Phosphoinositide 3-Kinase Dependent Regulation of Kv Channels in Dendritic Cells

Ekaterina Shumilina*, Naima Zahir*, Nguyen Thi Xuan and Florian Lang

Department of Physiology, University of Tübingen, *shared first authorship

Key Words

LPS • Toll-like receptors • Ion channels • Interleukin 12 • Wortmannin

Abstract

The phosphoinositide 3 (PI3) kinase plays a pivotal role in the regulation of dendritic cells (DCs), antigen-presenting cells that are able to initiate primary immune responses and to establish immunological memory. PI3 kinase is an endogenous suppressor of interleukin 12 (IL-12) production in DCs that is triggered by Toll-like receptor signaling. Inhibition of IL-12 production limits T helper 1 (Th1) polarization. On the other hand, PI3 kinase is an important regulator of various ion channels. The present study aimed to explore whether ion channels in DCs are regulated by PI3 kinase and whether they are important for DC function. To this end, DCs were isolated from murine bone marrow and ion channel activity was determined by patch clamp. As a result, DCs express voltage-gated K⁺ channels (Kv), which are blocked by *Stichodactyla helianthus* toxin (ShK, 2.5 nM). A significant upregulation of Kv currents was observed upon maturation of DCs as induced by stimulation of the cells with lipopolysaccharide (LPS,

0.1 µg/ml, 48 h). A dramatic increase of Kv current amplitude was observed following preincubation of the cells with LY294002 (100 nM), a specific inhibitor of PI3 kinase. PI3 kinase inhibitor wortmannin (100 nM) similarly increased Kv current. LY294002 treatment was further followed by a significant increase of IL-12 production. ShK (100 nM) significantly blunted the stimulation of IL-12 release by LPS but not when the cells were first pretreated with LY294002. The observations point to Kv channel sensitive and Kv channel insensitive regulation of DC function.

Copyright © 2007 S. Karger AG, Basel

Introduction

Dendritic cells (DCs) are antigen-presenting cells, central for the development of optimal T cell immunity, that are able to initiate primary immune responses and to establish immunological memory [1, 2]. Upon stimulation of Toll-like receptors (TLR) by microbial products such as lipopolysaccharides (LPS), DCs undergo maturation, express costimulatory molecules and MHC class II [3-6] and produce proinflammatory chemokines and

cytokines, including interleukin 12 (IL-12), which is an important inducer of Th1 responses [7-9].

The function of DCs is under the control of the phosphoinositide 3 kinase (PI3 kinase) pathway [10-13]. PI3 kinase is considered to be an endogenous suppressor of IL-12 production triggered by Toll-like receptor signalling and limits excessive Th1 polarization [14]. Inhibition of PI3 kinase was also shown to enhance IFN- β synthesis downstream of TLR3 and TLR4 [15]. Together with two other kinases, such as IRAK-M and SOCS-1, PI3 kinase belongs to the gate-keeping system, preventing excessive innate immune responses. PI3 kinase is stimulated by most of the activators of DCs that induce IL-12 production at an early phase and interferes at the first encounter to pathogens, providing a negative feedback regulation of IL-12 production in DCs [12, 14]. The downstream targets of the PI3 kinase pathway include ion channels [16-20]. However, little is known about the role of ion channels in the regulation of DC function.

DCs were shown to express voltage-gated outward K^+ currents [21]. Recently Kv1.3 and Kv1.5 channels were discovered in human blood-derived DCs [22]. These channels seem to play a role in the maturation process of DCs, since costimulatory molecule expression and cytokine production is decreased in DCs matured in the presence of Kv blockers [22]. Kv1.3 and Kv1.5 channels are highly expressed within cerebral inflammatory infiltrates of multiple sclerosis brain and are partially localized in cells belonging to the DC subtype [22, 23].

The present study has thus been performed to elucidate whether Kv channels in DCs are regulated by PI3 kinase dependent signaling and whether PI3 kinase sensitive ion channels participate in the regulation of DC functions.

Materials and Methods

Animals

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

Culture of bone marrow dendritic cells

Dendritic cells were isolated from bone marrow of 8-12 week old male and female 129/SvJ, 129/SvP₂ mice. The cells were cultured for 7 days in RPMI 1640 (GIBCO, Carlsbad) containing 10% FCS, 1% penicillin/streptomycin, 35 ng/ml GM-CSF (Preprotech Tebu). Cells were stimulated at day 7 with 0.1 μ g/ml lipopolysaccharide (LPS, Sigma, Taufkirchen, Germany) and analyzed 48 h afterwards. In some experiments a specific PI3 kinase inhibitor LY294002 (100 nM, Sigma, Taufkirchen,

Germany) or Kv channel inhibitor *Stichodactyla helianthus* toxin (ShK, Bachem AG, Weil am Rhein, Germany, 100 nM) were added to the medium 2 h before addition of LPS.

Cytokine quantification in cell supernatants

Cell culture supernatant was collected and stored at -20°C until used for ELISA. For analysis of IL-12p70 concentrations, a commercially available ELISA kit (BD PharMingen, Heidelberg, Germany) was used according to the manufacturer's instructions.

Immunostaining and flow cytometry

Cells (4×10^5) were incubated in 100 μ l PBS containing fluorochrome-conjugated antibodies at a concentration of 10 μ g/ml. A total of 1×10^4 cells were analyzed. The following antibodies (all from BD Pharmingen, Heidelberg, Germany) were used for staining: FITC-conjugated anti-mouse CD11c, clone HL3 (Armenian Hamster IgG₁, λ 2), PE-conjugated anti-mouse CD86, clone GL1 (Rat IgG_{2a}, κ) and PE-conjugated rat anti-mouse I-A/I-E, clone M5/114.15.2 (IgG2b, κ).

Patch clamp

Patch clamp experiments have been performed at room temperature in voltage-clamp, fast-whole-cell mode according to Hamill et al. [24]. The cells were continuously superfused through a flow system inserted into the dish. The bath was grounded via a bridge filled with NaCl Ringer solution. Borosilicate glass pipettes (1-3 MOhm tip resistance; GC 150 TF-10, Clark Medical Instruments, Pangbourne, UK) manufactured by a microprocessor-driven DMZ puller (Zeitz, Augsburg, Germany) were used in combination with a MS314 electrical micromanipulator (MW, Märzhäuser, Wetzlar, Germany). The currents were recorded by an EPC-9 amplifier (Heka, Lambrecht, Germany) using Pulse software (Heka) and an ITC-16 Interface (Instrutech, Port Washington, N.Y., USA). Whole-cell currents were elicited by 200 msec square wave voltage pulses from -90 to +90 mV in 20 mV steps delivered at 20 sec intervals from a holding potential of -70 mV. The currents were recorded with an acquisition frequency of 10 kHz and 3 kHz low-pass filtered. Since the time constant of activation decreased with the applied depolarizing voltage step and inactivation occurred especially during strong depolarization, the current was analyzed at its maximal amplitude value (peak current).

The cells were superfused with a bath solution containing (in mmol/l): 140 NaCl, 5 KCl, 1 MgCl₂, 2 CaCl₂, 10 glucose and 10 HEPES, pH 7.4 (NaOH). The patch clamp pipettes were filled with an internal solution containing (in mmol/l): 80 KCl, 60 K⁺-gluconate, 1 MgCl₂, 1 Mg-ATP, 1 EGTA, 10 HEPES, pH 7.2 (KOH). When indicated *Stichodactyla helianthus* toxin (ShK, Bachem AG, Weil am Rhein, Germany, 2.5 nM) was added to the bath solution.

The offset potentials between both electrodes were zeroed before sealing. The potentials were corrected for liquid junction potentials as estimated according to Barry & Lynch [25]. The original whole-cell current traces are depicted without filtering (acquisition frequency of 5 kHz) and currents of the individual voltage square pulses are superimposed. The applied voltages refer to the cytoplasmic face of the membrane with respect to

Fig. 1. Maturation of bone marrow derived DCs: Flow cytometric analysis of immature (control) and mature (48 h after stimulation with LPS, 0.1 µg/ml) DCs. Representative FACS histograms depicting the expression of MHC class II (A) and CD86 (B) surface molecules.

Fig. 2. Voltage gated (Kv) K⁺ channels in cultured bone marrow-derived DCs: A. Representative whole-cell depolarization-activated K⁺ currents in DCs under control conditions (control) and after 48 h stimulation of the cells with LPS (0.1 µg/ml). The currents were recorded from the cells with similar capacitance elicited by 200 ms depolarizing pulses ranging from -90 to +90 mV in 20 mV and 20 sec increments from a holding potential of -70 mV. B. Whole-cell current traces recorded from a LPS-stimulated DC before (LPS) or after (LPS+ShK) application of 2.5 nM ShK. The currents were recorded as in A. C. Current-voltage (I-V) relations of mean peak currents from LPS-stimulated DCs in paired experiments before (LPS, filled triangles, n=5) and after (LPS+ShK, open squares) application of 2.5 nM ShK. D. I-V relation of the current fraction inhibited by ShK as calculated from C by subtracting the currents obtained after application of ShK from those obtained before the application.

the extracellular space. The inward currents, defined as flow of positive charge from the extracellular to the cytoplasmic membrane face, are negative currents and depicted as downward deflections of the original current traces.

Statistics

Data are provided as means $\pm$ SEM, *n* represents the number of independent experiments. All data were tested for significance using Student's unpaired two-tailed *t*-test, one sample *t*-test or ANOVA (Dunnets test), where applicable, and only results with *p* < 0.05 were considered statistically significant.

Results

Mouse bone marrow derived dendritic cells (DCs) were stimulated with lipopolysaccharide (LPS, 0.1 µg/ml, 48 h) to obtain mature DCs and the expression of the DC surface markers MHC class II and CD86 was determined by FACS analysis (Fig. 1). LPS treatment led to a high expression of MHC class II and CD86.

Whole cell patch clamp experiments disclosed the presence of voltage-gated K⁺ (Kv) currents in mouse bone marrow derived DCs (Fig. 2A). Comparison of DCs

Fig. 3. Kv channels in DCs are markedly upregulated by PI3 kinase inhibition: A. Representative whole-cell Kv currents in DCs under control conditions (control), after 48 h stimulation of the cells with LPS (0.1 μg/ml) in the absence (LPS) or in the presence (LPS+LY294002) of PI3 kinase inhibitor LY294002 (100 nM, 2 h before addition of LPS to the culture). B. I-V relations of mean peak Kv current amplitude normalized to the cell capacitance (mean peak current density) from DCs under control conditions in the absence (control, filled triangles, n = 17) or in the presence (control+LY294002, filled diamonds, n = 9) of PI3 kinase inhibitor LY294002 (100 nM, 2h) and after LPS (0.1 μg/ml, 48 h) stimulation in the absence (LPS, open squares, n=10) or in the presence (LPS+ LY294002, open circles, n = 11) of LY294002 (100 nM, 2 h before addition of LPS to the culture). C. Bar graphs showing the mean peak current density at +90 mV in DCs after the same conditions as in B. * (p<0.05) indicates significant difference from control; ### (p<0.001) indicates significant difference from LPS+LY294002, ANOVA. D. Bar graphs showing the mean peak current density at +90 mV in DCs under control conditions in the absence (n = 11) or in the presence (n = 8) of PI3 kinase inhibitor wortmannin (100 nM, 2h) and after LPS (0.1 μg/ml, 48 h) stimulation in the absence (n = 11) or in the presence (n = 10) of wortmannin (100 nM, 2 h before addition of LPS to the culture).# (p<0.05), ## (p<0.01) and ### (p<0.001) indicate significant difference from LPS+wortmannin, ANOVA.

of different maturation stages disclosed the presence of low Kv currents in immature DCs and significant upregulation of Kv currents upon maturation as induced by stimulation with lipopolysaccharide (LPS, 0.1 μg/ml, 48 h, Fig. 2A, 3). Under control conditions in immature DCs mean peak current density measured at +90 mV was (13.2 ± 1.3) pA/pF (n = 17, Fig. 3C,D) and upon

maturation following LPS stimulation 37.7 ± 5.7 pA/pF, (n = 10, Fig. 3C,D). The reversal potential was -36.8 ± 2.3 mV (n = 17) under control conditions and significantly more negative (-45.5 ± 3.1 mV, n=10) upon LPS stimulation. No voltage oscillations were observed when measured in current clamp mode.

The mean activation time constant (τ_{act}) calculated

Fig. 4. IL-12 secretion is upregulated by inhibition of PI3 kinase: Arithmetic means ($\pm$ SE, $n = 12$) of normalized IL-12 secretion in DCs under control conditions (control) and after stimulation with LPS (0.1 μ g/ml, 48 h) in the absence (LPS) or presence (LPS+LY294002) of 100 nM LY294002. The IL-12 secretion under control conditions and after LPS stimulation in the presence of LY294002 was normalized in each experiment to the corresponding value after LPS stimulation in the absence of the inhibitor (LPS, open bar). The standard error bar of the LPS (open bar) indicates the normalized scatter of the absolute values between the individual experiments. ** ($p < 0.01$) and *** ($p < 0.001$) indicate significant difference from 1 (one sample t -test).

Fig. 5. Inhibition of IL-12 secretion by ShK in DCs: Arithmetic means ($\pm$ SE, $n = 19$) of normalized IL-12 secretion in DCs under control conditions (control) and after stimulation with LPS (0.1 μ g/ml, 48 h) in the absence (LPS) or presence (LPS+ShK) of 100 nM ShK. The IL-12 secretion under control conditions and after LPS stimulation in the presence of ShK was normalized in each experiment to the corresponding value after LPS stimulation in the absence of the inhibitor (LPS, open bar). The standard error bar of the LPS (open bar) indicates the normalized scatter of the absolute values between the individual experiments. *** indicates significant difference from 1 ($p < 0.0001$; one sample t -test).

from a single exponential fit at +50 mV was 2.7 ± 0.2 msec ($n = 12$) for immature cells and 2.1 ± 0.3 msec ($n = 10$) in LPS-stimulated cells. $V_{1/2}$ for activation of the currents defined by fitting the conductance/voltage relations to the Boltzmann equation was -34.1 ± 1.6 mV ($n = 12$) and -39.6 ± 1.2 mV ($n = 10$) in immature and mature cells, respectively. Experiments with different intervals between the voltage pulse trains demonstrated that the currents recovered within 20 sec from their use-dependent inactivation.

As illustrated in Fig. 2 (Fig. 2B-D), the currents were blocked by *Stichodactyla helianthus* toxin (ShK, 2.5 nM), a known specific inhibitor of Kv1.3 channels [26]. The biophysical and pharmacological properties of the observed K^+ currents resembled Kv1 (most probably Kv1.3) channels.

The Kv channels in both immature and mature DCs were down-regulated by PI3 kinase. Preincubation of

immature cells with LY294002 (100 nM, 2 h), a specific inhibitor of PI3 kinase led to upregulation of the current (39.4 ± 5.2 pA/pF at +90 mV, $n = 9$). A dramatic increase of Kv current amplitude was observed when LPS-stimulated cells matured in the presence of LY294002 (100 nM, 2 h before addition of LPS to the culture; Fig. 3A, B). Under these conditions the mean peak current density at +90 mV was 98.3 ± 11.9 pA/pF ($n = 11$, Fig. 3C). Another PI3 kinase inhibitor wortmannin (100 nM) led to similar upregulation of Kv currents in both immature and mature DCs (Fig. 3D). These results reveal a role of PI3 kinase as a negative regulator of Kv channels in DCs.

PI3 kinase dependent DC functions include interleukin 12 (IL-12) production [14]. Accordingly, IL-12 production is enhanced in PI3 kinase deficient mice [14]. Thus, we have explored, whether inhibition of PI3 kinase or of the K^+ channels affected IL-12 release.

As illustrated in Fig. 4, inhibition of PI3 kinase by

LY294002 (100 nM, 2 h before addition of LPS to the culture) was indeed followed by a significant increase in IL-12 production. Conversely, the Kv channel inhibitor ShK (100 nM) reduced significantly IL-12 secretion by LPS (0.1 µg/ml, 48 h)-stimulated DC cells (Fig. 5). Thus, both, PI3 kinase signaling and ShK sensitive Kv channels do modify the IL-12 release from DCs. DCs matured in the presence of both blockers ShK and LY294002 produced IL-12 to a similar extent (935 ± 224 , $n = 4$), as DCs matured in the presence of LY294002 alone (904 ± 213 , $n = 4$), suggesting that the LY294002-stimulated IL-12 secretion does not require Kv channel activity.

Discussion

The present study reveals that murine DCs derived from bone marrow express voltage-gated K⁺ channels. More importantly, the observations disclose that the channels are markedly upregulated by inhibition of PI3 kinase. Thus, the channels are apparently under tonic inhibition by the PI3 kinase pathway. The relevance of those observations for DC function is illustrated by the stimulating effect of the selective PI3 kinase blocker LY294002 and the inhibitory effect of the selective K⁺ channel blocker ShK on the release of IL-12. While IL-12 secretion is dependent on Kv channel activity, no effect of the Kv channel blocker was observed when DCs were matured in the presence of LY294002, suggesting that upon PI3 kinase inhibition IL-12 secretion is not dependent on Kv channel activity.

The present study did not aim to determine the molecular identity of the observed K⁺ channels. A most recent paper indicated that blood derived human DCs express Kv1.3 and Kv1.5 channels [22]. The electrical properties of those channels are virtually identical to those observed in this study.

Dual electrode voltage clamp in *Xenopus* oocytes suggests that the PI3 kinase dependent serum and glucocorticoid inducible kinase (SGK) isoforms enhance the K⁺ current induced by the voltage-gated K⁺ channels Kv1.3 [16, 27, 28], Kv1.5 [29] and Kv4.3 [30]. Similarly, overexpression of PDK1, SGK1, SGK2 or SGK3 in human embryonic kidney (HEK) cells leads to a marked upregulation of K⁺ channels with gating properties identi-

cal to Kv1.3 [16, 28, 31]. The same channels are activated by treatment of the HEK cells [16] and fibroblasts [32], with IGF1, an effect reversed by inhibition of PI3 kinase. The mechanisms accounting for the opposite regulation of Kv channels in DCs remain elusive.

In view of their sensitivity to the PI3 kinase pathways, the DC Kv channels may be regulated by known stimulators of the PI3 kinase pathway. The PI3 kinase is activated by IGF1 and insulin [16, 33–38].

K⁺ channel activity hyperpolarizes the cell membrane thus increasing Ca²⁺ entry through the Ca²⁺ release activated channel I_{CRAC} [39]. Accordingly, Ca²⁺ oscillations mediated by store-operated calcium channels are only maintained in the presence of proper functioning K⁺ channels. In particular, Kv channel blockers margatoxin and charybdotoxin decreased capacitative calcium entry in HEK cells [40]. However, in DCs calcium signaling antagonizes IL-12 production by mature DCs, negatively regulates pro-Th1 maturation and preferentially promotes Th2 responses, effects contrasting the stimulating effect of Kv channels on IL-12 secretion [11, 41]. Thus, the exact role of Kv channels in DC signaling still needs to be defined.

The present observation showed that the inhibition of PI3 kinase by LY294002 led to an increased IL-12 production which is in accordance to the data of Fukao et al. [14] who showed that DCs obtained from a PI3 kinase knock-out mouse or wild type DCs treated with wortmannin produced larger amounts of IL-12. In contrast, another study on murine DCs showed that PI3 kinase inhibitors suppressed LPS-induced secretion of IL-12 [10]. Those authors, however, used 500 times higher concentration of LY294002 (50 µM) than in our study.

In conclusion, LPS leads to a strong activation of Kv channels in the presence of PI3 kinase inhibitors in bone marrow derived DCs, an effect presumably participating in the stimulation of DC function.

Acknowledgements

This work was supported by grants from DFG and BMBF (F.L.). The authors gratefully acknowledge the meticulous preparation of the manuscript by Lejla Subasic.

References

- Banchereau J, Steinman RM: Dendritic cells and the control of immunity. *Nature* 1998;392:245-252.
- Dubsky P, Ueno H, Piqueras B, Connolly J, Banchereau J, Palucka AK: Human dendritic cell subsets for vaccination. *J Clin Immunol* 2005;25:551-572.
- Espuelas S, Roth A, Thumann C, Frisch B, Schuber F: Effect of synthetic lipopeptides formulated in liposomes on the maturation of human dendritic cells. *Mol Immunol* 2005;42:721-729.
- Hoarau C, Lagaraine C, Martin L, Velge-Roussel F, Lebranchu Y: Supernatant of *Bifidobacterium breve* induces dendritic cell maturation, activation, and survival through a Toll-like receptor 2 pathway. *J Allergy Clin Immunol* 2006;117:696-702.
- Katz J, Zhang P, Martin M, Vogel SN, Michalek SM: Toll-like receptor 2 is required for inflammatory responses to *Francisella tularensis* LVS. *Infect Immun* 2006;74:2809-2816.
- Warger T, Osterloh P, Rechtsteiner G, Fassbender M, Heib V, Schmid B, Schmitt E, Schild H, Radsak MP: Synergistic activation of dendritic cells by combined Toll-like receptor ligation induces superior CTL responses in vivo. *Blood* 2006;108:544-550.
- Carmona EM, Vassallo R, Vuk-Pavlovic Z, Standing JE, Kottom TJ, Limper AH: Pneumocystis cell wall beta-glucans induce dendritic cell costimulatory molecule expression and inflammatory activation through a Fas-Fas ligand mechanism. *J Immunol* 2006;177:459-467.
- Fulcher JA, Hashimi ST, Levroney EL, Pang M, Gurney KB, Baum LG, Lee B: Galectin-1-matured human monocyte-derived dendritic cells have enhanced migration through extracellular matrix. *J Immunol* 2006;177:216-226.
- Schakel K, von Kietzell M, Hansel A, Ebling A, Schulze L, Haase M, Semmler C, Sarfati M, Barclay AN, Randolph GJ, Meurer M, Rieber EP: Human 6-sulfo LacNAc-expressing dendritic cells are principal producers of early interleukin-12 and are controlled by erythrocytes. *Immunity* 2006;24:767-777.
- Bhattacharyya S, Sen P, Wallet M, Long B, Baldwin AS, Jr., Tisch R: Immunoregulation of dendritic cells by IL-10 is mediated through suppression of the PI3K/Akt pathway and of IkappaB kinase activity. *Blood* 2004;104:1100-1109.
- Caparros E, Munoz P, Sierra-Filardi E, Serrano-Gomez D, Puig-Kroger A, Rodriguez-Fernandez JL, Mellado M, Sancho J, Zubiaur M, Corbi AL: DC-SIGN ligation on dendritic cells results in ERK and PI3K activation and modulates cytokine production. *Blood* 2006;107:3950-3958.
- Fukao T, Koyasu S: PI3K and negative regulation of TLR signaling. *Trends Immunol* 2003;24:358-363.
- Luft T, Rodionova E, Maraskovsky E, Kirsch M, Hess M, Buchholtz C, Goerner M, Schnurr M, Skoda R, Ho AD: Adaptive functional differentiation of dendritic cells: integrating the network of extra- and intracellular signals. *Blood* 2006;107:4763-4769.
- Fukao T, Tanabe M, Terauchi Y, Ota T, Matsuda S, Asano T, Kadowaki T, Takeuchi T, Koyasu S: PI3K-mediated negative feedback regulation of IL-12 production in DCs. *Nat Immunol* 2002;3:875-881.
- Aksoy E, Vanden Berghe W, Detienne S, Amraoui Z, Fitzgerald KA, Haegeman G, Goldman M, Willems F: Inhibition of phosphoinositide 3-kinase enhances TRIF-dependent NF-kappa B activation and IFN-beta synthesis downstream of Toll-like receptor 3 and 4. *Eur J Immunol* 2005;35:2200-2209.
- Gamper N, Fillon S, Huber SM, Feng Y, Kobayashi T, Cohen P, Lang F: IGF-1 up-regulates K⁺ channels via PI3-kinase, PDK1 and SGK1. *Pflugers Arch* 2002;443:625-634.
- Helms MN, Liu L, Liang YY, Al Khalili O, Vandewalle A, Saxena S, Eaton DC, Ma HP: Phosphatidylinositol 3,4,5-trisphosphate mediates aldosterone stimulation of epithelial sodium channel (ENaC) and interacts with gamma-ENaC. *J Biol Chem* 2005;280:40885-40891.
- Li D, Wei Y, Babilonia E, Wang Z, Wang WH: Inhibition of phosphatidylinositol 3-kinase stimulates activity of the small-conductance K channel in the CCD. *Am J Physiol Renal Physiol* 2006;290:F806-F812.
- Lu Z, Jiang YP, Ballou LM, Cohen IS, Lin RZ: Galpha q inhibits cardiac L-type Ca²⁺ channels through phosphatidylinositol 3-kinase. *J Biol Chem* 2005;280:40347-40354.
- Storey NM, Gentile S, Ullah H, Russo A, Muessel M, Erxleben C, Armstrong DL: Rapid signaling at the plasma membrane by a nuclear receptor for thyroid hormone. *Proc Natl Acad Sci U S A* 2006;103:5197-5201.
- Fischer HG, Eder C: Voltage-gated K⁺ currents of mouse dendritic cells. *FEBS Lett* 1995;373:127-130.
- Mullen KM, Rozycka M, Rus H, Hu L, Cudrici C, Zafrańska E, Pennington MW, Johns DC, Judge SI, Calabresi PA: Potassium channels K(v)1.3 and K(v)1.5 are expressed on blood-derived dendritic cells in the central nervous system. *Ann Neurol* 2006;60:118-127.
- Rus H, Pardo CA, Hu L, Darrah E, Cudrici C, Niculescu T, Niculescu F, Mullen KM, Allie R, Guo L, Wulff H, Beeton C, Judge SI, Kerr DA, Knaus HG, Chandy KG, Calabresi PA: The voltage-gated potassium channel Kv1.3 is highly expressed on inflammatory infiltrates in multiple sclerosis brain. *Proc Natl Acad Sci U S A* 2005;102:11094-11099.
- Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ: Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch* 1981;391:85-100.
- Barry PH, Lynch JW: Liquid junction potentials and small cell effects in patch-clamp analysis. *J Membr Biol* 1991;121:101-117.
- Tudor JE, Pallaghy PK, Pennington MW, Norton RS: Solution structure of ShK toxin, a novel potassium channel inhibitor from a sea anemone. *Nat Struct Biol* 1996;3:317-320.
- Henke G, Maier G, Wallisch S, Boehmer C, Lang F: Regulation of the voltage gated K⁺ channel Kv1.3 by the ubiquitin ligase Nedd4-2 and the serum and glucocorticoid inducible kinase SGK1. *J Cell Physiol* 2004;199:194-199.
- Wärntges S, Friedrich B, Henke G, Duranton C, Lang PA, Waldegger S, Meyermann R, Kuhl D, Speckmann EJ, Obermüller N, Witzgall R, Mack AF, Wagner HJ, Wagner A, Broer S, Lang F: Cerebral localization and regulation of the cell volume-sensitive serum- and glucocorticoid-dependent kinase SGK1. *Pflugers Arch* 2002;443:617-624.
- Ullrich S, Berchtold S, Ranta F, Seeböhm G, Henke G, Lupescu A, Mack AF, Chao CM, Su J, Nitschke R, Alexander D, Friedrich B, Wulff P, Kuhl D, Lang F: Serum- and glucocorticoid-inducible kinase 1 (SGK1) mediates glucocorticoid-induced inhibition of insulin secretion. *Diabetes* 2005;54:1090-1099.
- Baltaev R, Strutz-Seeböhm N, Korniyshuk G, Myssina S, Lang F, Seeböhm G: Regulation of cardiac shal-related potassium channel Kv 4.3 by serum- and glucocorticoid-inducible kinase isoforms in *Xenopus* oocytes. *Pflugers Arch* 2005;450:26-33.
- Gamper N, Fillon S, Feng Y, Friedrich B, Lang PA, Henke G, Huber SM, Kobayashi T, Cohen P, Lang F: K(+) channel activation by all three isoforms of serum- and glucocorticoid-dependent protein kinase SGK. *Pflugers Arch* 2002;445:60-66.
- Shumilina E, Lampert A, Lupescu A, Myssina S, Strutz-Seeböhm N, Henke G, Grahmmer F, Wulff P, Kuhl D, Lang F: Deranged Kv channel regulation in fibroblasts from mice lacking the serum and glucocorticoid inducible kinase SGK1. *J Cell Physiol* 2005;204:87-98.

- 33 Alessi DR, Andjelkovic M, Caudwell B, Cron P, Morrice N, Cohen P, Hemmings BA: Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J* 1996;15:6541-6551.
- 34 Alessi DR, Cohen P: Mechanism of activation and function of protein kinase B. *Curr Opin Genet Dev* 1998;8:55-62.
- 35 Divecha N, Banfic H, Irvine RF: The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. *EMBO J* 1991;10:3207-3214.
- 36 Kobayashi T, Cohen P: Activation of serum- and glucocorticoid-regulated protein kinase by agonists that activate phosphatidylinositol 3-kinase is mediated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) and PDK2. *Biochem J* 1999;339:319-328.
- 37 Kotani K, Yonezawa K, Hara K, Ueda H, Kitamura Y, Sakaue H, Ando A, Chavanieu A, Calas B, Grigorescu F, .: Involvement of phosphoinositide 3-kinase in insulin- or IGF-1-induced membrane ruffling. *EMBO J* 1994;13:2313-2321.
- 38 Park J, Leong ML, Buse P, Maiyar AC, Firestone GL, Hemmings BA: Serum and glucocorticoid-inducible kinase (SGK) is a target of the PI 3-kinase-stimulated signaling pathway. *EMBO J* 1999;18:3024-3033.
- 39 Parekh AB, Penner R: Store depletion and calcium influx. *Physiol Rev* 1997;77:901-930.
- 40 Tanneur V, Ilgaz D, Duranton C, Fillon S, Gamper N, Huber SM, Lang F: Time-dependent regulation of capacitative Ca²⁺ entry by IGF-1 in human embryonic kidney cells. *Pflugers Arch* 2002;445:74-79.
- 41 Faries MB, Bedrosian I, Xu S, Koski G, Roros JG, Moise MA, Nguyen HQ, Engels FH, Cohen PA, Czerniecki BJ: Calcium signaling inhibits interleukin-12 production and activates CD83(+) dendritic cells that induce Th2 cell development. *Blood* 2001;98:2489-2497.