-based methods of siRNA introduction with subsequent antibiotic selection are not practical in human basophils, an end-stage, nonproliferating cell. Therefore, we sought alternative approaches to introduce siRNA into basophils.

TABLE 1. Specific Data from SHIP-1 Knockdown Experiments in Cultured Basophils

Experiment	% Decrease in SHIP-1	% Histamine Release to HRF/TCTP	
		Control siRNA	SHIP-1 siRNA
1	55	31	41
2	69	15	25
3	49	1	9
4	34	17	17

Currently, the only published method for transfection of human basophils is nucleofection (Amaxa) with a transfection efficiency of 8% for plasmid DNA and 12% recovery of the starting cells [38]. Although useful for single-cell analysis, the limited recovery of basophils using this method will not provide the 5×10^5 basophils necessary for Western blot analysis for SHIP protein. Therefore, we attempted a Rhodamine-labeled, control siRNA (Qiagen) to test a variety of transfection methods for improved transfection efficiency and basophil recovery. Three lipid-based transfection reagents, namely, Oligofectamine (Invitrogen), Fugene (Roche, Indianapolis, IN, USA), and Lipofectamine 2000 (Invitrogen; reported to have low cell toxicity), have been tested. Lipofectamine 2000 provided only at best a 29% transfection efficiency and 25% recovery of the primary basophils. The other methods yielded <10% efficiency.

As basophils are end-stage cells, we were not optimistic about the ability to transfect a sufficient quantity of these cells. Even Tat protein, previously published to be successful with human eosinophils [39], was unsuccessful for basophils [38]. We considered retroviral transduction and magnetofection. However, an initial attempt using a lentivirus approach gave low yields. Therefore, we altered a method published previously known to produce cultured human mast cells [31] and have succeeded in generating a model of human basophils that is suitable for transfection studies. The success of the transfection studies may be a result of the fact that these cells are still growing and are not fully mature as primary end-stage basophils. Although we have shown that the transfection efficiency in these cultured basophils is less than that of cultured mast cells, the sensitivity of histamine release with respect to HRF/TCTP and anti-IgE as stimuli is increased markedly in the cultured basophils compared with the mast cells. Therefore, we conclude that cultured human mast cells and basophils are useful models for transfection studies. These data are novel in that CD34⁺ stem cells can be used to produce sufficient numbers of basophil-like cells that can be manipulated to explore allergic inflammation in other respects.

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