

Serum- and glucocorticoid-inducible kinase SGK1 regulates reorganization of actin cytoskeleton in mast cells upon degranulation

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Schmid E, Gu S, Yang W, Münzer P, Schaller M, Lang F, Stournaras C, Shumilina E. Serum- and glucocorticoid-inducible kinase SGK1 regulates reorganization of actin cytoskeleton in mast cells upon degranulation. *Am J Physiol Cell Physiol* 304: C49–C55, 2013. First published September 26, 2012; doi:10.1152/ajpcell.00179.2012.—Aggregation of the high-affinity IgE receptor (FcεRI) on mast cells (MCs) causes MC degranulation, a process that involves cortical F-actin disassembly. Actin depolymerization may be triggered by increase of cytosolic Ca²⁺. Entry of Ca²⁺ through the Ca²⁺ release-activated Ca²⁺ (CRAC) channels is under powerful regulation by the serum- and glucocorticoid-inducible kinase SGK1. Moreover, FcεRI-dependent degranulation is decreased in SGK1-deficient (*sgk1*^{−/−}) MCs. The present study addressed whether SGK1 is required for actin cytoskeleton rearrangement in MCs and whether modulation of actin architecture could underlie decreased degranulation of *sgk1*^{−/−} MCs. Confirming previous results, release of β-hexosaminidase reflecting FcεRI-dependent degranulation was impaired in *sgk1*^{−/−} MCs compared with *sgk1*^{+/+} MCs. When CRAC channels were inhibited by 2-aminoethoxydiphenyl borate (2-APB; 50 μM), MC degranulation was strongly decreased in both *sgk1*^{+/+} and *sgk1*^{−/−} MCs and the difference between genotypes was abolished. Moreover, degranulation was impaired by actin-stabilizing (phalloidin) and enhanced by actin-disrupting (cytochalasin B) agents to a similar extent in *sgk1*^{+/+} MCs and *sgk1*^{−/−} MCs, implying a regulatory role of actin reorganization in this event. In line with this, measurements of monomeric (G) and filamentous (F) actin content by FACS analysis and Western blotting of detergent-soluble and -insoluble cell fractions indicated an increase of the G/F-actin ratio in *sgk1*^{+/+} MCs but not in *sgk1*^{−/−} MCs upon FcεRI ligation, an observation reflecting actin depolymerization. In *sgk1*^{+/+} MCs, FcεRI-induced actin depolymerization was abolished by 2-APB. The observed actin reorganization was confirmed by confocal laser microscopic analysis. Our observations uncover SGK1-dependent Ca²⁺ entry in mast cells as a novel mechanism regulating actin cytoskeleton.

CRAC channel; store-operated Ca²⁺ entry; 2-APB

MAST CELLS (MCs) are IgE-activated histamine-providing effector cells of allergic inflammation, responsible for a variety of allergic diseases such as anaphylaxis, allergic rhinitis, and asthma (14). Beyond that, MCs have been shown to exert critical proinflammatory functions as well as potential immunoregulatory roles in various immune disorders through the release of their potent bioactive mediators (2). MCs release histamine, proteases, chemotactic factors, cytokines, and metabolites of arachidonic acid that act on the vasculature, smooth muscle, connective tissue, mucous glands, and inflammatory cells (2).

Release of MC mediators, triggered, e.g., upon aggregation of the high-affinity IgE receptor (FcεRI), the process called degranulation, involves reorganization of actin cytoskeleton (5). FcεRI aggregation triggers cortical F-actin disassembly, which allows access of the secretory vesicles to the plasma membrane (1, 8, 23, 28, 34). The process of F-actin disassembly was shown to be dependent on the increase of intracellular Ca²⁺ (16, 23). On the other hand, a powerful regulator of Ca²⁺ entry and exocytosis in MCs is the serum- and glucocorticoid-inducible kinase SGK1 (9, 10, 33). SGK1-deficient (*sgk1*^{−/−}) MCs have a decreased FcεRI-dependent Ca²⁺ entry and degranulation, and *sgk1*^{−/−} mice are thus protected against anaphylactic reaction, pointing to a strongly impaired MC function in vivo (33). We have recently demonstrated that SGK1 acts through upregulating the Ca²⁺ release-activated Ca²⁺ (CRAC) channel in the plasma membrane (9, 10), which in MCs consists of the pore-forming subunit Orai1 (36) and the endoplasmatic Ca²⁺-sensing subunit STIM1 (3). SGK1 is partially effective through phosphorylation of the ubiquitin ligase Nedd4-2 (neuronal precursor cells expressed developmentally downregulated), which ubiquitinates Orai1, thus preparing the channel protein for degradation (9). Moreover, SGK1 activates the transcription factor NF-κB, which is required for Orai1 and STIM1 transcription (10). However, although regulation of Ca²⁺-dependent actin cytoskeleton dynamics is a crucial step for MC degranulation, nothing is known about the role of SGK1 in the process of actin restructuring.

We therefore addressed the question whether SGK1-dependent Ca²⁺ entry through CRAC channels is required for actin cytoskeleton rearrangement in MCs and whether modulation of actin architecture could underlie decreased degranulation of *sgk1*^{−/−} MCs.¹

MATERIALS AND METHODS

Animals. All animal experiments were conducted according to the guidelines of the American Physiological Society as well as the German law for the welfare of animals and were approved by local authorities.

Culture of bone marrow-derived mast cells. Bone marrow cells derived from femur and tibia of 6- to 12-wk-old *sgk1*^{+/+} and *sgk1*^{−/−} naive mice were cultured for 4 wk in RPMI 1640 (GIBCO, Carlsbad, Germany) containing 10% fetal calf serum, 1% penicillin-streptomycin, 20 ng/ml interleukin-3 (Immunotools, Friesoythe, Germany), and 100 ng/ml of the c-kit ligand stem cell factor (SCF, Immunotools). Bone marrow-derived mast cell (BMMC) maturation was confirmed by flow cytometry (FACSCalibur, Becton Dickinson) using the following specific fluorescent-labeled antibodies: phycoerythrin-labeled

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anti-FcεRI (eBioscience, Frankfurt, Germany), allophycocyanin-labeled anti-CD117 (BD Biosciences, Heidelberg, Germany) and fluorescein-isothiocyanate-labeled anti-CD34 (BD Biosciences). For experiments, BMMCs were sensitized for 1 h with monoclonal mouse anti-dinitrophenyl mouse IgE (anti-DNP IgE, 10 μg/ml per 1×10^6 cells, clone SPE-7, Sigma-Aldrich, Munich, Germany) in culture medium and then challenged with antigen, dinitrophenyl-human serum albumin (DNP-HSA, 50 ng/ml, Sigma-Aldrich). In some experiments, the inhibitor of Ca^{2+} channels 2-aminoethoxydiphenyl borate (2-APB, 50 μM, Sigma-Aldrich) or the actin cytoskeleton-stabilizing/disrupting agents phalloidin (500 nM, Santa Cruz)/cytochalasin B (3 μM, Sigma-Aldrich) were used, as indicated.

Transmission electron microscopy. BMMCs were fixed with prewarmed Karnovsky's fixative for 10 min at 37°C and stored at 4°C. Cell pellets were embedded in 3.5% agarose at 37°C and coagulated at room temperature. Embedded cell pellets were fixed in Karnovsky's solution. Postfixation was based on 1.0% osmium tetroxide containing 1.5% K-ferrocyanide in aqua bidest for 2 h. Following standard methods, blocks were embedded in glycidic ether and cut using an ultra microtome (Ultracut, Reichert, Vienna, Austria). Ultrathin sections (30 nm) were mounted on copper grids and analyzed using a Zeiss LIBRA 120 transmission electron microscope (Zeiss, Oberkochen, Germany) operating at 120 kV.

Calcium measurements. BMMCs were sensitized with 10 μg/ml IgE in the absence (for the control group) or in the presence of 2-APB (50 μM, Sigma-Aldrich) at 37°C for 1 h and subsequently loaded with 5 μM Fura-2 acetoxymethylester (Invitrogen) in the presence of 0.2 μg/ml Pluronic F-127 (Biotium) at 37°C for 30 min. Loaded BMMCs, washed once and resuspended in standard Ringer solution containing 1 mM Ca^{2+} or Ca^{2+} -free Ringer (0 mM Ca^{2+} , 0.5 mM EGTA) with or without 2-APB (50 μM, Sigma-Aldrich), were stimulated with antigen (Ag, DNP-HSA, 50 ng/ml). Ca^{2+} responses were measured under stirring with a spectrofluorimeter (LS 55, PerkinElmer), at alternate excitation wavelengths of 340 and 380 nm (37°C). The 340/380 nm ratio values were converted into nanomolar concentrations of cytosolic Ca^{2+} ($[\text{Ca}^{2+}]_i$) by lysis with Triton X-100 (Sigma-Aldrich) and a surplus of EGTA.

β-Hexosaminidase release. For the β-hexosaminidase release assay, mature BMMCs (0.3×10^6 per well) were seeded in a 96-well plate in fresh medium and treated first with either 2-APB (50 μM, 30 min, Sigma-Aldrich) or phalloidin (500 nM, 5 min, Santa Cruz) or cytochalasin B (3 μM, 5 min, Sigma-Aldrich) at 37°C or left untreated and then sensitized with 10 μg/ml anti-DNP IgE antibody for 1 h at 37°C in the absence or in the presence of the respective drug. Afterward, cells were centrifuged two times and washed in PBS (GIBCO) containing 1 mM CaCl_2 and challenged with antigen (DNP-HSA, 50 ng/ml, 15 min) at 37°C. The cells treated with 2-APB were challenged with antigen again in the presence of 2-APB (50 μM). The cells were then centrifuged and 20 μl supernatant and 20 μl of 2 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma-Aldrich) diluted in 0.2 M citrate buffer, pH 5, were mixed and incubated for 2 h at 37°C. The reaction was terminated by the addition of 60 μl 1 M Tris buffer, pH 9.0, and the absorbance was measured at 405 nm in an ELISA microplate reader. The data are shown as percentage of total release (0.1% Triton X-100) corrected for spontaneous release.

Measurement of G/F-actin ratio. *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs (2×10^6) were treated first with either 2-APB (50 μM, 30 min, Sigma-Aldrich) at 37°C or left untreated and then sensitized with 10 μg/ml anti-DNP IgE antibody for 1 h at 37°C in the absence or in the presence of 2-APB (50 μM) and then challenged with antigen (DNP-HSA, 50 ng/ml, 15 min) again in the absence or in the presence of 2-APB (50 μM) at 37°C.

The actin dynamics and content were estimated by flow cytometry analysis as described previously (38). In brief, cells were fixed with paraformaldehyde (4%, 15 min, 20°C), washed three times with PBS, permeabilized with 0.1% Triton X-100 (5 min, 4°C), washed three times with PBS plus 0.1% FCS, and stained with 5 μg/ml DNaseI

Alexa-488 and phalloidin Alexa-647 (1:100) (Molecular Probes, Invitrogen). The G-actin/F-actin ratio was calculated using the mean fluorescent intensity measured by FACS (FACSCalibur).

The biochemical determination of the ratio of soluble actin to total actin was performed as previously described (27, 35). Briefly, cells were incubated for 5 min in 0.3% Triton X-100 lysis buffer (5 mM Tris, 2 mM EGTA, 300 mM sucrose, 400 μM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 2 μM phalloidin) and precipitated with equal volume of 6% perchloric acid (PCA; Ts cluster). Insoluble proteins were scraped and precipitated with 3% PCA (Ti cluster). Pellets were diluted in 0.1 N NaOH. Equal volumes of Ts and Ti clusters mixed with $2 \times$ sample buffer were analyzed by SDS-PAGE/Western blotting and band intensity of Ts cluster were divided with the summary (total) of the band intensity of Ts and Ti cluster. The monomeric (G) to filamentous (F)-actin ratio was determined as previously described (18).

Confocal laser scanning microscopy. *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs (0.1×10^6) were treated first with either 2-APB (50 μM, 30 min, Sigma-Aldrich) at 37°C or left untreated and then sensitized with 10 μg/ml anti-DNP IgE antibody for 1 h at 37°C in the absence or in the presence of 2-APB (50 μM) and then challenged with antigen (DNP-HSA, 50 ng/ml, 15 min) again in the absence or in the presence of 2-APB (50 μM) at 37°C.

Direct fluorescence microscopy of F-actin was performed as previously described (11, 12). In brief, cells were fixed with 4% paraformaldehyde in PBS for 15 min, permeabilized with 0.1% Triton X-100 in PBS (10 min), and incubated with rhodamine-phalloidin (1:100 dilution; Molecular Probes, Eugene, OR) for 40 min in the dark. All specimens were examined with a BH-2 microscope (Olympus, Lake Success, NY) equipped with epifluorescence illumination. Confocal microscopy was performed with a Zeiss LSM 5 EXCITER confocal laser-scanning module (Carl Zeiss), and images were analyzed with the instrument's software.

Statistics. Data are provided as means $\pm$ SE, and *n* represents the number of independent experiments. All data were tested for significance using Student's unpaired two-tailed *t*-test or ANOVA (Bonferroni test), where applicable. Results with *P* < 0.05 were considered statistically significant.

RESULTS

As shown previously, *sgk1*^{-/-} BMMCs have an impaired FcεRI-dependent degranulation if compared with *sgk1*^{+/+} cells (33). Electron microscopy analysis revealed that granules of *sgk1*^{-/-} BMMCs were not different from *sgk1*^{+/+} BMMCs (Fig. 1). The total granule content was also not altered in *sgk1*^{-/-} BMMCs, since when BMMCs were permeabilized with Triton X-100, the total amount of β-hexosaminidase was not significantly different between *sgk1*^{-/-} BMMCs (0.69 ± 0.02 , *n* = 20) and *sgk1*^{+/+} BMMCs (0.68 ± 0.02 , *n* = 20).

As illustrated in Fig. 2, *A* and *B*, intracellular Ca^{2+} increase upon antigen stimulation measured in the presence of extracellular Ca^{2+} was attenuated in *sgk1*^{-/-} BMMCs compared with *sgk1*^{+/+} BMMCs, a finding confirming earlier observations (33). In both *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs, the CRAC channel blocker 2-APB (50 μM) almost abrogated the increase of cytosolic Ca^{2+} $[\text{Ca}^{2+}]_i$ upon FcεRI stimulation (Fig. 2, *A* and *B*). No significant difference of residual $[\text{Ca}^{2+}]_i$ increase was observed between 2-APB-treated *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs. Impaired increase of $[\text{Ca}^{2+}]_i$ in *sgk1*^{-/-} BMMCs reflected an impaired Ca^{2+} entry in those cells, since when BMMCs were stimulated with antigen in the absence of extracellular Ca^{2+} no difference was observed between genotypes (Fig. 2C). Moreover, 2-APB had no effect on antigen-induced Ca^{2+} release (Fig. 2C).

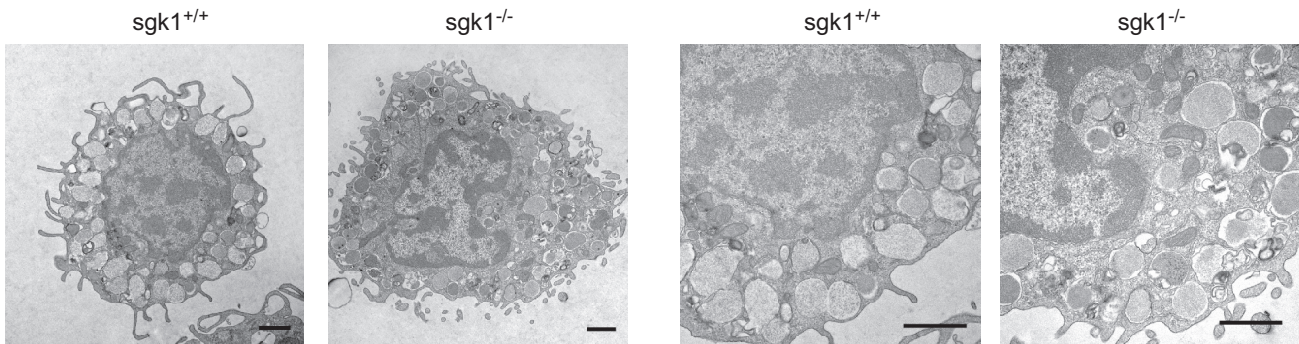


Fig. 1. Transmission electron microscopy of bone marrow-derived mast cells from serum- and glucocorticoid-inducible kinase 1 (SGK1)-positive (*sgk1*^{+/+}) and -deficient (*sgk1*^{-/-}) mice. Bars, 1 μ m.

When Ca^{2+} entry through CRAC channels was inhibited by 2-APB, MC degranulation was strongly decreased in both *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs and the difference between genotypes was abolished (Fig. 3).

As illustrated in Fig. 3, degranulation of both *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs was sensitive to actin-stabilizing (phalloidin) or actin-disrupting (cytochalasin B) agents. Release of β -hexosaminidase was impaired by phalloidin in *sgk1*^{+/+} BMMCs and enhanced by cytochalasin B in both *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs. Again, no significant difference in degranulation between genotypes could be observed in phalloidin- or cytochalasin-treated cells (Fig. 3A). These results were supported by electron microscopy analysis (Fig. 3B). The basal degranulation was not different between *sgk1*^{+/+} (0.024 ± 0.003 , $n = 13$) and *sgk1*^{-/-} (0.023 ± 0.004 , $n = 13$) BMMCs,

and neither treatment with 2-APB (0.022 ± 0.001 , $n = 3$ in *sgk1*^{+/+} BMMCs and 0.030 ± 0.013 , $n = 3$ in *sgk1*^{-/-} BMMCs), nor with cytochalasin B (0.028 ± 0.012 , $n = 3$ in *sgk1*^{+/+} BMMCs and 0.020 ± 0.001 , $n = 3$ in *sgk1*^{-/-} BMMCs), nor with phalloidin (0.025 ± 0.003 , $n = 5$ in *sgk1*^{+/+} BMMCs and 0.019 ± 0.002 , $n = 5$ in *sgk1*^{-/-} BMMCs) led to any significant change.

These data reveal that modification of actin architecture affects degranulation and its sensitivity to SGK1. Accordingly, additional experiments were performed to explore whether SGK1 influences monomeric (G) versus filamentous (F) actin equilibrium. For this purpose, BMMCs were stained with DNase1 Alexa-488 and phalloidin Alexa-647 that selectively bind to G- and F-actin, respectively. The fluorescence was measured by FACS, and the G/F-actin ratio was calculated.

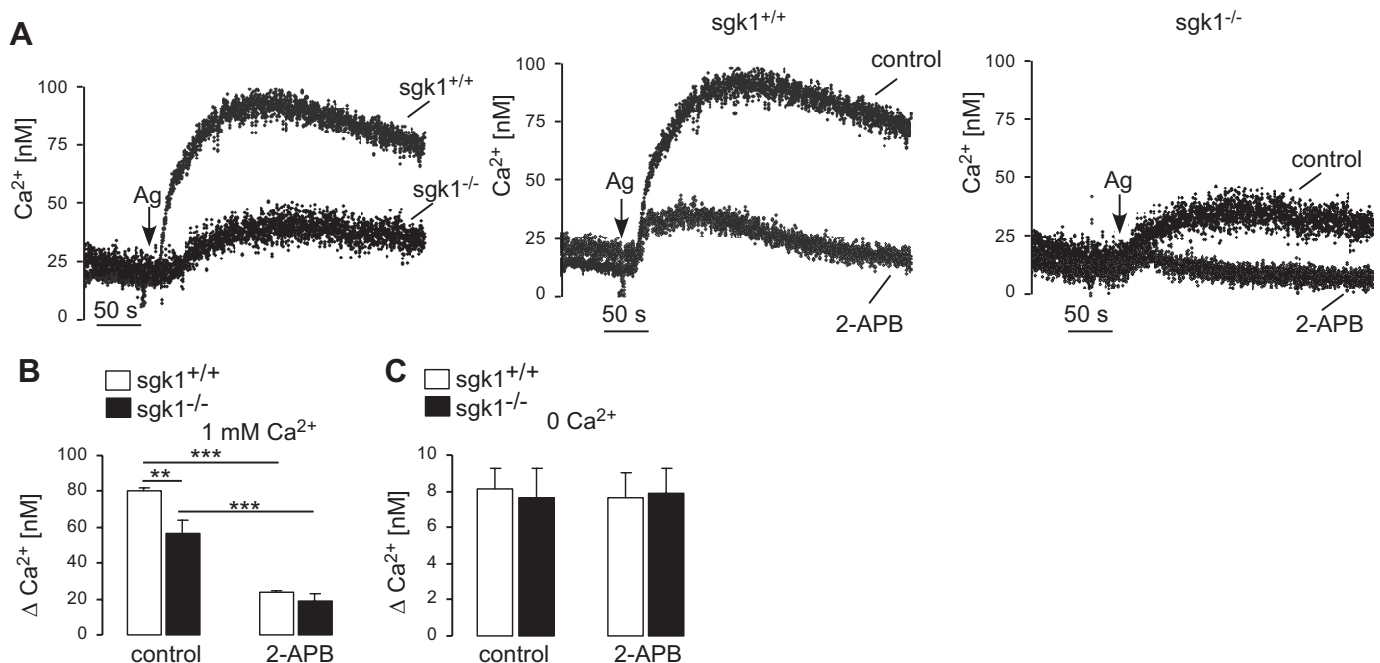


Fig. 2. Antigen-induced Ca^{2+} response in *sgk1*^{+/+} and *sgk1*^{-/-} mast cells. A: representative tracings of Fura-2-fluorescence reflecting cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) of IgE (10 μ g/ml, 1 h)-sensitized *sgk1*^{+/+} and *sgk1*^{-/-} bone marrow-derived mast cells (BMMCs) in the absence (for the control group) or in the presence of 2-aminoethoxydiphenyl borate (2-APB; 50 μ M) before and following acute stimulation with 50 ng/ml antigen (Ag) in Ca^{2+} -containing (1 mM) Ringer in the absence (control) or in the presence of 2-APB (50 μ M). B: arithmetic means of maximal $[\text{Ca}^{2+}]_i \pm \text{SE}$ ($n = 5$ per group) as measured in A. $**P < 0.01$, $***P < 0.001$, ANOVA. C: arithmetic means of maximal $[\text{Ca}^{2+}]_i \pm \text{SE}$ ($n = 9$ per group) of IgE (10 μ g/ml, 1 h)-sensitized *sgk1*^{+/+} (open bars) and *sgk1*^{-/-} (closed bars) BMMCs in the absence (for the control group) or in the presence of 2-APB (50 μ M) before and following acute stimulation with 50 ng/ml antigen in Ca^{2+} -free (0 mM Ca^{2+} , 0.5 mM EGTA) Ringer in the absence (control) or in the presence of 2-APB (50 μ M).

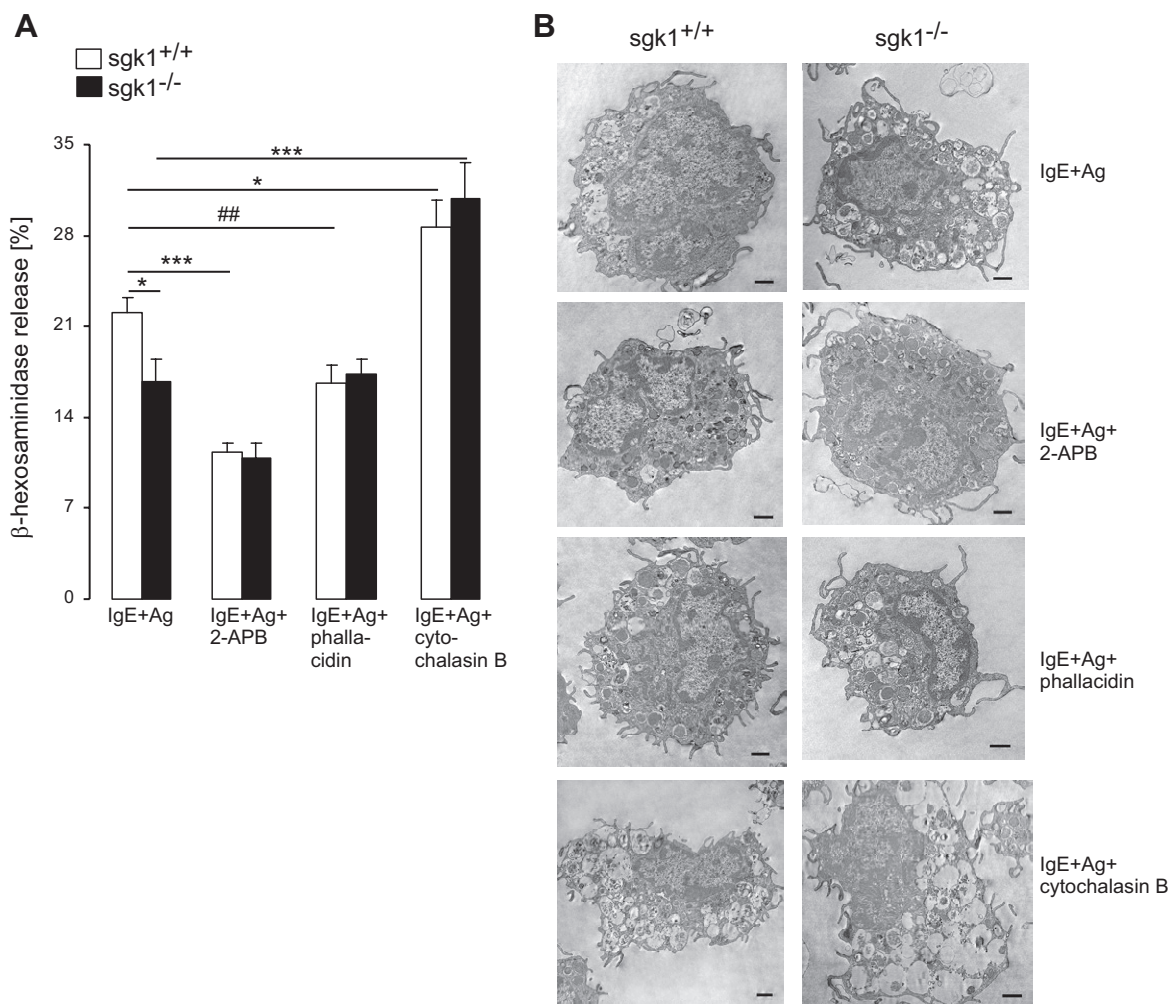


Fig. 3. β -Hexosaminidase release from *sgk1*^{+/+} and *sgk1*^{-/-} mast cells. **A**: mean β -hexosaminidase release ($\pm$ SE, $n = 11$ –23 individual experiments) from cultured *sgk1*^{+/+} (open bars) and *sgk1*^{-/-} (closed bars) BMMCs treated first with either 2-APB (50 μ M, 30 min) or phalloidin (500 nM, 5 min) or cytochalasin B (3 μ M, 5 min) or left untreated, then sensitized with IgE (10 μ g/ml, 1 h) in the absence or in the presence of the respective inhibitor and then stimulated for 15 min with 50 ng/ml antigen. When 2-APB-treated cells were stimulated with antigen, then 2-APB (50 μ M) was also added to the medium. The stimulated β -hexosaminidase release in each experiment was corrected for the spontaneous release. * $P < 0.05$, *** $P < 0.001$, ANOVA; ## $P < 0.01$, significant only with two-tailed unpaired t -test. **B**: transmission electron microscopy of degranulating *sgk1*^{+/+} and *sgk1*^{-/-} BMMCs under the same conditions as in **A**. Scale bars, 1 μ m.

Upon Fc ϵ RI ligation the G/F-actin ratio increased in *sgk1*^{+/+} BMMCs, reflecting F-actin depolymerization (Fig. 4A). This effect was abolished upon inhibition of Ca²⁺ entry through CRAC channels by 2-APB, showing that Ca²⁺ entry is required for actin depolymerization (Fig. 4A). Interestingly, in *sgk1*^{-/-} BMMCs the G/F-actin ratio remained unchanged upon antigen stimulation and no significant impairment of this ratio was detected upon 2-APB treatment (Fig. 4A).

These results were further confirmed by quantifying G/F-actin ratio using Western blot analysis of detergent-soluble and -insoluble cell fractions to monitor the cellular soluble G-actin and insoluble F-actin levels. As shown in Fig. 4, B and C, quantitative immunoblot analysis of Triton X-100-insoluble cytoskeletal pellets and corresponding supernatants revealed a significant increase of the Triton-soluble (monomeric) over Triton-insoluble (filamentous) actin ratio, indicating actin depolymerization in *sgk1*^{+/+} BMMCs stimulated with antigen. This effect was reversed by the CRAC channel inhibitor 2-APB. Again, no actin depolymerization upon antigen stimulation was observed in *sgk1*^{-/-} BMMCs and no effect on

actin dynamics was observed in *sgk1*^{-/-} BMMCs treated with 2-APB (Fig. 4, B and C).

The quantitative data on actin reorganization were fully supported by confocal laser scanning microscopic analysis showing disassembly of cortical F-actin upon Fc ϵ RI stimulation in *sgk1*^{+/+} but not in *sgk1*^{-/-} BMMCs (Fig. 5). Inhibition of CRAC channels with 2-APB prevented Fc ϵ RI-mediated fragmentation of the F-actin ring in *sgk1*^{+/+} BMMCs (Fig. 5).

DISCUSSION

The present study establishes for the first time the role of SGK1 in promoting F-actin disassembly upon mast cell (MC) stimulation via Fc ϵ RI receptor. Actin cortex has been suggested to act as a barrier, preventing the access of secretory vesicles to the plasma membrane (1, 6, 8, 23, 25, 28, 34). The disassembly of the actin cortex depends on the increase of cytosolic Ca²⁺ (26, 34). SGK1 stimulates Orai1 and STIM1 transcription (10) and prolongs Orai1 lifespan in the plasma membrane (9), being therefore an indispensable regulator of

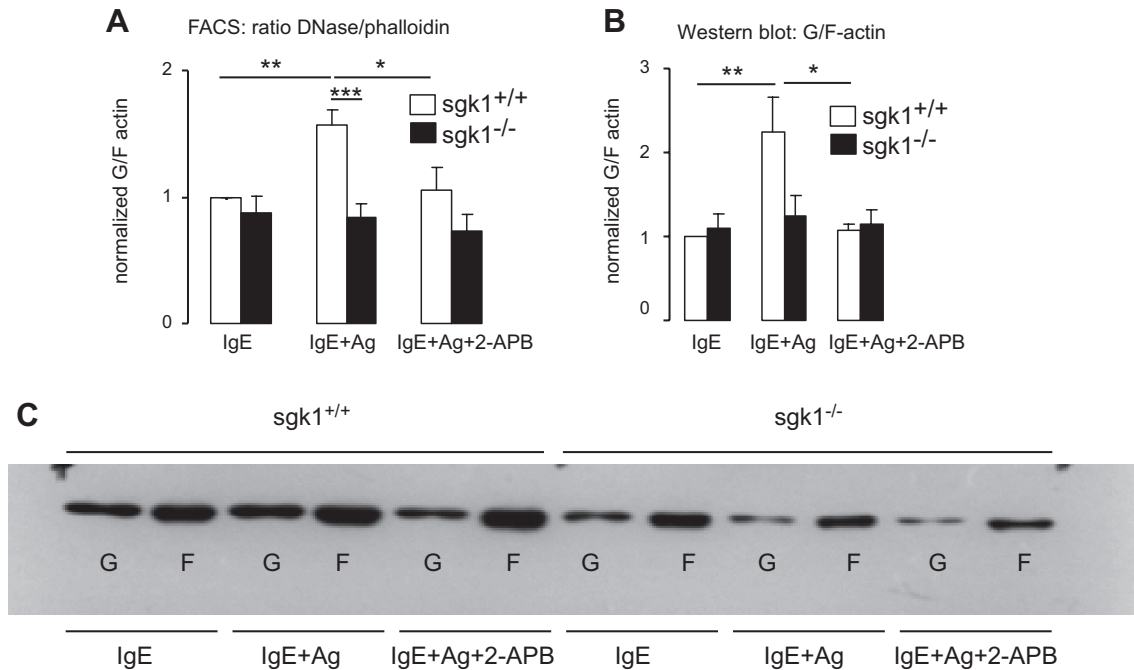


Fig. 4. G/F-actin ratio upon stimulation of *sgk1*^{+/+} and *sgk1*^{-/-} mast cells upon antigen-IgE-induced stimulation. Mean G/F-actin ratio ($\pm$ SE) of *sgk1*^{+/+} (open bars) and *sgk1*^{-/-} (closed bars) BMMCs, sensitized with IgE (10 μ g/ml, 1 h), challenged with antigen (50 ng/ml) in the absence (IgE + Ag) or in the presence (IgE + Ag + 2-APB) of 2-APB (50 μ M). **A**: cells were costained with DNaseI Alexa-488 (G-actin) and phalloidin Alexa-647 (F-actin), and the G/F-actin ratio was calculated from the mean fluorescence intensity obtained by FACS ($n = 7-11$). **B**: G/F-actin ratio was measured by quantitative immunoblot analysis after Triton X-100 subcellular fractionation ($n = 4-7$). **C**: one representative blot is shown. The data were normalized to the G/F-actin ratio of *sgk1*^{+/+} IgE-sensitized cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ANOVA.

store-operated Ca^{2+} entry. Accordingly, SGK1-deficient mast cells have decreased transcript levels of Orail and STIM1 (9, 10). The present study demonstrates that SGK1-regulated Ca^{2+} entry upon Fc ϵ RI receptor cross-linkage of bone marrow-derived MCs (BMMCs) triggers cortical actin depolymerization.

Mast cells develop normally in *sgk1*^{-/-} mice (33), and there were no changes in the granules of SGK1-deficient MCs, as revealed by electron microscopy in the present study. Similarly, there was no difference in granule content between the two genotypes, since the amount of β -hexosaminidase obtained upon

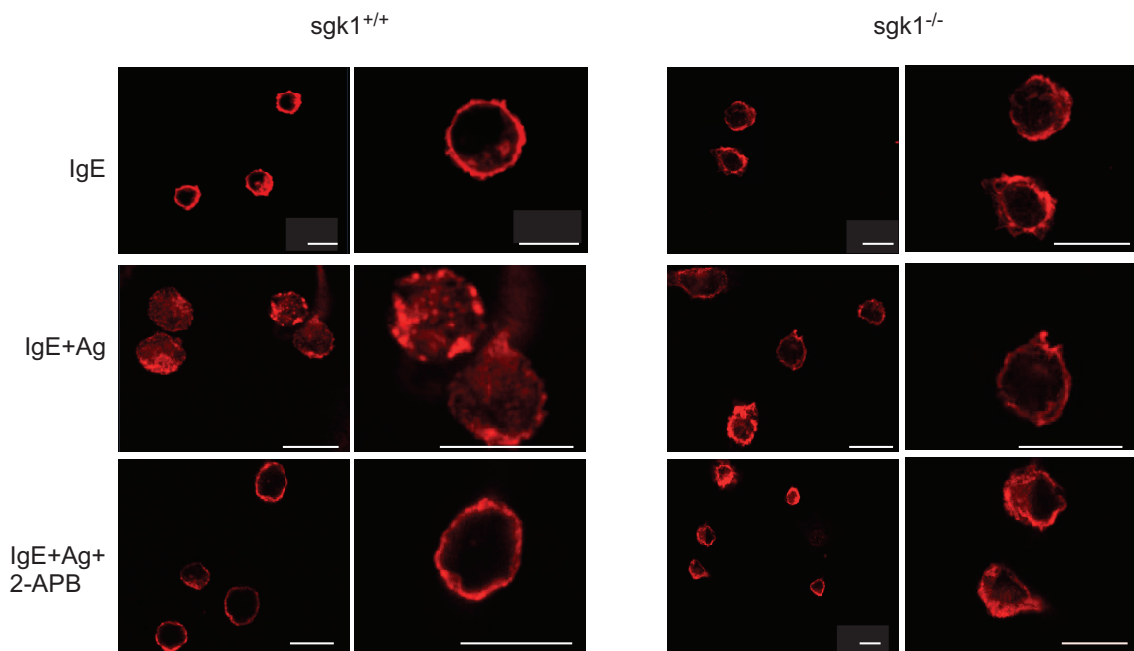


Fig. 5. Actin cytoskeleton rearrangement upon antigen-IgE-induced stimulation of *sgk1*^{+/+} and *sgk1*^{-/-} mast cells. Confocal laser scanning microscopic analysis of F-actin in *sgk1*^{+/+} (left) and *sgk1*^{-/-} (right) BMMCs, sensitized with IgE (10 μ g/ml, 1 h, top), challenged with antigen (50 ng/ml) in the absence (IgE + Ag, middle) or in the presence (IgE + Ag + 2-APB, bottom) of 50 μ M 2-APB. Scale bars, 10 μ m.

BMMC permeabilization was not different between genotypes. Moreover, impaired degranulation of *sgk1*^{-/-} BMMCs could be restored to the levels observed in *sgk1*^{+/+} BMMCs by cytochalasin B, an F-actin-disrupting agent. This indicates that modulation of actin architecture might underlie the severe phenotype of SGK1-deficient BMMCs.

The present study provides a link between SGK1-dependent Ca²⁺ entry and actin depolymerization, required for MC degranulation. However, it does not exclude other SGK1- and Ca²⁺-dependent mechanisms affecting MC degranulation. The present paper did not address the point of convergence between SGK1-regulated Ca²⁺ entry and depolymerization of actin cortex. It may involve Ca²⁺-activated proteins regulating actin dynamics. Gelsolin and villin are prominent Ca²⁺-dependent actin severing and capping proteins that regulate actin reorganization (7, 15). While gelsolin is expressed in bone marrow-derived MCs, villin expression is undetectable (unpublished observations). These preliminary findings imply that gelsolin could be a candidate governing actin disassembly upon SGK1-dependent Ca²⁺ entry in mast cells. In addition, it has been shown that myosin II-based contractility is required for the cortical F-actin disassembly in mast cells (34). The following pathway was proposed: Ca²⁺ → calmodulin → myosin light chain kinase (MLCK) → myosin-II activation → contraction → disassembly (34), pointing to calmodulin as a possible molecular link. However, the results of Sullivan et al. (34) suggest a modulatory rather than an essential role of myosin in the process of degranulation. Interestingly, the late steps of mast cell degranulation may be modulated by unconventional myosin (34). On the other hand, myosin light chain function acting downstream of Rho/ROCK signaling is involved as well in actin cytoskeleton reorganization regulated by bone morphogenetic protein signaling (17). Clearly, further work will be required to fully elucidate the mechanisms linking cytosolic Ca²⁺ and actin cytoskeleton disassembly in MCs.

Interestingly, MCs from p21-activated kinase Pak1-deficient mice (1) display a very similar phenotype of actin depolymerization and mast cell degranulation as observed in the present study with SGK1-deficient MCs. Pak1-deficient MCs are similarly unable to undergo F-actin disassembly following cross-linkage of the FcεRI receptor, an effect resulting from impaired Ca²⁺ entry. However, how Pak1 is involved in Ca²⁺ mobilization is unclear. Since Pak1 is regulating actin cytoskeleton, one intriguing possibility would be that Ca²⁺ influx itself is influenced by the modulation of cytoskeletal elements. Indeed, different ion channels have been shown to be regulated by cytoskeleton dynamics. Thus, the activity of a Na⁺ channel in human myeloid leukemia K562 cells is extremely sensitive to the state of actin cytoskeleton (19, 21, 22, 30, 31). Depolymerization of actin results in Na⁺ channel activation whereas application of G-actin under conditions promoting its rapid polymerization abolishes Na⁺ channel activity (19, 21, 22). Moreover, Na⁺ channel activity could be manipulated through capping of actin filaments and modulating the filament length, indicating that actin dynamics (capping-uncapping, filament growth-shrinkage) can be a powerful regulator of ion channel activity (31). In addition, Na⁺/P_i cotransport activation was shown to rapidly modify cytoskeletal protein polymerization dynamics in opossum kidney cells (25), an effect that was shown to be regulated by PLC-γ1 signaling and Ca²⁺-dependent villin activation (26).

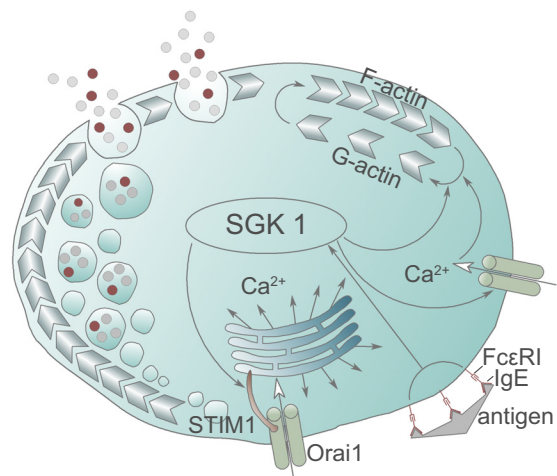


Fig. 6. Schematic depicting the model of SGK1-dependent reorganization of actin cytoskeleton.

However, CRAC channel activation seems to be insensitive to actin cytoskeleton rearrangement. Thus, in the RBL-1 rat basophilic cell line, the widely used model system for mast cells, stabilization as well as disassembly of actin microfilaments fails to alter the rate or extent of activation of CRAC channel (4). Moreover, in T cells, CRAC channels are similarly unaffected by actin stabilization or disruption (20). On the other hand, the microtubule cytoskeleton participates in the maintenance of Ca²⁺ influx upon MC degranulation (24). It has been suggested that microtubules play a facilitative role in store-operated Ca²⁺ entry pathway by optimizing the localization of STIM1 (32). On the other hand, microtubule distribution in MCs itself depends on STIM1 activity and Ca²⁺ influx (13), closing a positive feedback loop.

SGK1 has been shown to induce microtubule depolymerization in hippocampal neurons by a direct action of SGK1 on microtubules and by the SGK1-mediated phosphorylation of tau (37). On the other hand, SGK1 requires interaction with F-actin to phosphorylate the transient receptor potential vanilloid 4 (TRPV4) cation channel (29). Therefore it is tempting to speculate that SGK1 and actin cytoskeleton mutually interact in MCs. In any case, SGK1-dependent regulation of actin cytoskeleton rearrangement demonstrated in the present study broadens the scope of SGK1 as cytoskeleton regulator.

In conclusion, our studies provide new insights into the role of SGK1 in mast cell biology and identify a novel mechanism of actin cytoskeleton regulation through SGK1-dependent Ca²⁺ entry (Fig. 6).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Evi.S., C.S., and Ek.S. conception and design of the research; Evi.S., S.G., W.Y., P.M., and M.S. performed the experiments; Evi.S., S.G., W.Y., P.M., M.S., and Ek.S. analyzed the data; Evi.S., S.G., C.S., and Ek.S. interpreted the results of the experiments; Evi.S. and Ek.S. prepared the figures; Evi.S., C.S., and Ek.S. drafted the manuscript; Evi.S., F.L., C.S., and Ek.S. edited and revised the manuscript; Evi.S., C.S., and Ek.S. approved the final version of the manuscript.

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