Large-conductance calcium-activated potassium channel activity is absent in human and mouse neutrophils and is not required for innate immunity

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Large-conductance Ca2+ activated K+ (BK) channels are reported to be essential for NADPH oxidase-dependent microbial killing and innate immunity in leukocytes. Using human peripheral blood and mouse bone marrow neutrophils, pharmacological targeting, and BK channel gene-deficient (BK−/−) mice, we stimulated NADPH oxidase activity with 12-O-tetradecanoylphorbol-13-acetate (PMA) and performed patch-clamp recordings on isolated neutrophils. Although PMA stimulated NADPH oxidase activity as assessed by O2− and H2O2 production, our patch-clamp experiments failed to show PMA-activated BK channel currents in neutrophils. In our studies, PMA induced slowly activating currents, which were insensitive to the BK channel inhibitor iberiotoxin. Instead, the currents were blocked by Zn2+, which indicates activation of proton channel currents. BK channels are gated by elevated intracellular Ca2+ and membrane depolarization. We did not observe BK channel currents, even during extreme depolarization to +140 mV and after elevation of intracellular Ca2+ by N-formyl-I-leucyl-I-leucyl-Iphenylalanine. As a control, we examined BK channel currents in cerebral and tibial artery smooth muscle cells, which showed characteristic BK channel current pharmacology. Iberiotoxin did not block killing of Staphylococcus aureus or Candida albicans. Moreover, we addressed the role of BK channels in a systemic S. aureus and Yersinia enterocolitica mouse infection model. After 3 and 5 days of infection, we found no differences in the number of bacteria in spleen and kidney between BK−/− and BK+/+ mice. In conclusion, our experiments failed to identify functional BK channels in neutrophils. We therefore conclude that BK channels are not essential for innate immunity.

K+ channels in neutrophils are not as well studied as those in neuronal or muscle cells (26) and even in other leukocytes such as T and B cells (2, 20). The most comprehensive study of K+ channels in neutrophils was published in 1990 by Krause and Welsh (29). They did not find BK channels in human neutrophils but found two separate K+ currents: a voltage-dependent current and a Ca2+-activated current. Neither current was sensitive to charybdotoxin. Subsequently, Ca2+-activated intermediate-conductance K+ channels were identified in cultured HL-60-derived neutrophils (47). ATP-sensitive K+ channels, which may play a role in neutrophil migration and chemotaxis in the inflammatory response, may...
also be present in neutrophils (10). BK channels had been detected in macrophages but had not been described in neutrophils (15, 18).

Given the proposed importance of BK channels for innate immunity (1), we used BK channel gene-deleted (BK−/−) mice to study the role of the BK channel in the immune system. We failed to find that PMA stimulated BK channel activity in mouse and human neutrophils. Iberiotoxin, a specific blocker of the BK channel, did not decrease ROS production and microorganism killing in human neutrophils. Survival of bacteria was not increased in mice after genetic depletion of the BK channel. Thus our results are in agreement with the data obtained by Femling et al. (17), who also could not confirm an essential role of BK channels in neutrophil function.

METHODS

Mice. All the experiments were performed with 2- to 3-month-old male and female wild-type (BK+/+) and BK−/− mice. BK−/− mice were generated as described elsewhere (42). BK+/+ and BK−/− mice were of the hybrid SV129/C57BL6 background. Litter- or age-matched mice were also infected intravenously with 107 S. aureus O:8 SE. Statistical analysis was performed by one-way analysis of variance and paired t-test.

Confocal imaging. For Ca2+ measurements, cells were loaded with 5 μM fluo 4-AM (Molecular Probes) and 0.01% pluronic acid (Calbiochem) for 30 min at 35°C in HEPES-SSS (in mM: 134 NaCl, 6 KCl, 1 MgCl2, 2 CaCl2, 10 HEPES, and 10 glucose, with pH adjusted to 7.2 with KOH), and the pipette solution contained (in mM) 110 K+-aspartate, 30 KCl, 2 MgCl2, 10 HEPES, and 10 glucose (with pH adjusted to 7.4 with NaOH) and a high-K+-pipette solution containing (in mM) 140 KCl, 10 NaCl, 2 MgCl2, 0.7 CaCl2, 1 EGTA, and 10 HEPES (with pH adjusted to pH 7.3 with KOH) to record currents. In another set of experiments, we used a high-Na+-extracellular solution containing (in mM) 134 NaCl, 6 KCl, 1 MgCl2, 2 CaCl2, 10 HEPES, and 10 glucose (with pH adjusted to 7.4 with NaOH) and a high-K+-pipette solution containing (in mM) 110 K+-aspartate, 30 KCl, 1 NaCl, 1 MgCl2, 10 HEPES, and 0.05 EGTA (with pH adjusted to 7.2 with KOH), which is routinely used in our laboratory to record BK channel currents (37, 41).

We performed some experiments using symmetrical high-K+- aspartate solutions. The bath solution contained (in mM) 110 K+-aspartate, 30 KCl, 10 NaCl, 1 MgCl2, 2 CaCl2, 10 HEPES, and 10 glucose (with pH adjusted to 7.2 with KOH), and the pipette solution contained (in mM) 110 K+-aspartate, 30 KCl, 10 NaCl, 1 MgCl2, 10 HEPES, and 0.05 EGTA (with pH adjusted to 7.2 with KOH). In another set of experiments, the symmetrical high-K+- aspartate solutions were supplemented with NH4 (14) to facilitate the measurements of proton currents. The extracellular solution contained (in mM) 80 K+-aspartate, 25 (NH4)2SO4, 2 MgCl2, 2 CaCl2, 10 HEPES, and 1 EGTA (with pH adjusted to 7.0 with KOH), and the pipette solution contained (in mM) 80 K+-aspartate, 25 (NH4)2SO4, 2 MgCl2, 2 CaCl2, 10 HEPES, and 1 EGTA (with pH adjusted to 7.0 with KOH).

Values are means ± SE. Statistical analysis was performed by one-way analysis of variance and paired t-test.
diphenylene iodonium for 15 min at 37°C and then activated with 0.025 or 1 μg/ml PMA or buffer control. Experiments were done in duplicate. Samples were incubated in 96-well plates at 37°C for up to 60 min, and the absorption of samples with and without 300 U/ml SOD was scanned repetitively at 550 nm using a Microplate Autoreader (Molecular Devices, Munich, Germany). The final ferricytochrome c concentration was 250 μM, and the final cell concentration was 3.75 × 10^6/ml.

Measurement of cellular oxidant stress by dihydrorhodamine oxidation. The generation of reactive oxygen radicals was additionally assessed using dihydrorhodamine-1,2,3 (DHR), as described previously (28). Briefly, prewarmed neutrophils [1 × 10^7/ml HBSS with Ca^{2+} and Mg^{2+} (HBSS^{++}, Biochrom)] were loaded with 1 μM DHR for 10 min at 37°C and then incubated with 100 nM iberiotoxin for 15 min at 37°C. Cells were activated with 0.025 or 1 μg/ml PMA or buffer control at 37°C. After 45 min, the reactions were stopped by addition of ice-cold 1% BSA-PBS. Samples were analyzed using a FACScan (Becton Dickinson, Heidelberg, Germany). Data were collected from 10,000 cells per sample. The shift of green fluorescence in the FL-1 mode was determined, and the mean fluorescence intensity (representing the amount of generated rhodamine 123) is reported.

Killing of *C. albicans* by human neutrophils. *C. albicans* was as assessed as previously described (12, 35). *C. albicans* were selected from single colonies grown on Sabouraud-agar plates, inoculated into Sabouraud broth, and grown overnight at 30°C. The microorganisms were washed twice in HBSS^{++}-HSA and adjusted to a density of 5 × 10^7 cells/ml. Pooled human serum (Sigma) was added to a final concentration of 10%, and microorganisms were opsonized for 10 min at 37°C. Neutrophils were isolated from peripheral blood as described above and resuspended in HBSS^{++} containing 0.05% human serum albumin (HSA) and 10% pooled human serum. Neutrophils were preincubated for 15 min with 100 nM iberiotoxin at

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**Fig. 1.** Big-conductance Ca^{2+}-activated K^{+} (BK) channel activity is absent in human and mouse neutrophils. A: representative currents in human and mouse neutrophils BK^{+/+} and BK^{−/−} neutrophils recorded in the absence (Ctr) and presence of 1 μg/ml phorbol 12-myristate 13-acetate (PMA, 5–7 min after application), in the presence of 1 μg/ml PMA + 100 nM iberiotoxin (Ibtx, 10–15 min after application), and in the presence of 1 μg/ml PMA + 3 mM ZnCl2 (Zn, 1–3 min after application). Iberiotoxin did not inhibit PMA-induced currents, indicating that BK channels are not present. Effects of PMA and Zn^{2+} were significant, since only small currents were induced by “sham bath change” within 25 min. Amphotericin-perforated cells were voltage clamped at a holding potential of –30 mV and pulsed for 300 ms from –100 to +140 mV in 20-mV increments every 2 s. High-Na^{+} external solution and high-K^{+} internal solution were used (1).

B: mean current amplitudes recorded in human and mouse BK^{+/+} and BK^{−/−} mouse neutrophils in response to step depolarization to +140 mV in the absence and presence of 1 μg/ml PMA, in the presence of 1 μg/ml PMA + 100 nM iberiotoxin, and in the presence of 1 μg/ml PMA + 3 mM ZnCl2. Numbers of cells are indicated above bars. *P < 0.05. ns, Not significant.
37°C. Opsonized microorganisms were added at a microorganism-to-neutrophil ratio of 2:1. A sample without neutrophils served as a control. The samples were shaken for 90 min at 37°C, and incubation was stopped by addition of 2 ml of ice-cold distilled water to disrupt the neutrophils. Aliquots (25 µl) were spread on Sabouraud agar plates, and colonies were counted after 24 h of incubation at 30°C. The percent killing was calculated as follows: (CFU sample (microorganisms) – CFU sample (neutrophils + microorganisms))/CFU sample (microorganism) × 100.

Killing of S. aureus by human neutrophils. Neutrophils were isolated from peripheral blood of healthy volunteers as described previously (43) and resuspended in HBSS containing 0.05% HSA. To prepare bacteria, basic medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose, and 0.1% K2HPO4) was inoculated with 1:100 dilution of an overnight culture and shaken at 37°C until midlogarithmic phase. The bacteria were washed twice in 10 mM potassium phosphate (KPi) buffer (pH 7.0) containing 0.01% HSA and adjusted to 5 × 10^7 bacteria/ml. Bacterial and neutrophil suspen-

Fig. 2. Currents in symmetrical high-K⁺ aspartate solutions were not sensitive to iberiotoxin. A: representative currents in BK⁺/+ and BK⁻/⁻ mouse neutrophils recorded in the absence and presence of 1 µg/ml PMA (5–7 min after stimulation) and in the presence of 1 µg/ml PMA + 100 nM iberiotoxin (10–15 min after application). Amphotericin-perforated cells were voltage clamped at a holding potential of 0 mV and pulsed for 300 ms from −100 to +100 mV in 20-mV increments every 2 s. Symmetrical high-K⁺ aspartate solutions were used in bath and pipette. B: mean current amplitudes recorded in BK⁺/+ and BK⁻/⁻ mouse neutrophils in response to step depolarization to +100 mV in the absence and presence of 1 µg/ml PMA and in the presence of 1 µg/ml PMA + 100 nM iberiotoxin. Numbers of cells are indicated above bars.
sions were mixed to final concentrations of $5 \times 10^6$/ml and $2.5 \times 10^6$/ml, respectively. Bacteria were opsonized by addition of pooled human serum (Sigma) to a final concentration of 10%. Samples (500 μl) with 100 nM iberiotoxin and without iberiotoxin were shaken at 37°C. Incubation was stopped by dilution of aliquots in ice-cold, distilled water. The neutrophils were disrupted by vigorous vortexing. Appropriate sample volumes were plated on basic medium agar distilled water. The neutrophils were disrupted by vigorous vortexing.

**RESULTS**

Membrane currents in human and mouse neutrophils are not inhibited by iberiotoxin. Iberiotoxin is a potent and specific blocker of the BK channel (19). We did not observe iberiotoxin-sensitive currents in human blood and mouse bone marrow neutrophils (Fig. 1A), even after stimulation with PMA. We used the amphotericin B perforated-patch mode of the patch-clamp technique to measure currents in isolated neutrophils. We used the same solutions and protocols routinely used for BK channel current measurements (37, 41) and the solutions and protocols used by Ahluwalia et al. (1) but failed to find iberiotoxin-sensitive currents in neutrophils. Representative currents measured in cells clamped at $-30$ mV and depolarized for 300 ms from $-100$ to $+140$ mV in 20-mV increments in the absence and presence of 1 μg/ml PMA, in the presence of 1 μg/ml PMA + 100 nM iberiotoxin, and in the presence of 1 μg/ml PMA + 3 mM ZnCl2 are shown in Fig. 1A. Depolarization-evoked current amplitudes increased on stimulation with PMA and were not inhibited by iberiotoxin (Fig. 1B) but were blocked by Zn2+, which abolishes proton currents (17). The BK channel can be also blocked by 1 mM TEA (30, 48). TEA, at 1 mM, had no effect on depolarization-evoked currents in human neutrophils ($n = 6$ cells; data not shown).

Next, using symmetrical high-K+ aspartate solutions, we attempted to find BK channel currents. Representative currents measured in mouse neutrophils clamped at 0 mV and depolarized for 300 ms from $-100$ to $+100$ mV in 20-mV increments in the absence and presence of 1 μg/ml PMA (5–6 min) and in the presence of 1 μg/ml PMA + 100 nM iberiotoxin (10–12 min) are shown in Fig. 2A. Depolarization-evoked current amplitudes tended to increase on stimulation with PMA and were not inhibited by iberiotoxin (Fig. 2B). External application of 1 mM Ba2+ blocked the amplitude of the inward component of the current significantly but did not affect the outward component ($n = 3$ cells each for BK+/− and BK−/− mice; data not shown), indicating the presence of inwardly rectifying K+ currents previously reported in newt blood neutrophils (27).

The effects of PMA, a well-known PKC activator (39), on BK channel activity are not clear: inhibition (46) and activation have been reported (6). Ahluwalia et al. (1) found that PMA increased [Ca2+]i in human neutrophils and, therefore, activated BK channels that are Ca2+ sensitive. We also found that
PMA induced a $Ca^{2+}$ increase in human neutrophils (Fig. 3A). Changes in $[Ca^{2+}]_i$ were measured in fluo-4-AM-loaded neutrophils using Nipkow disk confocal microscopy. The time course of changes in fluo-4 fluorescence intensity evoked by 1 $\mu$M PMA averaged from five cells in a representative experiment is shown in Fig. 3A. The relative fluorescence $(F/F_0)$ increase in the presence of PMA was <20% but was, nonetheless, statistically significant. To achieve a more significant increase in $[Ca^{2+}]_i$, we used $N$-formyl-$L$-methionyl-$L$-leucyl-phenylalanine (fMLP). $[Ca^{2+}]_i$ in neutrophils was increased much more effectively by 1 $\mu$M fMLP than by PMA at the same concentration. Despite a significant increase in $[Ca^{2+}]_i$, fMLP failed to induce appropriate BK channel activity in human neutrophils (Fig. 3B).

As a control, we measured BK currents in smooth muscle cells isolated from mouse tibial and cerebral arteries (Fig. 4). In contrast to the neutrophils, in arterial smooth muscle cells, depolarization-evoked currents were effectively blocked by 100 nM iberiotoxin. BK channel activity in $BK^{+/+}$ cerebral smooth muscle cells was abolished by iberiotoxin (Fig. 4A). In contrast, BK channel currents were absent in $BK^{-/-}$ cells. BK channel currents in $BK^{+/+}$ tibial smooth muscle cells were effectively blocked by iberiotoxin (Fig. 4B). In tibial and cerebral smooth muscle cells, iberiotoxin also blocked spontaneous transient outward currents,

Fig. 4. BK channel activity in arterial smooth muscle cells. A: BK channel activity in a smooth muscle cell isolated from a cerebral artery of a $BK^{+/+}$ mouse (top trace) was blocked by 100 nM iberiotoxin (10 min after application, middle trace). BK channel activity was absent in a smooth muscle cell isolated from a cerebral artery of a $BK^{-/-}$ mouse (bottom trace). Amphotericin-perforated cells were voltage clamped at a holding potential of $-20$ mV. High-Na$^+$ external and high-K$^+$ internal pipette solutions were used (41). Data were filtered at 1 kHz and sampled at 2 kHz. Arrow indicates coordinated openings of several BK channels, which are known as spontaneous transient outward current (STOC) (37). Similar results were obtained in 3 other $BK^{+/+}$ and 3 other $BK^{-/-}$ cerebral smooth muscle cells (see Ref. 41 for our previous recordings). B: 100 nM iberiotoxin blocked STOCs and whole cell K$^+$ current in a smooth muscle cell isolated from the tibial artery of a $BK^{+/+}$ mouse. Amphotericin-perforated cell was voltage clamped at $-40$ mV, and linear voltage ramps at 0.5 V/s from $-100$ to $+100$ mV were applied every 10 s. High-Na$^+$ external and high-K$^+$ internal pipette solutions were used (41). The 6 superimposed records are shown in the absence and presence of 100 nM iberiotoxin (10 min after application). Similar results were obtained in 3 other cells.
which represent coordinated openings of a cluster of BK channels caused by Ca\(^{2+}\) sparks (37, 41).

As another control, proton-like currents were recorded in human and mouse neutrophils. Symmetrical high-K\(^+\) aspartate solutions were supplemented with NH\(_4\) (14). Representative current traces are shown in Fig. 5. Amphotericin-perforated cells were voltage clamped at a holding potential of \(-60\) mV and pulsed for 8 s from \(-60\) to \(+60\) mV in 20-mV increments every 20 s. PMA-stimulated currents were not sensitive to iberiotoxin. However, the currents were almost completely blocked by Zn\(^{2+}\). Iberiotoxin (10–15 min after application), and in the presence of 1 mM ZnCl\(_2\) (1–3 min after application). Amphotericin-perforated cells were voltage clamped at a holding potential of \(-60\) mV and pulsed for 8 s from \(-60\) to \(+60\) mV in 20-mV increments every 20 s. Recordings were performed in symmetrical high-K\(^+\) aspartate solutions supplemented with NH\(_4\) (14). PMA-stimulated currents were not sensitive to iberiotoxin. However, they were blocked by Zn\(^{2+}\). Similar results were obtained in 4 other human and 3 other BK\(^{+/+}\) and 3 other BK\(^{-/-}\) mouse neutrophils.

Presented by Ahluwalia et al. (1) but in agreement with other reports (11, 17).

Iberiotoxin does not inhibit killing of human neutrophils. Ahluwalia et al. (1) reported that iberiotoxin is able to inhibit neutrophil killing. We tested the effect of iberiotoxin on the ability of human neutrophils to kill \(S.\) \(aureus\) and \(C.\) \(albicans\) (Fig. 6, A and B). Survival of \(S.\) \(aureus\) at the end of 15, 30, and 60 min of incubation with neutrophils at 37°C is shown in Fig. 6A. The ratio of bacteria to neutrophils was taken as 2:1. Almost all bacteria were killed at 60 min. Bacterial survival was not significantly changed by 100 nM iberiotoxin at 15, 30, or 60 min. The ability of neutrophils to kill \(C.\) \(albicans\) at the end of 90 min of incubation with neutrophils at 37°C is shown in Fig. 6B. Opsonized microorganisms were added at a microorganism-to-neutrophil ratio of 2:1. The ~40% of \(C.\) \(albicans\) killed in control was not decreased by 100 nM iberiotoxin. Therefore, our data indicate that iberiotoxin does not inhibit killing of \(S.\) \(aureus\) and \(C.\) \(albicans\) by neutrophils, in contrast to the data presented by Ahluwalia et al. (1) but in agreement with other reports (11, 17).

Iberiotoxin does not inhibit ROS production in human neutrophils. Effective killing of \(S.\) \(aureus\) and \(C.\) \(albicans\) requires NADPH oxidase activity and generation of ROS (4, 24). We tested the effect of iberiotoxin on generation of ROS in human neutrophils with two independent assays. \(O_2^-\) was measured using the assay of SOD-inhibitable reduction of ferricytochrome \(c\) (Fig. 6E). At 25 ng/ml–1 \(\mu g/ml\), PMA, a known NADPH oxidase activator (8), stimulated \(O_2^-\) production. Iberiotoxin (100 nM) did not block \(O_2^-\) production in neutrophils in the absence or presence of PMA. Similar results were obtained with the DHR oxidation assay. The generation of reactive oxidants was stimulated by 25 ng/ml and 1 \(\mu g/ml\) PMA and was not inhibited by 100 nM iberiotoxin (Fig. 6F).

In contrast to iberiotoxin, diphenylene iodonium, a classical inhibitor of NADPH-dependent ROS production (9), strongly reduced the oxidant generation in PMA-stimulated cells (\(n = 6;\) data not shown). Therefore, our data show that ROS production was not inhibited by iberiotoxin. The data are in agreement with the absence of an inhibitory effect of iberiotoxin on killing activity of human neutrophils and confirm data obtained in previous studies (1, 17).

BK channel knockout does not reduce resistance to \(S.\) \(aureus\) and \(Y.\) \(pestis\) infection in mice. If BK channels are essential for innate immunity, a reasonable expectation would be that BK\(^{-/-}\) mice are less resistant to infections than BK\(^{+/+}\) mice. We performed experiments with \(S.\) \(aureus\) and \(Y.\) \(pestis\) infected mice to explore this possibility (Fig. 6C). BK\(^{-/-}\) and

![Fig. 5. Proton-like currents in human and mouse neutrophils were not sensitive to iberiotoxin but were blocked by Zn\(^{2+}\). Currents in human and BK\(^{+/+}\) and BK\(^{-/-}\) mouse neutrophils were recorded in the absence and presence of 1 \(\mu g/ml\) PMA (5–7 min after stimulation), in the presence of 1 \(\mu g/ml\) PMA + 100 nM iberiotoxin (10–15 min after application), and in the presence of 1 \(\mu g/ml\) PMA + 3 mM ZnCl\(_2\) (1–3 min after application). Amphotericin-perforated cells were voltage clamped at a holding potential of \(-60\) mV and pulsed for 8 s from \(-60\) to \(+60\) mV in 20-mV increments every 20 s. Recordings were performed in symmetrical high-K\(^+\) aspartate solutions supplemented with NH\(_4\) (14). PMA-stimulated currents were not sensitive to iberiotoxin. However, they were blocked by Zn\(^{2+}\). Similar results were obtained in 4 other human and 3 other BK\(^{+/+}\) and 3 other BK\(^{-/-}\) mouse neutrophils.](http://ajpcell.physiology.org/)
BK\(^{-/-}\) mice were intravenously infected with S. aureus or Y. enterocolitica. Three days after infection with S. aureus and 5 days after infection with Yersinia, the mice were killed, and the number of viable bacteria recovered from spleen and kidney was determined. The number of viable Yersinia and S. aureus was not increased by the absence of BK channels in mice (Fig. 6, C and D). Thus the data do not support the idea that BK channels are essential for innate immunity and, thus, for protection against bacterial infections.

**DISCUSSION**

We did not find that PMA stimulated BK channel activity in human or mouse neutrophils. For a positive control, we recorded iberiotoxin-sensitive BK channel currents in arterial muscle cells, PMA stimulated PKC and, thereby, activated BK channels via cGMP-dependent protein phosphorylation. For example, in pulmonary arterial smooth muscle cells, PMA stimulated PKC and, thereby, activated BK channels via cGMP-dependent protein kinase (6), which directly phosphorylates the pore-forming channel \(\alpha\)-subunits (3). Also, the increase in \(\text{Ca}^{2+}\) in neutrophils is not the commonly observed effect of PMA (33). We detected the PMA-induced increase in neutrophil \(\text{Ca}^{2+}\); however, the response we observed was much more modest than that reported by Ahluwalia et al. On the other hand, a similar concentration of fMLP increased \(\text{Ca}^{2+}\) significantly but failed to stimulate BK channel activity.

Although BK channel activity was not detected in neutrophils in our study and in the report by Femling et al. (17), these cells successfully kill S. aureus and C. albicans (17) (Fig. 6, A and B). Iberiotoxin, a specific blocker of the BK channel, did not decrease the ability of neutrophils to eliminate microor-
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organisms. The killing assays were performed in two independent laboratories (Berlin and Tübingen) using different experimental methods and conditions. Although Fleming et al. (17) also obtained their results in two separate laboratories, four independent sources report no essential role for BK channels in the neutrophil killing function. Our experiments with BK−/− mice also do not support the idea that BK channels are essential for innate immunity. BK−/− mice were not less resistant to S. aureus and Yersinia infection than their BK+/+ littermates. The notion that neutrophil function via BK channel activity should be revised.

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