Blunted IgE-mediated activation of mast cells in mice lacking the serum- and glucocorticoid-inducible kinase SGK3

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Submitted 7 December 2009; accepted in final form 30 July 2010

Zemtsova IM, Heise N, Fröhlich H, Qadri SM, Kucherenko Y, Boini KM, Pearce D, Shumilina E, Lang F. Blunted IgE-mediated activation of mast cells in mice lacking the serum- and glucocorticoid-inducible kinase SGK3. Am J Physiol Cell Physiol 299: C1007–C1014, 2010. First published August 4, 2010; doi:10.1152/ajpcell.00539.2009.—Previous studies have shown that pharmacological inhibition of the phosphoinositol-3 (PI3) kinase disrupts the activation of mast cells. Through phosphoinositol-3-dependent kinase PKD1, PI3 kinase activates the serum- and glucocorticoid-inducible kinase 3 (SGK3). The present study explored the role of SGK3 in mast cell function. Mast cell numbers in the ear conch were similar in both genotypes. Stimulation with IgE and cognate antigen triggered the release of intracellular Ca2+ and entry of extracellular Ca2+ into BMMCs. Influx of extracellular Ca2+ but not Ca2+ release from intracellular stores was significantly blunted in sgk3−/− BMMCs compared with sgk3+/+ BMMCs. Antigen stimulation further led to a rapid increase of a K+-selective conductance in sgk3−/− BMMCs, an effect again blunted in sgk3−/− BMMCs. In contrast, the Ca2+ ionophore ionomycin activated K+ currents to a similar extent in sgk3−/− and in sgk3+/+ BMMCs. β-Hexosaminidase release, triggered by antigen stimulation, was also significantly decreased in sgk3−/− BMMCs. IgE-dependent anaphylaxis measured as a sharp decrease in body temperature upon injection of DNP-HSA antigen was again significantly blunted in sgk3−/− compared with sgk3+/+ mice. Serum histamine levels measured 30 min after induction of an anaphylactic reaction were significantly lower in sgk3−/− than in sgk3+/+ mice. In conclusion, both in vitro and in vivo function of BMMCs are impaired in gene targeted mice lacking SGK3. Thus SGK3 is critical for proper mast cell function.

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MATERIALS AND METHODS

Mice. All animal experiments were conducted according to the German law for the welfare of animals and were approved by local authorities.

The targeting strategy for disruption of the Sgk3 gene has been described earlier (58). To generate mice homozygous for the targeted allele, the resulting heterozygote (sgk3+/−) males and females were...
interbred to yield SGK3-deficient mice (sgk3<sup>−/−</sup>) and their wild-type littermates (sgk3<sup>+/+</sup>.

**Culture of bone marrow-derived mast cells.** Mast cells were isolated from femoral bone marrow of 6- to 8-wk-old naive sgk3<sup>+/+</sup> and sgk3<sup>−/−</sup> mice and cultured for 4 wk in RPMI 1640 (Invitrogen Life Technologies) containing 10% FCS, 1% penicillin-streptomycin, 20 ng/ml IL-3 (R&D Systems), and 100 ng/ml of the c-kit ligand stem cell factor (PeproTech). Bone marrow mast cell (BMMC) maturation was confirmed by flow cytometry (FACSCalibur; BD Biosciences) using the following specific fluorescent-labeled Abs: PE-labeled anti-FceRI (eBioscience), allophycocyanin-labeled anti-CD117 (BD Pharmingen), and FITC-labeled anti-CD34 (BD Pharmingen). Cells were kept in culture 4–6 wk before the experiments. For experiments, BMMCs were sensitized for 1 h with monoclonal mouse antidinitrophenyl (DNP) mouse IgE (anti-DNP IgE, 5–10 µg/ml per 1 × 10⁶ cells, clone SPE-7; Sigma-Aldrich) in culture medium and challenged with DNP-human serum albumin (DNP-HSA; 50 ng/ml; Sigma-Aldrich).

**Determination of mast cell numbers in the ear conches.** Anesthetized mice were euthanized by cervical dislocation, and the skin was cleansed with 70% ethanol. Ear conches were cut off at the base, fixed in 4% paraformaldehyde overnight, and finally embedded in paraffin. Tissue sections (4-µm thick) taken from the middle of the conches were prepared, deparaffinized, and stained with toluidine blue. Mast cell numbers of 10 different areas on different slices per conch of 4 sgk3<sup>+/+</sup> and 3 sgk3<sup>−/−</sup> mice were determined using a Zeiss Axiovert 200 microscope with a LD Achroplan ×20 lens in brightfield mode.

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**Fig. 1.** Maturation of bone marrow mast cells (BMMCs) from sgk3<sup>+/+</sup> and sgk3<sup>−/−</sup> mice. A: original dot plots of CD117-, CD34-, and FcεRI-positive BMMCs from sgk3<sup>+/+</sup> and sgk3<sup>−/−</sup> mice. Numbers depict the percentage of cells in the respective quadrant, acquired within the mast cell gate. B: frequency of mast cells in primary culture. Mean percent (± SE; n = 6 individual BMMC cultures) of sgk3<sup>+/+</sup> (open bars) and sgk3<sup>−/−</sup> (closed bars) BMMCs acquired within the mast cell gate. C: ear conche sections of sgk3<sup>+/+</sup> (top) and sgk3<sup>−/−</sup> (bottom) mice stained with toluidine blue for mast cell detection (mast cells are indicated by black arrows). D: number of mast cells (±SE) in skin, analyzed by staining of ear conche sections with toluidine blue. Mean mast cell numbers of toluidine blue-positive cells in one area (×200 magnification) as calculated from 10 different areas on different slices per conch of 4 sgk3<sup>+/+</sup> (open bar) and 3 sgk3<sup>−/−</sup> (closed bar) mice (P = 0.43, two-tailed unpaired t-test).
Patch clamp. Patch-clamp experiments were performed at room temperature in voltage-clamp, fast-whole cell mode (41). BMMCs were continuously superfused by a flow system inserted into the dish. The bath was grounded via a bridge filled with NaCl-Ringer solution containing (in mM) 145 NaCl, 5 KCl, 1.2 MgCl₂, 1 CaCl₂, 10 glucose, and 10 HEPES/NaOH (pH 7.4, 300 mosM). Borosilicate glass pipettes (2–4 MΩ) with pipette tips manufactured by a microprocessor-driven DMZ puller (Zeitz, Augsburg, Germany) were used in combination with a MS314 electrical micromanipulator (MW, Märzhäuser, Wet zlar, Germany). The currents were determined as 10 successive 200-ms square pulses from HEKA (Lambrecht, Germany) using Pulse software (HEKA) and an EPC-9 amplifier with a MS314 electrical micromanipulator (MW, Märzhäuser, Wet zlar, Germany) manufactured by a microprocessor-driven DMZ puller (Zeitz, Augsburg, Germany) were used in combination with an inverted phase-contrast microscope (Axiovert 100, Zeiss, Oberkochen, Germany). Cells were excited alternatively at 340 and 380 nm, and the light was deflected by a dichroic mirror into either the objective (Fluar ×40/1.30 oil, Zeiss, Oberkochen) or a camera. Emitted fluorescence intensity was recorded at 505 nm and data acquisition was performed by using specialized computer software (Metaflour, Universal Imaging, Downingtown). Intracellular Ca²⁺ was measured and following addition of DNP-HSA to IgE-sensitized BMMCs in the absence or presence of extracellular Ca²⁺.

As a measure for the increase of cytosolic Ca²⁺ activity, the slope and peak of the changes in the 340/380 nm ratio were calculated for each experiment. For intracellular calibration purposes, ionomycin (10 μM) was applied at the end of each experiment. Experiments were performed with Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1.2 MgSO₄, 2 CaCl₂, 2 Na₂HPO₄, 32 HEPES, and 5 glucose, pH 7.4.

Measurement of degranulation. Mature BMMCs were seeded on 96-well plates in fresh medium with anti-DNP IgE antibody (5 μg/ml) for 1 h. Afterwards cells were washed in Tyrode salt solution (Sigma-Aldrich) and challenged with DNP-HSA (50 ng/ml). Twenty microliters of supernatant and 20 μl of 2 mM 4-nitrophenyl α-D-glucosaminide (Sigma-Aldrich), diluted in 0.2 M citrate buffer, pH 4.5, were added to each well of the 96-well plate, and color was developed for 2 h at 37°C. The reaction was terminated with 1 M Tris solution. As a measure for the increase of cytosolic Ca²⁺ activity, the slope and peak of the changes in the 340/380 nm ratio were calculated for each experiment. For intracellular calibration purposes, ionomycin (10 μM) was applied at the end of each experiment. Experiments were performed with Ringer solution containing (in mM) 125 NaCl, 5 KCl, 1.2 MgSO₄, 2 CaCl₂, 2 Na₂HPO₄, 32 HEPES, and 5 glucose, pH 7.4.

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ELISA microplate reader. The data are expressed as the percentage of the total release (Triton X-100 0.1%) and are corrected for spontaneous release.

Passive systemic anaphylaxis/antigen-induced anaphylaxis and serum histamine concentrations. Mice were sensitized with 30 μg/250 μl anti-DNP IgE by intraperitoneal application. Five hours later, mice were challenged with either DNP-HSA (100 μg/200 μl) or PBS. Body temperature was monitored before and every minute after antigen challenge with an 8-Channel USB Thermometer (Tübingen, Germany) during the midportion of the light phase of the light cycle. Mice were placed with the tail raised, and the Vaseline-covered probe was inserted a standardized distance of 2 cm until a stable temperature reading was obtained. Baseline temperature was measured after mice were habituated to rectal probe insertion. Ambient room temperature was 23°C, and the animals were exposed to a 12-h light and 12-h dark cycle (7 AM to 7 PM). Data are provided as means ± SE; n represents the number of animals/independent experiments. All data were tested for significance using Student’s unpaired two-tailed t-test or ANOVA (Dunnet’s test), where applicable. P < 0.05 was considered to indicate statistical significance.

**RESULTS**

Cells were derived from the bone marrow (BMMCs) of SGK3 knockout mice (sgk3<sup>−/−</sup>) and their wild-type littermates (sgk3<sup>+/+</sup>), and expression of the mast cell surface markers CD117, CD34, and FcεRI (Fig. 1A) was determined. No significant difference in the abundance of any of the three markers for mast cell maturation was observed between BMMCs of the two genotypes (Fig. 1B).

The number of mast cells in the skin, analyzed by staining of ear conch sections with toluidine blue (Fig. 1, C and D), was similar in sgk3<sup>+/+</sup> and sgk3<sup>−/−</sup> mice (P = 0.43).

Stimulation with IgE and cognate antigen was followed by a sharp increase of cytosolic Ca<sup>2+</sup> in sgk3<sup>+/+</sup> cells, an effect significantly blunted in sgk3<sup>−/−</sup> cells (Fig. 2, A and B). Before addition of antigen the basal Ca<sup>2+</sup> level was not different between sgk3<sup>+/+</sup> and sgk3<sup>−/−</sup> BMMCs (fluorescence ratio: 1.40 ± 0.07 in sgk3<sup>+/+</sup> vs. 1.35 ± 0.14 in sgk3<sup>−/−</sup> BMMCs). To further assess the effect of SGK3 on Ca<sup>2+</sup> mobilization, the cells were sensitized with IgE and challenged with antigen in the absence of extracellular Ca<sup>2+</sup> (Fig. 2C). As a result, in the nominal absence of extracellular Ca<sup>2+</sup>, the exposure to IgE and antigen was followed by a transient increase in intracellular Ca<sup>2+</sup>, an effect not significantly different between sgk3<sup>+/+</sup> and

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![Figure 3](http://ajpcell.physiology.org/)

Fig. 3. Antigen-induced K<sup>+</sup> currents are reduced in sgk3<sup>−/−</sup> BMMCs. A: representative whole cell currents from sgk3<sup>+/+</sup> (left) and sgk3<sup>−/−</sup> (right) BMMCs elicited by 200-ms pulses ranging from −115 to +65 mV in 20-mV increments from a holding potential of −35 mV. Currents were recorded in standard NaCl bath solution 5 min after stimulation with either Ag (50 ng/ml, top) or ionomycin (1 μM, bottom). The dotted line indicates the zero current value. B: mean current-voltage relationships (± SE, n = 7) in sgk3<sup>+/+</sup> (open symbols) and sgk3<sup>−/−</sup> (closed symbols) BMMCs before (control, squares) and 3 min after stimulation with antigen (Ag, 50 ng/ml, triangles). C: mean whole cell conductance (± SE) of sgk3<sup>+/+</sup> (open bars) and sgk3<sup>−/−</sup> (closed bars) BMMCs as recorded in B before (control) and after stimulation with either Ag (50 ng/ml) or ionomycin (1 μM). Data were calculated by linear regression between −55 and +5 mV. *P < 0.05, significant difference between sgk3<sup>+/+</sup> and sgk3<sup>−/−</sup> cells (ANOVA); ###P < 0.001, significant difference from sgk3<sup>+/+</sup> cells under control conditions (ANOVA); ##P < 0.01, significant difference from sgk3<sup>−/−</sup> cells under control conditions (ANOVA).
sgk3−/− cells (Fig. 2D). Thus lack of SGK3 predominantly impaired the entry of extracellular Ca2+.

BMMCs are known to express Ca2+-activated K+ channels KCa3.1, which are important amplifiers of Ca2+ entry upon IgE-antigen-dependent stimulation (69). The K+ currents of BMMCs upon receptor stimulation were measured in patch-clamp experiments (Fig. 3). Addition of antigen to the bath solution resulted in a rapid increase of a K+–selective conductance. The current amplitude was growing after antigen application and reached its maximum in about 3 min. The maximal amplitude was significantly blunted in sgk3−/− if compared with sgk3+/+ cells (Fig. 3). However, when the cells were stimulated with a Ca2+ ionophore ionomycin, no difference in measured K+ currents was detected between the genotypes (Fig. 3C). Accordingly, the surface expression and maximal activity of the Ca2+-activated K+ channel did not differ between sgk3+/+ and sgk3−/− cells; i.e., SGK3 deficiency primarily decreased the Ag-stimulated Ca2+ entry.

Decreased Ca2+ entry in sgk3−/− BMMCs could result in decreased antigen-induced mediator release. To determine whether SGK3 deficiency influences mast cell degranulation, the release of β-hexosaminidase was measured in sgk3+/+ and sgk3−/− cells. As shown in Fig. 4, β-hexosaminidase release was significantly reduced in sgk3−/− BMMCs.

To determine whether this defect in Ag-stimulated Ca2+ entry into sgk3−/− BMMCs affects mast cell function in vivo, we tested sgk3+/+ and sgk3−/− mice for passive systemic anaphylaxis (Fig. 5). Mice were sensitized with anti-DNP IgE intraperitoneally, and after 5 h rest, they received DNP–HSA antigen or saline as a control by intraperitoneal injection, and body temperature was monitored over time. The measured drop in body temperature following antigen treatment was reduced in sgk3−/− mice (Fig. 5, A and B). Serum histamine levels measured 30 min after induction of the anaphylactic reaction were significantly lower in sgk3−/− than those levels in sgk3+/+ mice (Fig. 5C), thus pointing to an impairment of sgk3-deficient mast cell function in vivo.

**DISCUSSION**

The present study unravels a novel function of the serum- and glucocorticoid-inducible kinase SGK3. Specifically, Ca2+ entry, Ca2+-activated K+ channel activity, and degranulation are blunted in BMMCs from SGK3 knockout mice (sgk3−/−) compared with BMMCs from their wild-type littermates (sgk3+/+). Accordingly, sgk3−/− mice appear to be more resistant to anaphylactic shock.

As shown earlier (6, 20, 22, 25, 28, 29, 44, 57, 72), Ca2+ entry through Ca2+ channels is critically important for the regulation of mast cell degranulation. The Ca2+ entry depends on the potential difference across the cell membrane (63) and
is thus influenced by the activity of Ca\(^{2+}\)-activated K\(^+\) channels (19, 20, 27, 55, 69).

The stimulation of K\(^+\) channels is blunted, but not completely inhibited, in the nominal absence of extracellular Ca\(^{2+}\) (71). Accordingly, the activation of K\(^+\) channels largely depends on extracellular Ca\(^{2+}\). However, the present data do not rule out additional mechanisms involved in the regulation of K\(^+\) channels.

In mast cells, PI3 kinase has been shown to regulate cell proliferation, adhesion, and migration, as well as antigen-IgE-induced degranulation and cytokine release (2). Moreover, PI3 kinase was suggested to target the TRPV2 Ca\(^{2+}\) channel in these cells (75). In the Xenopus oocyte heterologous expression system, SGK1 and SGK3 have previously been shown to increase the cell membrane abundance and activity of the Ca\(^{2+}\) channel TRPV5 (32) and TRPV6 (17). It is conceivable that SGK1 has a similar stimulating effect on TRPV2 and/or other Ca\(^{2+}\) channels important for Ca\(^{2+}\) entry into mast cells.

In the Xenopus oocyte system, SGK3 has been shown to stimulate the activity of several ion channels including the epithelial Na\(^+\) channel EnaC (34, 62), the renal and cochlear Cl\(^-\) channel complex CIC-Ka/barttin (30), the cell volume-regulated Cl\(^-\) channel CIC2 (61), the cardiac voltage-gated Na\(^+\) channel SCN5A (16), the cardiac K\(^+\) channels KCNE1/KCNQ1 (31) and HERG (54), the glutamate receptor GluR1 (73) as well as the voltage-gated K\(^+\) channels Kv1.3 (37, 42, 80), Kv1.5 (77), and Kv4.3 (7). SGK3 further stimulates the activity of a wide variety of transporters including the Na\(^+\)/H\(^+\) exchanger NHE3 (71), the Na\(^+\)/H\(^+\) exchanger NHE4 (9), the Na\(^+\)/H\(^+\) exchanger NHE5 (11), the creatine transporter CreaT (SLC6A8) (14), EAAT3 (67), and EAAT5 (15), the dicarboxylate cotransporter SLC22A13 (83), the myoinositol transporter SMIT (46), and the Na\(^+\)-K\(^+\)-ATPase activity (43). Both, SGK1 and SGK3 regulate the function of channels and transporters by influencing expression, trafficking, and degradation of the channel and transport proteins (50).

SGK3 has been shown in vitro to confer cell survival (52, 83), an effect that may be related to the effect of SGK3 on Kv1.3 channel activity. In human embryonic kidney cells and Jurkat lymphocytes, Kv1.3 is involved in the regulation of cell proliferation (37, 51) and apoptosis (40, 51, 74). The antipapoptotic effect may further be secondary to phosphorylation of forkhead transcription factors (21, 52, 83). Moreover, SGK3 has been shown to phosphorylate and thus inactivate Bad (52, 83). Phosphorylated Bad binds to the chaperone 14-3-3 and is thus prevented from traveling to the mitochondria, where it triggers apoptosis (53).

At first glance, it may be surprising that both the knockout of SGK1 (71) and knockout of SGK3 (this study) disrupt antigen-induced Ca\(^{2+}\) entry into and activation of mast cells. Accordingly, even though both kinases obviously serve similar functions in mast cells, they cannot fully replace each other and lack of one of the two kinases disrupts mast cell function. It must be kept in mind that SGK3 is constitutively expressed, while SGK1 is genomically upregulated following cell stress. Thus SGK3 may be required for the stimulation of channel or carrier expression in unstressed conditions; i.e., before antigen exposure, while SGK1 may be required for the full-blown entry of Ca\(^{2+}\) during stimulation.

In conclusion, SGK3 participates in antigen-stimulated Ca\(^{2+}\) entry and subsequent activation of Ca\(^{2+}\)-activated K\(^+\) channels. The latter leads to augmentation of Ca\(^{2+}\) entry and thus similarly participates in the stimulation of mast cell degranulation. Those cellular effects are critical for anaphylactic response in vivo.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the meticulous preparation of the manuscript by Tanja Loch, Sari Rübe, and Lejla Subasic.

GRANTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, SFB 766) and NIH R01-DK56695 (to D. Pearce).

DISCLOSURES

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

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