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Functional $K_{Ca}3.1$ K^+ channels are required for human lung mast cell migration

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ABSTRACT

Mast cell recruitment and activation is critical for the initiation and progression of inflammation and fibrosis. Mast cells infiltrate specific structures in many diseased tissues such as the airway smooth muscle in asthma. This microlocalisation of mast cells is likely to be key to disease pathogenesis. Human lung mast cells (HLMC) express the Ca^{2+}-activated K^+ channel, $K_{Ca}3.1$, which modulates mediator release, and is proposed to facilitate the retraction of the cell body during migration of several cell types. We have tested the hypothesis that blockade of $K_{Ca}3.1$ would attenuate HLMC proliferation and migration.

HLMC were isolated and purified from lung resected for bronchial carcinoma. HLMC proliferation was assessed by cell counts at various time points following drug exposure. HLMC chemotaxis was assayed using standard Transwell® chambers (8 µm pore size). Ion currents were measured using the single cell patch clamp technique.

$K_{Ca}3.1$ blockade with TRAM-34 did not inhibit HLMC proliferation and clotrimazole had cytotoxic effects. In contrast, HLMC migration towards the chemokine CXCL10, the chemoattractant SCF, and the supernatants from TNFα-stimulated asthmatic ASM was markedly inhibited with both the non-selective $K_{Ca}3.1$ blocker charybdotoxin and the highly specific $K_{Ca}3.1$ blocker TRAM-34 in a dose-dependent manner. Although $K_{Ca}3.1$ blockade inhibits HLMC migration, $K_{Ca}3.1$ is not opened by the chemotactic stimulus. Thus $K_{Ca}3.1$ must be involved downstream of the initial receptor-ligand interactions.

Since modulation of $K_{Ca}3.1$ can inhibit HLMC chemotaxis to diverse chemoattractants, the use of $K_{Ca}3.1$ blockers such as TRAM-34 could provide novel therapeutic strategies for mast cell-mediated diseases such as asthma.

INTRODUCTION

Mast cells are major effector cells in many diverse inflammatory and fibrotic diseases including asthma, pulmonary fibrosis, and rheumatoid arthritis.[1] In these diseases, there is evidence of increased mast cell numbers within specific tissue structures,[2] [3] where they contribute to the immunopathology through the release of a plethora of pleiotropic autacoids, proteases and cytokines (Reviewed by Bradding & Holgate 1999).[1] For example, in asthma mast cells infiltrate the airway smooth muscle (ASM) bundles in the airways of asthmatic subjects, but not those of either patients with eosinophilic bronchitis or normal subjects.[2] This event is likely to be key in the development of the disordered ASM physiology of asthma. Similarly, in rheumatoid disease there is accumulation of mast cells in the pannus at the edge of articular cartilage erosion.[3] It follows from this that targeting mast cell migration to these sites may offer a novel means of treating diseases in which mast cells play a role.

We have recently demonstrated that ASM cell-derived CXCL10 (also known as IFNγ-inducible protein of 10 kDa [IP-10]) induces human lung mast cell (HLMC) chemotaxis.[4] CXCL10 induces HLMC migration by binding to the chemokine receptor CXCR3 which is preferentially expressed on mast cells within the ASM of asthmatic subjects.[5] Thus inhibition of the CXCL10/CXCR3-axis could provide a novel and effective treatment of asthma. However, in addition to CXCL10, the ASM produces several other mast cell chemoattractants including stem cell factor (SCF),[6] transforming growth factor-β (TGFβ),[7] and the chemokines CXCL8, CXCL12 and CCL11.[5] A more general approach to preventing mast cell migration may therefore demonstrate better efficacy in the treatment of asthma and other mast cell-mediated diseases than targeting of specific chemokines.

Ion channels are emerging as interesting therapeutic targets in both inflammatory and structural non-excitable cells.[8] [9] HLMC, human bone marrow-derived and human peripheral blood-derived mast cells express the intermediate conductance Ca^{2+}-activated K^+ channel, $K_{Ca}3.1$ (also known as $IK_{Ca}1$).[10][11][12] This channel maintains a negative membrane potential during cell activation thus increasing the driving potential for Ca^{2+} influx through store operated calcium channels.[13] Thus blockade of $K_{Ca}3.1$ attenuates HLMC degranulation while opening it enhances it.[10] [11] In addition to their role in cell activation, ion channels carrying K^+ and Cl^- have been implicated in many diverse cellular processes including proliferation, chemotaxis and apoptosis. K_{Ca} channels are required for lysophosphatidic acid-induced microglial cell migration [14] and evidence also exists for the involvement of $K_{Ca}3.1$ in both T and B cell proliferation.[15]

In this study we have examined the hypothesis that $K_{Ca}3.1$ is important for HLMC migration and proliferation. To test this, we have examined the effects of $K_{Ca}3.1$ blockade with the relatively non-selective $K_{Ca}3.1$ blocker charybdotoxin and the highly specific $K_{Ca}3.1$ blocker triarylmethane-34 (TRAM-34) on CXCL10-, SCF-, and ASM-induced HLMC migration. We have also studied the effect of the $K_{Ca}3.1$ blockers TRAM-34 and clotrimazole on HLMC proliferation.

MATERIALS AND METHODS

Reagents

The following were purchased: recombinant human (rh)SCF, rhIL-6, rhIL-10, rhCXCL10 (R&D, Abingdon, U.K.); 1-ethyl-2-benzimidazolinone (1-EBIO), charybdotoxin, iberiotoxin (Sigma, Poole, Dorset, U.K.); mouse IgG_1 mAb YB5.B8 (anti-CD117) (Cambridge Bioscience, Cambridge, U.K.); sheep anti-mouse IgG_1 Dynabeads® (Dynal, Wirral, UK); DMEM/Glutamax/Hepes, antibiotic/antimycotic solution, MEM non-essential amino acids and foetal calf serum (FCS) (Life technologies, Paisley, Scotland, U.K.). TRAM-34 was a generous gift from Dr Heike Wulff (University of California Irvine, California, USA).

Human lung mast cell purification

All human subjects gave written informed consent, and the study was approved by the Leicestershire Research Ethics Committee. HLMC were dispersed enzymatically from macroscopically normal lung obtained within 1 hr of resection for lung cancer and purified using immunoaffinity magnetic selection (Dynabeads®) as described previously.[10] The final mast cell purity, assessed using metachromatic staining, was >99% with cell viability >98% (monitored by exclusion of trypan blue). HLMC were cultured in DMEM/Glutamax/Hepes containing 1% antibiotic/antimycotic solution, 1% non-essential amino acids, 10% FCS, 100 ng/ml SCF, 50 ng/ml IL-6, and 10 ng/ml IL-10.

HLMC proliferation assay

To monitor proliferation, following purification HLMC were resuspended in DMEM/Glutamax/Hepes containing SCF (100ng/ml), IL-6 (50ng/ml) and IL-10 (10ng/ml) at a concentration of 0.25×10^6 cells/ml as described previously.[16] Clotrimazole and TRAM-34 were added in the concentration range 10 to 1000 nM. Control wells containing either 0.1% dimethyl sulphoxide (DMSO) or culture medium alone were also established. Metachromatic cells were counted after 1 and 4 weeks in culture using Kimura stain.

Human lung mast cell chemotaxis

HLMC chemotaxis assays were performed using the Transwell® system (BD Biosciences, Oxford, UK) with 24 well plates as described previously.[4] [5] CXCL10 or SCF was placed in the lower wells (omitted in negative control) at a concentration of 100 ng/ml. 50 μl of 2x the final concentration of TRAM-34 (final concentration 20 or 200 nM), charybdotoxin or iberiotoxin (final concentration of 100 nM) was added to the upper chambers immediately prior to the addition of 1×10^5 HLMC per well (50 μl). The vehicle for TRAM-34 was DMSO and the final concentration of DMSO was 0.1% in all wells including a control. After incubating the cells for 3h at 37°C, we counted the number of HLMC in the bottom well using Kimura stain in a haemocytometer. HLMC migration was calculated as the fold increase of migrated cells in the test wells compared to the negative control (no chemoattractant in the lower well) as described previously.[4] [5]

ASM cells (passage 3–7) from subjects with asthma (n = 4) were plated into six-well plates (9.6×10^4 cells/2 ml DMEM, 10% foetal calf serum), grown for 1 week, then growth was arrested for 48 hours with serum-deprived medium and stimulated with tumour necrosis factor (TNF)-α (10 ng/ml) for 24 hours.[5] The supernatants were removed and stored at –80°C before use in the chemotaxis experiments.

Patch clamp electrophysiology

The whole-cell variant of the patch clamp technique was used.[10] Patch pipettes were made from boro-silicate fibre-containing glass (Clark Electromedical Instruments, Reading, UK), and their tips heat polished resulting in resistances of typically 4-6 MΩ. The standard pipette solution contained (in mM): KCl, 140; $MgCl_2$, 2; HEPES, 10; Na^+ATP, 2; GTP, 0.1; pH 7.3. The standard external solution contained (in mM): NaCl, 140; KCl, 5., $CaCl_2$, 2; $MgCl_2$,1; HEPES, 10; pH 7.3. For recording, mast cells were placed in 35mm dishes containing standard external solution.

Whole cell currents were recorded using an Axoclamp 200A amplifier (Axon Instruments, Foster City, CA, USA), and currents evoked by applying voltage commands to a range of potentials (120 to +130 mV) in 10mV steps from a holding potential of -20 mV. The currents were digitised (sampled at a frequency of 10kHz), stored on computer and subsequently analysed using pClamp software (Axon Instruments). Capacitance transients were minimized using the capacitance neutralization circuits on the amplifier. Correction for series resistance was not routinely applied. In some experiments, continuous membrane currents were recorded at a constant holding potential of +40 mV, data being digitized at 200Hz and recorded using Axoscope (Axon Instruments). Experiments were performed at 27°C, temperature being controlled by a Peltier device. Experiments were performed with a perfusion system (Automate Scientific Inc, San Francisco, CA, USA) to allow solution changes, although drugs were added directly to the recording chamber.

Data presentation and statistical analysis

Data is presented as the mean ± SEM from separate donors performed in duplicate. Confidence intervals were calculated at 95% using Graphpad Prism 4 software. The inhibition of chemotaxis is presented as the percentage migration compared to the positive control after the subtraction of the negative control from all conditions. Differences between data sets were analysed using a paired two-tailed Student's t-test (Microsoft Excel v2003). A p value <0.05 was considered statistically significant.

RESULTS

HLMC proliferation

HLMC proliferate in long-term culture.[16] Clotrimazole, a blocker of $K_{Ca}3.1$ (K_d 70 nM) and inhibitor of cytochrome P450, produced a dose-dependent reduction in HLMC number by 4 weeks in culture, with complete cell death evident at 1000 nM (Figure 1A). In contrast, TRAM-34, a highly specific blocker of $K_{Ca}3.1$ (K_d 20 nM), without any effect on cytochrome P450, had no effect on HLMC at 1 or 4 weeks (Figure 1B). This suggests that $K_{Ca}3.1$ is not involved in HLMC proliferation and that the effects of clotrimazole were "toxic" and independent of its effect on $K_{Ca}3.1$. This is in contrast to the role of $K_{Ca}3.1$ in human T lymphocyte proliferation.[15]

HLMC chemotaxis

Due to its apparent toxic effect on HLMC, clotrimazole was not used in the chemotaxis assays. For blockade of $K_{Ca}3.1$ during chemotaxis we used TRAM-34, and another $K_{Ca}3.1$ blocker charybdotoxin (K_d 5 nM).

The migration of HLMC in response to 100 ng/ml of CXCL10 was 2.2 ± 0.2 fold greater than that of the control (no CXCL10) (n=6) (95% CI, 1.7-2.7, p=0.001) (Figure 2), in keeping with previous experiments.[4] CXCL10-induced migration was inhibited dose-dependently by TRAM-34. Thus with 200 nM TRAM-34, migration was reduced by 80 ± 7 % (n=6) (95% CI, 62-99%, p=0.0001) (Figure 3). Consistent with this, CXCL10-induced HLMC migration was inhibited by 78 ± 14 % with the addition of 100 nM charybdotoxin (n=6) (95% CI, 42-113%, p=0.002) (Figure 3).

In order to determine whether the inhibition of HLMC migration by $K_{Ca}3.1$ blockade was restricted to an interaction with G-protein-coupled chemoattractants or a general phenomenon, we next tested the effects of TRAM-34 and charybdotoxin on SCF-induced migration. SCF (100 ng/ml) induced HLMC migration to a similar extent as CXCL10 in the majority of donors with mean migration of 2.0 ± 0.2 fold compared to control (n=4) (95% CI, 1.3-2.6, p=0.020) (Figure 2). Both charybdotoxin (100 nM) and TRAM-34 (200 nM) inhibited SCF-induced migration by 92 ± 8 % (n=4) (95% CI, 69-116%, p=0.001), and 76 ± 11 % (n=4) (95% CI, 42-110%, p=0.006) respectively (Figure 4). As a further control, we also examined the effects of the specific large conductance Ca^{2+}- activated K^+ channel ($K_{Ca}1.1$, also known as BK_{Ca}) blocker iberiotoxin (100 nM). Since HLMC do not express $K_{Ca}1.1$ currents or mRNA,[10] [22] iberiotoxin should be ineffective. Indeed, iberiotoxin did not attenuate the migration of HLMC to either CXCL10 or SCF (2.0 ± 0.6 fold in the control compared to 1.8 ± 0.5 fold with 100 nM iberiotoxin). Background control migration was not inhibited by charybdotoxin or TRAM-34.

Since HLMC migration to SCF and CXCL10 was inhibited with similar efficacy, we next examined the effects of $K_{Ca}3.1$ blockade on HLMC migration towards the complex milieu of chemoattractants present in cell supernatants from stimulated asthmatic ASM. The ASM supernatants induced HLMC migration greater than either CXCL10 or SCF alone, with mean migration of 2.6 ± 0.4 fold compared to control (n=4) (95% CI, 1.5-3.8, p=0.022) (Figure 2). Both charybdotoxin (100 nM) and TRAM-34 (200 nM) inhibited HLMC migration to ASM supernatants with similar efficacy as with CXCL10 or SCF alone, with 87 ± 12 % inhibition of migration with charybdotoxin (n=4) (95% CI, 47-126%, p=0.006), and 97 ± 8 % inhibition with TRAM-34 (n=4) (95% CI, 72-123%, p=0.001) (Figure 5). In contrast, iberiotoxin did not attenuate the migration of HLMC towards ASM supernatants (2.6 ± 0.4 fold in the

control compared to 2.4 ± 0.5 fold with 100 nM iberiotoxin) (n=4) (95% CI, 0.9-4.0, p=0.538) (Figure 5).

Effects of CXCL10 on HLMC $K_{Ca}3.1$ activation

We have recently demonstrated that the $G_{\alpha s}$-coupled β_2-adrenoceptor influences $K_{Ca}3.1$ gating.[17] To assess whether the $G_{\alpha i}$-coupled CXCL10-receptor CXCR3 influences $K_{Ca}3.1$ function we attempted to record $K_{Ca}3.1$ currents following the addition of 100 ng/ml CXCL10 to HLMC (n=23 cells from 4 separate donors). In spite of being able to record $K_{Ca}3.1$ currents in these cells following the addition of the $K_{Ca}3.1$-specific channel opener 1-Ethyl-2-benzimidazolinone (1-EBIO), we did not see any evidence of either the transient or sustained opening of $K_{Ca}3.1$ in response to CXCL10 (Figure 6).

DISCUSSION

In this study, we demonstrate for the first time that blockade of the K^+ channel $K_{Ca}3.1$ markedly attenuates the chemotactic response of *ex vivo* HLMC to the chemokine CXCL10, the mast cell growth factor SCF, and conditioned media from asthmatic ASM. In contrast to its effects on acute mitogen-induced lymphocyte proliferation, $K_{Ca}3.1$ does not appear to be important for HLMC proliferation in long-term culture.

The redistribution of mast cells within human tissues is likely to be key in the initiation and propagation of a variety of diseases.[2] [3] [5] Inhibiting their migration within tissues may therefore offer a truly novel approach for the treatment of mast cell-driven pathobiology. As an example, we have recently demonstrated that the CXCL10/CXCR3 axis may be a critical determinant of the mast cell distribution within the human lung.[5] Thus, inhibition of CXCL10/CXCR3-induced chemotaxis is an attractive target for asthma therapy. However, while targeting CXCL10/CXCR3 may prove fruitful, the ASM produces several other chemoattractants including SCF, TGFβ and several further chemokines.[5][6][7] These may play their own roles in mast cell recruitment by ASM under certain conditions, and in addition, are probably important for the microlocalisation of mast cells within other tissue compartments in unrelated diseases such as tubulointerstitial renal disease and rheumatoid arthritis.[2] [3] [18] Thus the ability of $K_{Ca}3.1$ blockade to profoundly inhibit mast cell migration in response to two diverse chemoattractants, CXCL10 which activates the G-protein-coupled receptor CXCR3, and SCF which activates the tyrosine kinase receptor CD117, suggests that $K_{Ca}3.1$ has great potential as a therapeutic target for mast cell-mediated disease in humans. Moreover, blockade of $K_{Ca}3.1$ almost completely attenuates HLMC migration towards the complex milieu of chemoattractants present in TNFα-stimulated asthmatic ASM cell supernatants, strengthening the hypothesis that functional $K_{Ca}3.1$ channels are an absolute requirement for HLMC migration.

To inhibit HLMC migration we used charybdotoxin and TRAM-34, two distinct molecules which block the $K_{Ca}3.1$ pore at different sites.[19] [20] Charybdotoxin is a 37 amino acid peptide derived from the venom of the scorpion *Leiurus quinquestriatus* and blocks $K_{Ca}3.1$ with a K_d for channel block of 5-10 nM.[19] [21] It also blocks the voltage-gated K^+ channel $K_v1.3$ and the large conductance K_{Ca}, $K_{Ca}1.1$. However, HLMC do not express $K_v1.3$ or $K_{Ca}1.1$ mRNA or their electrical currents.[10] [22] TRAM-34 is a highly specific small molecule blocker of $K_{Ca}3.1$ (K_d 20 nM),[23] which was derived from the $K_{Ca}3.1$ blocker and antifungal agent clotrimazole. However, unlike clotrimazole, TRAM-34 does not interfere with cytochrome P450.[23] Charybdotoxin blocks $K_{Ca}3.1$ by binding to the external pore with high affinity,[19] while the highly lipophilic TRAM-34 binds to residues within the internal vestibule of the channel.[20] We used TRAM-34 and charybdotoxin at concentrations up to 10x the K_d because it has been estimated previously that for complete channel block to be achieved, drugs need to be present at 5-10x the K_d.[15] Thus the ability of these two potent but pharmacologically distinct $K_{Ca}3.1$ blockers to inhibit HLMC migration to a similar extent at 10x the K_d indicates that the mechanism behind this is indeed $K_{Ca}3.1$ blockade. In addition, the $K_{Ca}1.1$ blocker iberiotoxin was without effect, further suggesting that the effects observed are specific to $K_{Ca}3.1$.

The role of $K_{Ca}3.1$ in HLMC and T cell mediator secretion is to maintain the negative membrane potential during cell activation, counteracting the tendency for Ca^{2+} influx to depolarise the cell membrane. Thus $K_{Ca}3.1$ increases the driving force for Ca^{2+} influx because store-operated Ca^{2+} channels conduct larger currents at

negative membrane potentials.[10] [11] [13]. However, the role of this channel in cell migration is predicted to be different. A K_{Ca} channel with some properties of $K_{Ca}3.1$ has been demonstrated in glial cells and has been proposed to facilitate the retraction of the rear body of the migrating cell,[24] [25] despite appearing to be more highly expressed at the leading edge of lamellipodia in migrating MDCK-F (transformed renal epithelial cells), NIH-3T3 fibroblasts and human melanoma cells.[26] The mechanism controlling their seemingly selective activation at the rear body of the cell is unclear although the localised Ca^{2+} concentration is likely to be critical. In addition, migration appears to require the opening and closing of the channels, most probably due to calcium oscillations, since both $K_{Ca}3.1$ channel blockers and the opener 1-EBIO all inhibit chemotaxis in these cells.[14][24] Thus, the intermittent activation of the $K_{Ca}3.1$-like channel in these cells is believed to facilitate the swelling and shrinking of the cell body required for cell migration [25] and would explain why functional $K_{Ca}3.1$ are important for migration in HLMC.

CXCL10 increases intracellular Ca^{2+} transiently in HLMC through the release of Ca^{2+} from internal stores but does not activate Ca^{2+} influx from the extracellular fluid.[4] We have shown in this study that CXCL10 does not directly open $K_{Ca}3.1$, which is in keeping with our previous data demonstrating that influx of extracellular Ca^{2+} is the critical requirement for $K_{Ca}3.1$ opening during HLMC activation.[10] In addition, SCF does not open $K_{Ca}3.1$.[10] This indicates that the gating of $K_{Ca}3.1$ during HLMC migration is downstream of the chemoattractant stimulus, most likely related to adhesive signals required for the migratory process. This data also demonstrates that, while the $K_{Ca}3.1$ channel in HLMC is known to be directly coupled to the $G_{\alpha s}$-dependent β_2-adrenoceptor,[17] it is not coupled to the $G_{\alpha i}$-dependent CXCR3 receptor.

We have demonstrated previously that HLMC proliferate in long-term culture. Following an initial decrease in cell number over the first week, HLMC proliferate so that by 4 weeks there can be up to 4 times the starting number.[16] Interestingly, in contrast to published observations in T and B cells [27] [28] and endothelial cells,[29] TRAM-34 had no significant effect on HLMC proliferation. TRAM-34 inhibits mitogenesis of preactivated human T cells at concentrations similar to those required for channel blocking.[27] In contrast to TRAM-34, clotrimazole did inhibit proliferation, and at 1000 nM killed HLMC, suggesting therefore that this effect was independent of $K_{Ca}3.1$ blockade (Figure 1A). Clotrimazole has many diverse effects on cells other than channel blocking, such as reducing the expression of G_1-phase cyclins,[30] modulation of cytochrome P450 activity [31] and inhibition of cellular glycolysis,[32] which could all contribute to the anti-proliferative effects we have presented. These data suggest that SCF-induced HLMC proliferation does not involve $K_{Ca}3.1$ unlike acute T cell mitogenic stimulation with anti-CD3.[15]

Blockade of $K_{Ca}3.1$ has shown promise in several diseases/disease models. Clotrimazole was very effective in ameliorating active human rheumatoid arthritis but caused unacceptable side effects due to inhibition of cytochrome P450.[33] TRAM-34 prevents vascular restenosis after balloon angioplasty in rats through its ability to inhibit neointimal vascular smooth muscle proliferation, and without any undue toxicity.[34] Another specific $K_{Ca}3.1$ blocker 4-phenyl-4*H*-pyran (K_d 8 nM) reduces infarct volume in a rat model of subdural haematoma, suggesting a possible use in the management of traumatic brain injury.[35] Clinical trials are also underway studying the effects of $K_{Ca}3.1$ inhibition in sickle cell anaemia. These studies of $K_{Ca}3.1$ blockade are encouraging in that the lack of obvious toxicity of TRAM-34 suggests real therapeutic potential for human disease. However, with respect to

mast cell-mediated disease, further mechanistic studies of $K_{Ca}3.1$ blockade utilising rodent models are unlikely to be informative because $K_{Ca}3.1$ currents have never been observed in rodent mast cells.[8]

In summary, we demonstrate for the first time that blockade of the K^+ channel $K_{Ca}3.1$ markedly attenuates the chemotactic response of *ex vivo* HLMC to both the chemokine CXCL10 and the mast cell growth factor SCF, as well as TNFα-stimulated asthmatic ASM supernatants. This suggests that blocking $K_{Ca}3.1$ has great potential as a target for the treatment of asthma and other inflammatory diseases in which mast cells play a role.

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COMPETING INTEREST STATEMENT
The authors have no competing interests to declare.

ETHICS APPROVAL
All human subjects gave written informed consent, and the study was approved by the Leicestershire Research Ethics Committee.

FIGURE LEGENDS

Figure 1
HLMC proliferation in long-term culture. **A**) HLMC proliferation was attenuated dose-dependently with the addition of clotrimazole. Higher concentrations (μM) of clotrimazole killed the cells. **B**) Conversely, TRAM-34 had no significant effect on HLMC proliferation. Drug concentrations are given in nanomoles/L and data is the mean ± SEM from three separate donors. Donors in A are different to those in B. * $p<0.05$.

Figure 2
SCF (n=4), CXCL10 (n=6) and supernatants from TNFα-stimulated asthmatic ASM (n=4) induce HLMC migration. Data is presented as the mean ± SEM from at least four individual donors. * $p<0.05$ ** $p<0.01$.

Figure 3
Attenuation of CXCL10-induced HLMC migration with $K_{Ca}3.1$ blockade. TRAM-34 attenuates CXCL-10-induced HLMC migration in a dose-dependent manner. The pharmacologically distinct $K_{Ca}3.1$ blocker charybdotoxin attenuates CXCL10-induced HLMC migration with similar efficacy to TRAM-34. Data is the mean ± SEM from six separate donors. * $p<0.005$ ** $p<0.001$.

Figure 4
Attenuation of SCF-induced HLMC migration with $K_{Ca}3.1$ blockade. Blockade of $K_{Ca}3.1$ attenuates SCF-induced HLMC migration with similar efficacy to CXCL10-induced HLMC migration. Data is the mean ± SEM from four separate donors. * $p<0.05$ ** $p<0.01$.

Figure 5
Attenuation of HLMC migration towards TNFα-stimulated asthmatic ASM supernatants with $K_{Ca}3.1$ blockade. TRAM-34 attenuates HLMC migration to ASM supernatants in a dose-dependent manner. Charybdotoxin inhibits migration with similar efficacy to TRAM-34. Iberiotoxin, which is structurally related to charybdotoxin but does not block $K_{Ca}3.1$, has no significant effect on HLMC migration. Data is presented as the mean ± SEM from four separate donors. * $p<0.05$, ** $p<0.01$, n.s. = not significant.

Figure 6
Whole cell electrophysiological recording of electrical currents in HLMC at rest and following the addition of CXCL10 and then 1-EBIO. **A**) Continuous recording of current at +40 mV in a HLMC demonstrating a stable baseline with minimal basal current. No response to the addition of CXCL10, where indicated, but a rapid increase in outward current within 90 sec of adding 1-EBIO to the recording chamber was observed. **B**) Current-voltage curves from another cell at rest, 3 min post addition of CXCL10 and then 3 min post addition of 1-EBIO, demonstrating development of a typical $K_{Ca}3.1$ whole cell current following the latter.

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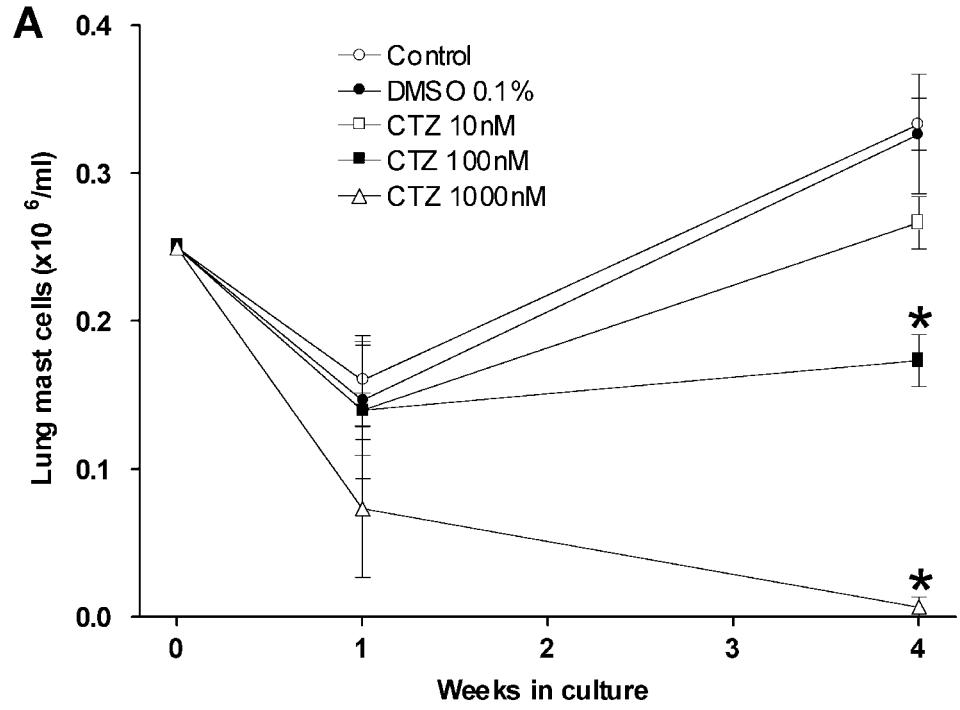


Figure 1A

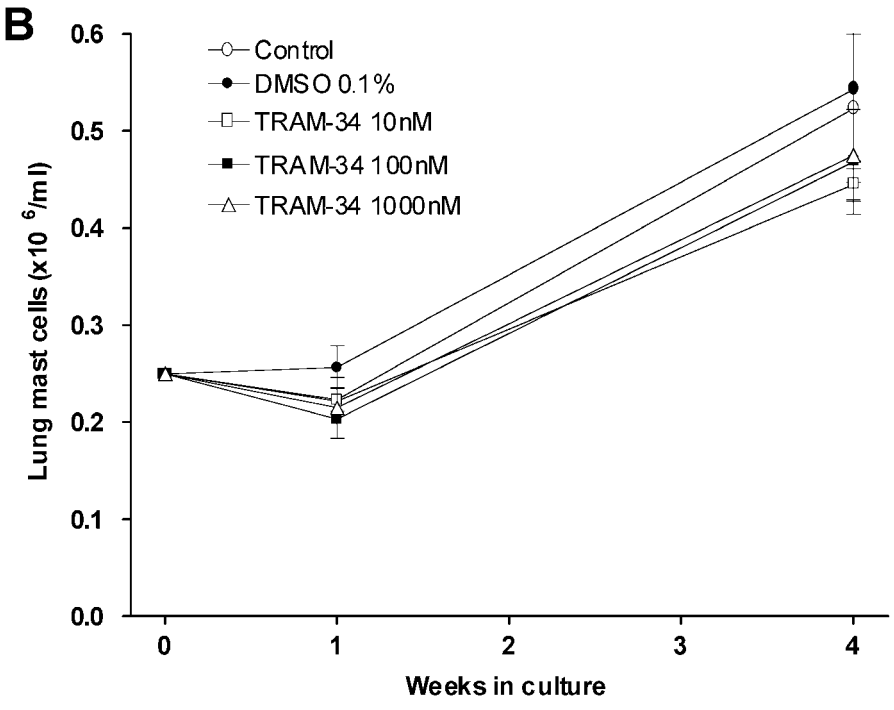
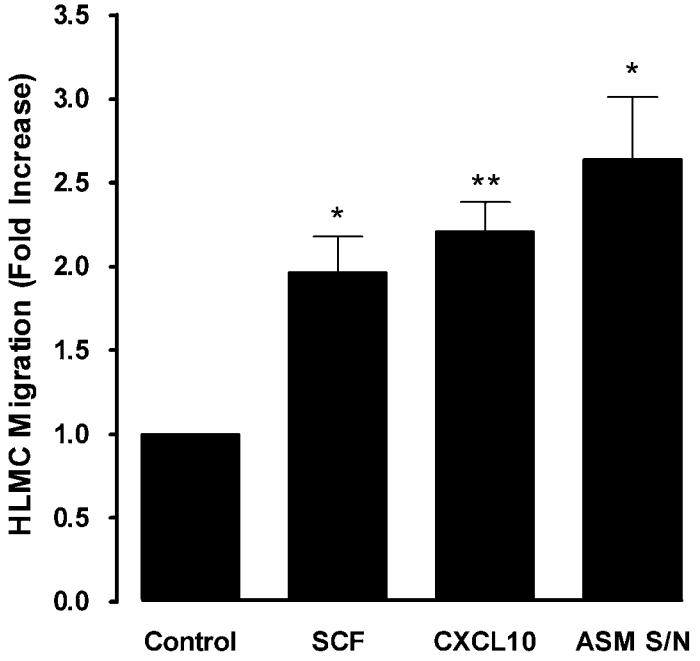


Figure 1B



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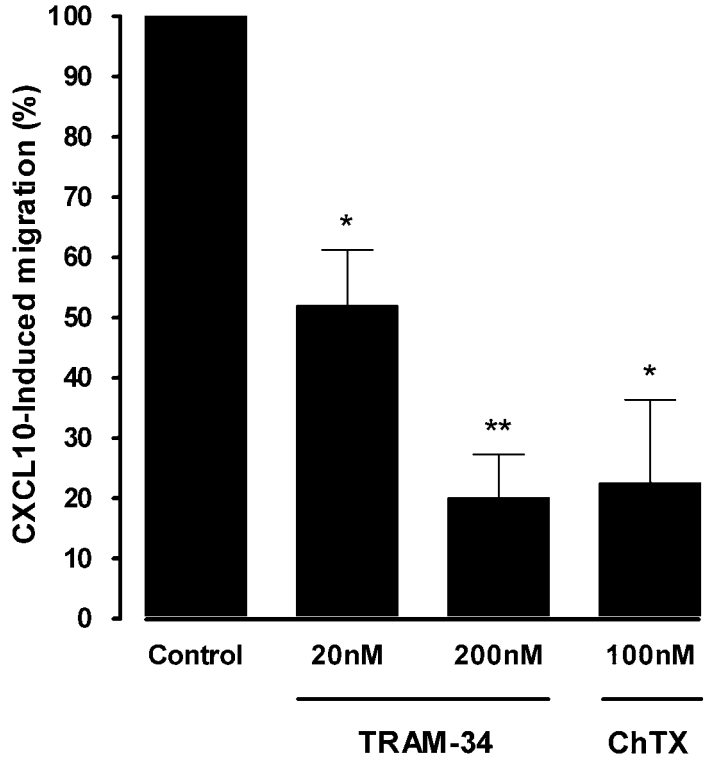

Figure 3

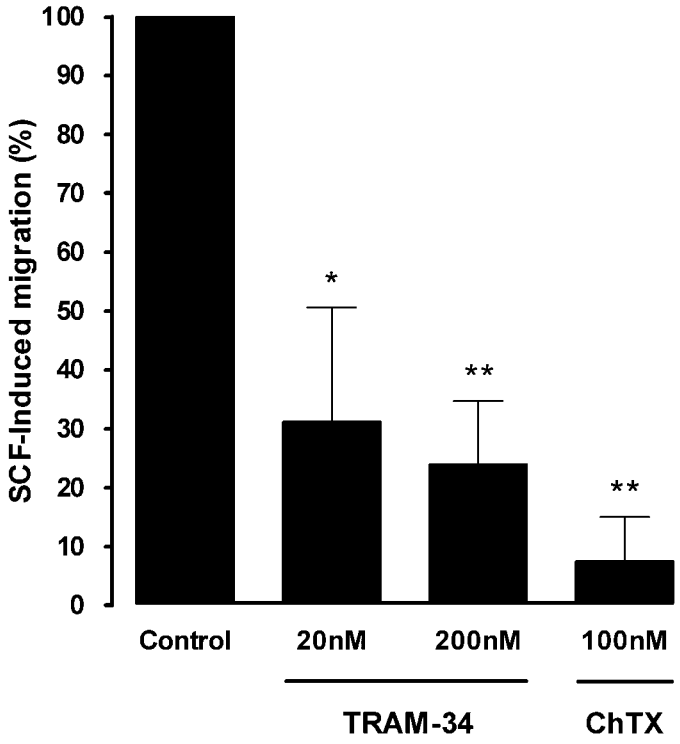

Figure 4

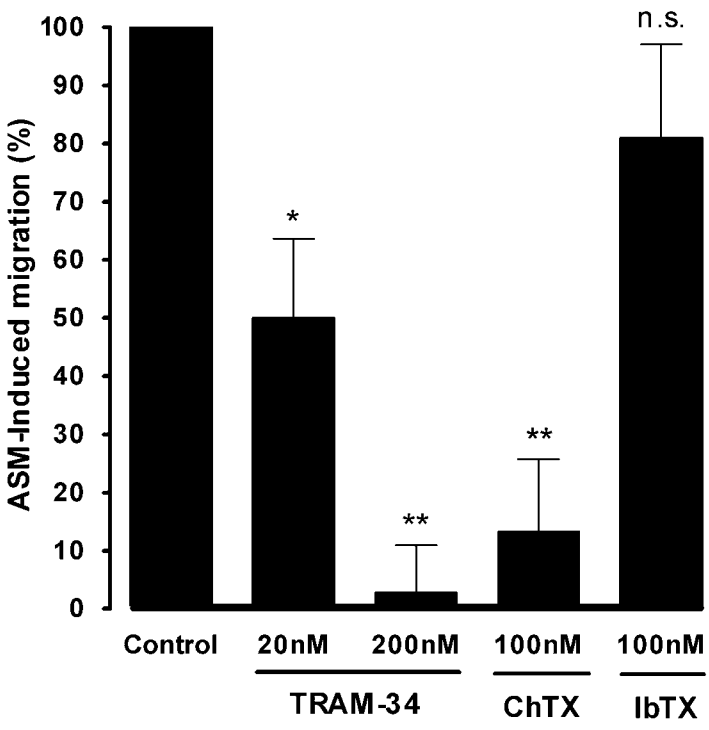

Figure 5

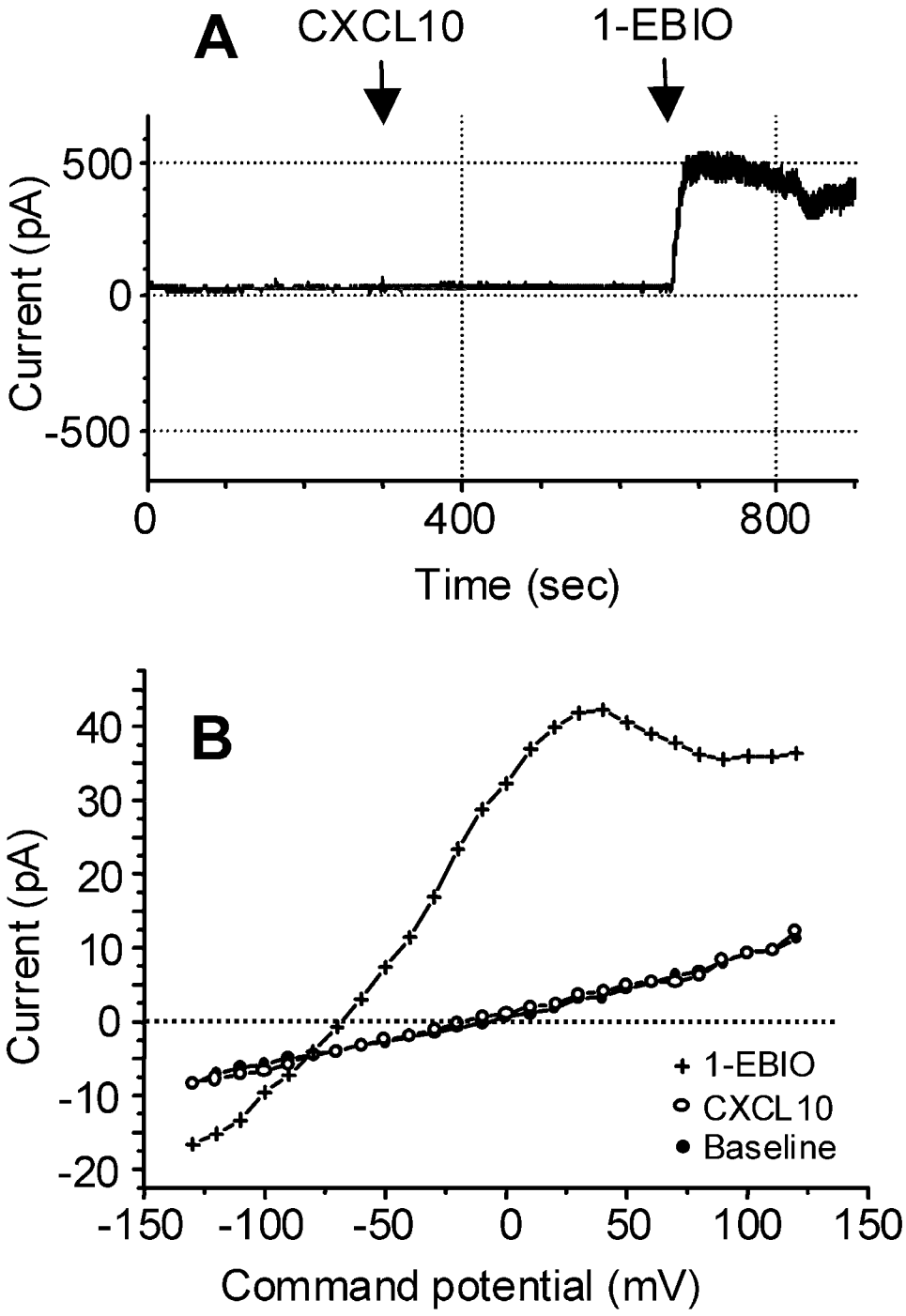

Figure 6