Calcium-Activated Potassium Channel KCa3.1 in Lung Dendritic Cell Migration

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Migration to draining lymph nodes is a critical requirement for dendritic cells (DCs) to control T-cell-mediated immunity. The calcium-activated potassium channel KCa3.1 has been shown to be involved in regulating cell migration in multiple cell types. In this study, KCa3.1 expression and its functional role in lung DC migration were examined. Fluorescence-labeled antigen was intranasally delivered into mouse lungs to label lung Ag-carrying DCs. Lung CD11c^{high}CD11b^{low} and CD11c^{low}CD11b^{high} DCs from PBS-treated and ovalbumin (OVA)-sensitized mice were sorted using MACS and FACS. Indo-1 and DiBAC4(3) were used to measure intracellular Ca²⁺ and membrane potential, respectively. The mRNA expression of KCa3.1 was examined using real-time PCR. Expression of KCa3.1 protein and CCR7 was measured using flow cytometry. Migration of two lung DC subsets to lymphatic chemokines was examined using TransWell in the absence or presence of the KCa3.1 blocker TRAM-34. OVA sensitization up-regulated mRNA and protein expression of KCa3.1 in lung DCs, with a greater response by the CD11c^{high}CD11b^{low} than CD11c^{low}CD11b^{high} DCs. Although KCa3.1 expression in Ag-carrying DCs was higher than that in non-Ag-carrying DCs in OVA-sensitized mice, the difference was not as prominent. However, Ag-carrying lung DCs expressed significantly higher CCR7 than non-Ag-carrying DCs. CCL19, CCL21, and KCa3.1 activator 1-EBIO induced an increase in intracellular calcium in both DC subsets. In addition, 1-EBIO-induced calcium increase was suppressed by TRAM-34. *In vitro* blockade of KCa3.1 with TRAM-34 impaired CCL19/CCL21-induced transmigration. In conclusion, KCa3.1 expression in lung DCs is up-regulated by OVA sensitization in both lung DC subsets, and KCa3.1 is involved in lung DC migration to lymphatic chemokines.

Keywords: allergic airway inflammation; antigen uptake; asthma; calcium-activated potassium channel; dendritic cell

Chemokine-induced dendritic cell (DC) migration is calcium dependent (1, 2). Lymphatic chemokine CCL19 and CCL21 bind to their receptor CCR7, a G protein-coupled receptor, whose activation induces calcium mobilization from intracellular store via the IP3 pathway (3), whereas extracellular calcium influx is primarily through calcium release-activated calcium channel activity in bone marrow-derived DCs (4). Consequently, a small increase in intracellular free calcium leads to the activation of calcium-activated potassium channels that subsequently exert an impact on DC functions. Although the involvement of calcium in cell migration is well recognized (4–7), little is known regarding the role of calcium-activated potassium channel in DC biology.

CLINICAL RELEVANCE

The present study enhances our knowledge on the underlying cellular and molecular mechanisms involving the KCa3.1 channel in the migration of dendritic cells during immune response in the lung. The findings from this study will be important in designing better therapeutic approaches in the control of allergic airway inflammation and asthma.

KCa3.1 (EC₅₀ of 95 nM Ca²⁺) (8) belongs to the small- and intermediate-conductance-calcium-activated potassium channel subfamily. It is expressed in almost all types of migrating cells (9), including airway smooth muscle cells (10), vascular smooth muscle cells (11–13), T cells (14), macrophages (15), and mast cells (16). Functionally, KCa3.1 plays a key role in calcium-dependent cell functions, such as proliferation, activation, and migration, in a broad range of cell types. KCa3.1 regulates the proliferation of T lymphocytes (17), transformed cells (18), airway and vascular smooth muscle cells (12, 19–22), and vascular endothelial cells (23). The selective blockade of KCa3.1 largely inhibits endothelial cell proliferation, suggesting that KCa3.1 is a potential target for angiogenesis disorders (23). In addition, KCa3.1 is involved in the cell activation and migration of macrophages (24) and smooth muscle cells (12) and in the migration of mast cells (16, 25) and epithelial cells (26), particularly under pathophysiological conditions. However, no in-depth investigation has revealed the mechanisms by which KCa3.1 regulates cell migration. The speculation for the mechanism of KCa3.1 in cell migration includes cell volume regulation, orchestrating membrane resting potential to maintain the calcium fluctuation, and regulating polymerization or depolymerization of the actin cytoskeleton (9).

We have recently reported two functionally distinct lung DC subsets—CD11c^{high}CD11b^{low} DCs with regulatory properties and CD11c^{low}CD11b^{high} DCs with immunogenic properties—in a mouse model of allergic airway inflammation (27). These two DC subsets differ in the patterns of migration and antigen uptake in response to OVA sensitization. The immunogenic CD11c^{low}CD11b^{high} DC subset has a better ability to migrate to draining lymph nodes, and its antigen uptake activity is more significantly potentiated by OVA sensitization than the regulatory CD11c^{high}CD11b^{low} DC subset (28). Based on previous reports on the role of KCa3.1 in cell biology, we hypothesized that calcium-activated potassium channel KCa3.1 is expressed in lung DCs and plays a role in lung DC migration. In this study, the expression of KCa3.1 in mouse lung DCs was examined, and its role in lung DC migration was determined.

MATERIALS AND METHODS

Experimental Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of Creighton University. Female Balb/c mice (4–5 wk old) (Harlan Laboratories, Indianapolis, IN) were sensitized

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according to the protocol (Figure 1A) described previously (27, 28). In parallel, 30 μ l (150 μ g in PBS) AlexaFluor647-conjugated OVA or DQ-OVA (Invitrogen, Carlsbad, CA) was intranasally delivered into the lungs of OVA-sensitized mice as described previously (28). On Day 47, 48 hours after the intranasal antigen delivery, mouse lungs were collected.

Flow Cytometry

AutoMACS-separated CD11c⁺ cells (27, 28) were labeled with cocktail comprising the following antibodies: CD11cPE-Cy7, CD11bPE-Cy5, and CCR7-AlexaFluor700 (eBioscience, San Diego, CA). Ag-carrying cells were defined as BODIPY FL-positive (wavelength 530 nm) or AlexaFluor647-positive (wavelength 660 nm) cells. DCs isolated from the mice not receiving labeled antigen served as controls to define non-Ag-carrying cells. To detect KCa3.1 expression, cell fixation and permeabilization were performed using a Cytfix/Cytoperm kit (BD Biosciences, San Jose, CA) followed by incubation with polyclonal rabbit anti-KCa3.1 antibody and then FITC-conjugated goat antirabbit IgG antibody (Abcam, Cambridge, MA). FITC-conjugated rabbit antimouse IgG1 (eBioscience) was used as isotype control. KCa3.1 expression levels were normalized according to the formula (MFI of KCa3.1 – MFI of isotype control).

Intracellular Free Calcium and Measurement of Membrane Potential

CD11c⁺ cells stained with CD11cPE-Cy7 and CD11bPE-Cy5 were loaded with 5 μ M Indo-1 or 1 μ M DiBAC4 (3) (Invitrogen) and resuspended in L15 medium (Sigma-Aldrich, St. Louis, MO) containing 2 mM free Ca²⁺. Baseline fluorescence signal was recorded over time by a FACSaria flow cytometer (BD Biosciences) before the addition of ionomycin, CCL19, CCL21, KCa3.1 opener 1-EBIO, and the KCa3.1 blocker TRAM-34. The ratios of fluorescence signal intensity at a wavelength of 390 versus 530 nm (bound indo-1/free indo-1) indicated the intracellular Ca²⁺ levels. DiBAC4 (3) fluorescence was measured at 530 nm with excitation at 488 nm.

Immunofluorescence Imaging

FACS-sorted CD11c^{low}CD11b^{high} DCs stained with tetramethylrhodamine-conjugated wheat germ agglutinin (Invitrogen), DAPI, and anti-KCa3.1 primary and secondary antibodies were visualized using the Olympus BX51 fluorescence microscope and an Olympus DP71 digital camera (Olympus, Japan).

Chemotaxis Assay

Chemotaxis in response to 100 ng/ml CCL19 and CCL21 (PeproTech, Rocky Hill, NJ) in the presence or absence of KCa3.1 blocker TRAM-

34 (200 nM) was examined using a TransWell system as described previously (28).

Quantitative RT-PCR

First-strand cDNA was synthesized using Improm II reverse transcriptase kit (Promega, Madison, WI). The sequences for KCa3.1 forward primer were 5'- AACTGGCATCGGACTCATGGTTCT-3', reverse primer, 5'- AGTCATGAACAGCTGGACCTCCTT-3'; and GAPDH forward primer, 5'- TCAACAGCAACTCCCACTCTTCCA-3', reverse primer, 5'- ACCCTGTTGCTGTAGCCGTATTCA-3'. Real-time PCR was performed on a CFX 96 Real Time PCR Machine (Bio-Rad, Hercules, CA). The value of 2^{- $\Delta\Delta$ CT} was calculated to indicate the fold changes of KCa3.1 mRNA in cells from OVA-sensitized mice relative to PBS-treated mice.

Statistical Analysis

Data were analyzed using Flowjo (Tree Star, Ashland, OR) and GraphPad (GraphPad Software, La Jolla, CA). Paired and unpaired Student's *t* test and one sample *t* test were used. A value of *P* < 0.05 was considered significant. Values are expressed as means $\pm$ SEM.

RESULTS

KCa3.1 Is Differentially Expressed in the Two Lung DC Subsets

Lung CD11c^{high} and CD11c^{low} and CD11c^{low}CD11b^{high} DC subsets expressed KCa3.1 protein as measured by flow cytometry (Figure 2A). Significantly higher levels of KCa3.1 protein expression were observed in DCs isolated from OVA-sensitized and OVA-challenged mice as compared with PBS-treated, nonsensitized mice (Figure 2B). However, the greatest changes were observed in the CD11c^{low}CD11b^{high} immunogenic DC subset in mRNA and protein expression (Figures 2B and 2C), indicating that OVA sensitization might exert more influence on KCa3.1 expression in the CD11c^{low}CD11b^{high} DC subset. The DCs isolated from OVA-sensitized and OVA-challenged mice demonstrated a significant up-regulation (4.37 $\pm$ 0.87-fold in the CD11c^{high} DC subset and 9.37 $\pm$ 0.39-fold in the CD11c^{low} DC subset, respectively; *n* = 4) in KCa3.1 mRNA relative to the DCs isolated from PBS-treated mice (Figure 2C). To confirm that the fluorescence is, at least in part, from membrane-bound antibody, fluorescence imaging was used to detect the localization of KCa3.1 expression. KCa3.1 expression

Figure 1. Sensitization protocol. (A [solid frame]) Ovalbumin (OVA) sensitization and PBS-treated controls. (B [dashed frame]) Identification of lung Ag-carrying cells. A total of 150 μ g of OVA-AlexaFluor647 in 30 μ l was intranasally delivered into lungs of OVA-sensitized mice on Day 45. Lungs were collected on Day 47 for the evaluation of antigen uptake by lung dendritic cell (DC) subsets.

(Figure 2D; FITC, *green*) is colocalized with cell membrane that was stained with wheat germ agglutinin (tetramethylrhodamine conjugate) (Figure 2D, *red*). The localization of KCa3.1 protein on cell membrane is a crucial structural basis for its functionality.

KCa3.1 Opener 1-EBIO Induces Membrane Hyperpolarization and Intracellular Ca^{2+} Increase

The efflux of potassium cation caused by opening of KCa3.1 results in cell membrane hyperpolarization, which serves as a driving force for further extracellular calcium influx. The addition of 1-EBIO induced membrane hyperpolarization as indicated by a decrease in DiBAC4 (3) fluorescence intensity at 530 nm, a consequence caused by potassium efflux upon activation of KCa3.1 (Figures 3A and 3B). We then measured the kinetics of the intracellular free Ca^{2+} in the presence of the KCa3.1 opener 1-EBIO. The addition of 1-EBIO (500 μM [Figure 3C] and 1,000 μM [Figure 3D]) induced an instantaneous increase in intracellular free Ca^{2+} in a dose-dependent manner as a consequence of membrane hyperpolarization. In 1-EBIO-treated lung DCs, the addition of the KCa3.1-specific blocker TRAM-34 (1 μM) immediately decreased intracellular free Ca^{2+} levels. These findings indicated that the 1-EBIO-induced Ca^{2+} increase and the TRAM-34-induced Ca^{2+} decrease are KCa3.1 mediated and further confirmed the expression of KCa3.1 in the two lung DC subsets. CD11c^{high}CD11b^{low} DCs exhibit relatively high indo-1 ration signal compared with CD11c^{low}CD11b^{high} DCs. This is possible due

to the different efficiency in dye loading between two DC subsets and does not exclude the involvement of other types of channels, such as the nonselective cation channel.

KCa3.1 Expression Is Higher in Ag-Carrying DCs than in Non-Ag-Carrying DCs

To examine the relationship between DC activation and KCa3.1 expression, the AlexaFluor647-conjugated OVA or DQ-OVA was intranasally delivered into mouse lungs. DCs that have taken up OVA antigen exhibited fluorescence at emission wavelength 660 nm (AlexaFluor647) or 510 nm (BODIPY FL) and thus can be identified by flow cytometry (Figure 4, *upper panel*). Ag-carrying cells expressed higher levels of KCa3.1 protein than non-Ag-carrying cells, but the degrees of the difference were not as much as those between sensitized and nonsensitized mice (Figures 1 and 4), suggesting that antigen uptake is not the only factor regulating KCa3.1 expression.

Ag-Carrying Lung DCs Express Higher Levels of CCR7 than Non-Ag-Carrying DCs

We have previously demonstrated that lung DCs in OVA-sensitized mice express higher levels of CCR7 than those in PBS-treated, nonsensitized mice and that the immunogenic lung DC subset has higher CCR7 expression than the regulatory DCs. To examine the relationship between antigen uptake and CCR7 expression, the DQ-OVA antigen was intranasally delivered into mouse lungs so that Ag-carrying DCs and non-Ag-carrying DCs could be detected using flow cytometry (Figure 5,

Figure 2. Expression of KCa3.1 in lung DC subsets. (A) The expression of KCa3.1 in lung DC subsets measured by immunostaining (FITC) and flow cytometry as compared with isotype controls are presented as a histogram of cell frequency versus mean fluorescence intensity (MFI). Data are representative of three independent experiments. (B) Quantitative analysis of KCa3.1 protein expression in lung DC subsets. Protein expression was calculated according to the formula $(\text{MFI}^{\text{KCa3.1}} - \text{MFI}^{\text{isotype}})$ ($n = 3$; error bars, 95% confidence interval [CI]). (C) Fold change of mRNA expression of KCa3.1 in lung DC subsets. The value of fold change between OVA-treated and PBS-treated mice was calculated according to the formula $2^{-(\Delta\text{CT}_{\text{OVA}} - \Delta\text{CT}_{\text{PBS}})}$. One sample t test was used to test statistical significance with respect to value 0 ($n = 4$; error bars, 95% CI). (D)

Localization of KCa3.1 on cell membrane. D1, KCa3.1 expression (FITC); D2, cell membrane (WGA-tetramethylrhodamine); D3, nuclear (DAPI); D4, merged image (cell was a MACS-separated-FACS-isolated lung CD11c^{low}CD11b^{high} DC, a representative example of at least three independent experiments; original magnification: $\times 60$).

Figure 3. –KCa3.1 activator 1-EBIO-induced membrane hyperpolarization and intracellular calcium increase. Auto-MACS-separated CD11c⁺ lung cells were loaded with membrane potential dye DiBAC4(3) (1 μM) or indo-1 (5 μM) at 37°C for 45 minutes. Signal acquisition was performed to obtain the baseline values. The KCa3.1 activator 1-EBIO was added to the CD11c⁺ cell suspension containing lung CD11c^{high}CD11b^{low} (upper panel) and CD11c^{low}CD11b^{high} (lower panel) DCs at 90 ± 5 seconds (A, B) and 45 ± 5 seconds (C, D), with a final concentration of 500 μM (A–C) and 1 mM (D). (E) TRAM-34 (1 μM) was added to 1-EBIO-treated lung DCs. Signal acquisition started 15 seconds after the addition of TRAM-34 and lasted for 300 seconds. Data were collected from three independent experiments using lung DCs pooled from four to six experimental animals for each experiment.

upper panel). Ag-carrying cells had significantly higher CCR7 expression than non-Ag-carrying cells in lung CD11c^{high} and CD11c^{low} and CD11c^{low}CD11b^{high} DC subsets (Figure 5, lower panel).

Lymphatic Chemokines Induce Intracellular Ca²⁺ Increase

Chemokine-induced cell migration is calcium dependent. Activation of CCR7, a G-protein-coupled receptor, induces calcium release from intracellular storage and subsequent calcium influx, which has been shown in human monocyte-derived DCs (2, 5) and in mouse bone marrow-derived DCs (1). The lung CD11c^{high}CD11b^{low} and CD11c^{low}CD11b^{high} DCs were isolated

from OVA-sensitized mice on Day 45 (see Figure 1). *In vitro*, the addition of the lymphatic chemokine CCL19 (1 μg/ml) or CCL21 (1 μg/ml) induced an increase of intracellular free Ca²⁺ (Figures 6A–6F). We used 1 μg/ml of CCL19 and CCL21 in calcium influx assay to achieve maximum amplitude of calcium influx. Kinetics analysis revealed gradually increased slope over 150 seconds from the addition of the chemokines until calcium levels reached a plateau, unlike the typical calcium influx observed in the presence of ionomycin. These data suggested that the CCR7 activation by CCL19 or CCL21 contributed to the activation of KCa3.1 by inducing intracellular Ca²⁺ increase.

Figure 4. Expression of KCa3.1 in Ag-carrying and non-Ag-carrying lung DCs. (A) Ag-carrying DCs were defined based on AlexaFluor647 signal intensity in the cells isolated from the mouse lungs not receiving labeled antigen. (B) KCa3.1 expression in the Ag-carrying and non-Ag-carrying DCs. (C) KCa3.1 expression levels were quantified and indicated by mean fluorescence intensity ($n = 3$; data obtained from three experimental animals and one control animal).

Figure 5. Expression of CCR7 in Ag-carrying and non-Ag-carrying lung DCs. (A) Ag-carrying DCs were defined based on DQ-OVA signal intensity in the cells isolated from the mouse lungs not receiving labeled antigen. (B) CCR7 expression in the Ag-carrying and non-Ag-carrying DCs. (C) Quantification of CCR7 expression indicated by mean fluorescence intensity ($n = 3$, data obtained from three experimental animals and one control animal).

Blockade of KCa3.1 Impairs CCL19/CCL21-Induced Lung DC Migration

The blockade of KCa3.1 by TRAM-34 (200 nM) significantly reduced the migration of CD11c^{high}CD11b^{low} and CD11c^{low}CD11b^{high} DCs in response to lymphatic chemokines CCL19 (100 ng/ml) and CCL21 (100 ng/ml) in a TransWell transmigration system as compared with the controls without TRAM-34 (Figures 7A and 7B). These findings added strong evidence that KCa3.1 was involved in lymphatic chemokine-induced DC migration.

DISCUSSION

Here, for the first time, we report the KCa3.1 expression in two functionally distinct lung DC subsets in PBS-treated and OVA-sensitized and OVA-challenged mice. In PBS-treated mice, the immunogenic CD11c^{low}CD11b^{high} DCs had significantly lower expression of KCa3.1 as compared with the regulatory CD11c^{high}CD11b^{low} DCs. OVA sensitization differentially up-regulated KCa3.1 expression in two lung DC subsets,

with the greatest up-regulation observed in immunogenic CD11c^{low}CD11b^{high} DCs. However, the final expression levels of KCa3.1 in two lung DC subsets in OVA-sensitized mice are similar. This explains why the blockade of KCa3.1 by its specific marker TRAM-34 did not demonstrate a differential impairing effect on lung DC migration in the two DC subsets. Additionally, although Ag-carrying DCs expressed higher levels of KCa3.1 than non-Ag-carrying DCs, the difference was not as prominent as the difference between PBS- and OVA-sensitized mice. This suggests that, under the condition of allergic airway inflammation, KCa3.1 expression in lung DCs is probably regulated by multiple factors other than antigen loading. Other factors that are present in the microenvironment, such as cytokines and growth factors, contribute to the consequential up-regulation of KCa3.1 expression.

The implications of these findings help to explain the different patterns of migration that we reported previously. OVA sensitization rapidly recruits immunogenic CD11c^{low}CD11b^{high} DCs, significantly enhances their migration to draining lymph node, and largely boosts their antigen uptake activity (28). This is well

Figure 6. CCL19/CCL21-induced intracellular calcium increase. Cells were labeled with CD11c and CD11b antibody and incubated with Indo-1 for 45 minutes. Intracellular calcium levels were recorded by flow cytometry before and after the addition of ionomycin (1 μ g/ml) (A, B), CCL19 (1 μ g/ml) (C, D) and CCL21 (1 μ g/ml) (E, F) in lung CD11c^{high}CD11b^{low} (upper panel) and CD11c^{low}CD11b^{high} (lower panel) DCs. Data were collected from three independent experiments using lung DCs pooled from four to six experimental animals for each experiment.

Figure 7. Effect of TRAM-34 on lung DC chemotaxis. Final concentration of chemokines and blocker: CCL19, 100 ng/mL; CCL21, 100 ng/mL; TRAM34, 200 nM ($n = 3$). *Upper panel:* CD11c^{high}CD11b^{low} DCs. *Lower panel:* CD11c^{low}CD11b^{high} DCs. The chemotactic index, a measure of the specificity of migration, was determined as follows: (number of cells migrating to chemokines)/(number of cells that migrated to medium alone).

correlated with a greater up-regulation of KCa3.1 by OVA sensitization in this DC subset, providing strong evidence that KCa3.1 is involved in OVA sensitization-mediated DC activation, with a greater effect in immunogenic CD11c^{low}CD11b^{high} DCs.

The linkage between KCa3.1 and lung DC migration appears to be CCR7-induced intracellular calcium release and the following store-operated calcium entry. This is evidenced by the coexpression of CCR7 and KCa3.1, CCR7 activation-induced calcium influx, and 1-EBIO-induced membrane hyperpolarization in mouse lung DCs. The blockade of KCa3.1 by TRAM-34 disrupted the temporal coupling between KCa3.1 and calcium influx and subsequently impaired CCR7-induced chemotaxis.

KCa3.1 activity in regulating cell proliferation, activation, and migration is calcium dependent. Only a small increase in intracellular Ca²⁺ is required to activate KCa3.1 and allow K⁺ efflux, which counteracts the depolarizing effect of Ca²⁺ influx (8, 29–31). The net result of a CCR7 activation-induced Ca²⁺ influx is membrane depolarization, provided that no other ion channel is involved. However, if potassium efflux couples with calcium influx temporally, the overall consequence could be depolarization or hyperpolarization, depending on the kinetics and conductance of the involved calcium and potassium channels. It has been recently reported that store-operated calcium

influx leads to cell membrane hyperpolarization in human monocyte-derived macrophages and that the outward cationic current is carried by KCa3.1 (31). In the context of cell proliferation, calcium influx permits cells in the G1 phase to pass through the G1-S checkpoint (32, 33). The role of KCa3.1 channels in cell migration is more complicated. Because KCa3.1 channels are not sensitive to voltage stimulation, they seem perfectly designed to maintain the resting membrane potential in the cells without excitable membranes, such as immune cells. In addition, KCa3.1 is responsible for concerting with calcium oscillation in migratory cells by acting in a fluctuating pattern to keep pace with cell protrusion and retraction (34). KCa3.1 is also a swelling-activated potassium channel in many cell types, and the activation of KCa3.1 can orchestrate the volume, regulate cytoskeleton organization, and facilitate transmembrane water transport (35, 36). However, sustained opening of KCa3.1 disrupts the coupling between calcium influx and the calcium-activated potassium channel and thus impairs the temporal dynamics of the calcium signal necessary for cell migration. In transformed renal epithelial cells, 1-EBIO reduces migration rate (26). Finally, TRAM-34 is a highly specific small molecule blocker of KCa3.1 that does not affect cytochrome P450. TRAM-34 has low toxicity and causes minimal cell death and apoptosis (10, 24). If the role of KCa3.1 in DC migration under inflammatory condition is further established in an *in vivo* study, TRAM-34 could be a potential drug that targets KCa3.1.

KCa3.1 seems to be preferentially involved in cell biology under pathological conditions. In the case of OVA allergen-induced acute airway inflammation, KCa3.1 regulates DC migration at two levels. First, CCR7 activation is linked to KCa3.1 activation via CCL19/CCL21-induced intracellular calcium release (1, 2). The high CCR7 expression in the immunogenic lung DC subset or under inflammation conditions creates a favorable condition for KCa3.1 activation, which facilitates further calcium influx for a rapid DC migration. Second, a higher KCa3.1 expression in lung DCs under allergic inflammation conditions warrants its greater involvement in DC migration. Knowing this will help define a new role of ion channels in the regulation of DC migration.

In conclusion, our data suggest that antigen sensitization up-regulates KCa3.1 expression, which may contribute to enhancing cell migration in response to lymphatic chemokines, particularly in the immunogenic lung DC subset.

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References

- Yanagihara S, Komura E, Nagafune J, Watarai H, Yamaguchi Y. EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation. *J Immunol* 1998;161:3096–3102.
- Chan VW, Kothakota S, Rohan MC, Panganiban-Lustan L, Gardner JP, Wachowicz MS, Winter JA, Williams LT. Secondary lymphoid-tissue chemokine (SLC) is chemotactic for mature dendritic cells. *Blood* 1999;93:3610–3616.
- Berridge MJ. Inositol trisphosphate and calcium signalling mechanisms. *Biochim Biophys Acta* 2009;1793:933–940.
- Hsu S, O'Connell PJ, Klyachko VA, Badminton MN, Thomson AW, Jackson MB, Clapham DE, Ahern GP. Fundamental Ca²⁺ signaling mechanisms in mouse dendritic cells: CRAC is the major Ca²⁺ entry pathway. *J Immunol* 2001;166:6126–6133.
- Scandella E, Men Y, Legler DF, Gillessen S, Prikler L, Ludewig B, Groettrup M. CCL19/CCL21-triggered signal transduction and migration of dendritic cells requires prostaglandin E2. *Blood* 2004;103:1595–1601.
- Partida-Sanchez S, Goodrich S, Kusser K, Oppenheimer N, Randall TD, Lund FE. Regulation of dendritic cell trafficking by the ADP-ribosyl

- cyclase CD38: impact on the development of humoral immunity. *Immunity* 2004;20:279–291.
7. Matzner N, Zemtsova IM, Nguyen TX, Duszenko M, Shumilina E, Lang F. Ion channels modulating mouse dendritic cell functions. *J Immunol* 2008;181:6803–6809.
 8. Joiner WJ, Wang LY, Tang MD, Kaczmarek LK. hSK4, a member of a novel subfamily of calcium-activated potassium channels. *Proc Natl Acad Sci USA* 1997;94:11013–11018.
 9. Schwab A, Nechyporuk-Zloy V, Fabian A, Stock C. Cells move when ions and water flow. *Pflugers Arch* 2007;453:421–432.
 10. Shepherd MC, Duffy SM, Harris T, Cruse G, Schuliga M, Brightling CE, Neylon CB, Bradding P, Stewart AG. Kca3.1 Ca²⁺-activated K⁺ channels regulate human airway smooth muscle proliferation. *Am J Respir Cell Mol Biol* 2007;37:525–531.
 11. Platoshyn O, Remillard CV, Fantozzi I, Mandegar M, Sison TT, Zhang S, Burg E, Yuan JX. Diversity of voltage-dependent K⁺ channels in human pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2004;287:L226–L238.
 12. Neylon CB, Lang RJ, Fu Y, Bobik A, Reinhart PH. Molecular cloning and characterization of the intermediate-conductance Ca²⁺-activated K⁺ channel in vascular smooth muscle: relationship between K(Ca) channel diversity and smooth muscle cell function. *Circ Res* 1999;85:e33–e43.
 13. Saito T, Fujiwara Y, Fujiwara R, Hasegawa H, Kibira S, Miura H, Miura M. Role of augmented expression of intermediate-conductance Ca²⁺-activated K⁺ channels in postischemic heart. *Clin Exp Pharmacol Physiol* 2002;29:324–329.
 14. Ghanshani S, Wulff H, Miller MJ, Rohm H, Neben A, Gutman GA, Cahalan MD, Chandy KG. Up-regulation of the IKCa1 potassium channel during T-cell activation: molecular mechanism and functional consequences. *J Biol Chem* 2000;275:37137–37149.
 15. Schmid-Antomarchi H, Schmid-Alliana A, Romey G, Ventura MA, Breittmayer V, Millet MA, Husson H, Moghrabi B, Lazdunski M, Rossi B. Extracellular ATP and UTP control the generation of reactive oxygen intermediates in human macrophages through the opening of a charybdotoxin-sensitive Ca²⁺-dependent K⁺ channel. *J Immunol* 1997;159:6209–6215.
 16. Cruse G, Duffy SM, Brightling CE, Bradding P. Functional KCa3.1 K⁺ channels are required for human lung mast cell migration. *Thorax* 2006;61:880–885.
 17. Wulff H, Miller MJ, Hansel W, Grissmer S, Cahalan MD, Chandy KG. Design of a potent and selective inhibitor of the intermediate-conductance Ca²⁺-activated K⁺ channel, IKCa1: a potential immunosuppressant. *Proc Natl Acad Sci USA* 2000;97:8151–8156.
 18. Rane SG. A Ca²⁺-activated K⁺ current in ras-transformed fibroblasts is absent from nontransformed cells. *Am J Physiol* 1991;260:C104–C112.
 19. Neylon CB, Avdonin PV, Larsen MA, Bobik A. Rat aortic smooth muscle cells expressing charybdotoxin-sensitive potassium channels exhibit enhanced proliferative responses. *Clin Exp Pharmacol Physiol* 1994;21:117–120.
 20. Kohler R, Wulff H, Eichler I, Kneifel M, Neumann D, Knorr A, Grgic I, Kampfe D, Si H, Wibawa J, et al. Blockade of the intermediate-conductance calcium-activated potassium channel as a new therapeutic strategy for restenosis. *Circulation* 2003;108:1119–1125.
 21. Tharp DL, Wamhoff BR, Turk JR, Bowles DK. Upregulation of intermediate-conductance Ca²⁺-activated K⁺ channel (IKCa1) mediates phenotypic modulation of coronary smooth muscle. *Am J Physiol Heart Circ Physiol* 2006;291:H2493–H2503.
 22. Tharp DL, Wamhoff BR, Wulff H, Raman G, Cheong A, Bowles DK. Local delivery of the KCa3.1 blocker, TRAM-34, prevents acute angioplasty-induced coronary smooth muscle phenotypic modulation and limits stenosis. *Arterioscler Thromb Vasc Biol* 2008;28:1084–1089.
 23. Grgic I, Eichler I, Heinau P, Si H, Brakemeier S, Hoyer J, Kohler R. Selective blockade of the intermediate-conductance Ca²⁺-activated K⁺ channel suppresses proliferation of microvascular and macrovascular endothelial cells and angiogenesis in vivo. *Arterioscler Thromb Vasc Biol* 2005;25:704–709.
 24. Toyama K, Wulff H, Chandy KG, Azam P, Raman G, Saito T, Fujiwara Y, Mattson DL, Das S, Melvin JE, et al. The intermediate-conductance calcium-activated potassium channel KCa3.1 contributes to atherogenesis in mice and humans. *J Clin Invest* 2008;118:3025–3037.
 25. Duffy SM, Cruse G, Cockerill SL, Brightling CE, Bradding P. Engagement of the EP2 prostanoid receptor closes the K⁺ channel KCa3.1 in human lung mast cells and attenuates their migration. *Eur J Immunol* 2008;38:2548–2556.
 26. Schwab A, Schuricht B, Seeger P, Reinhardt J, Dartsch PC. Migration of transformed renal epithelial cells is regulated by K⁺ channel modulation of actin cytoskeleton and cell volume. *Pflugers Arch* 1999;438:330–337.
 27. Shao Z, Bharadwaj AS, McGee HS, Makinde TO, Agrawal DK. Fms-like tyrosine kinase 3 ligand increases a lung DC subset with regulatory properties in allergic airway inflammation. *J Allergy Clin Immunol* 2009;123:917–924, e912.
 28. Shao Z, Makinde TO, McGee HS, Wang X, Agrawal DK. Fms-like tyrosine kinase 3 ligand regulates migratory pattern and antigen uptake of lung dendritic cell subsets in a murine model of allergic airway inflammation. *J Immunol* 2009;183:7531–7538.
 29. Khanna R, Chang MC, Joiner WJ, Kaczmarek LK, Schlichter LC. HSK4/hIK1, a calmodulin-binding KCa channel in human T lymphocytes. Roles in proliferation and volume regulation. *J Biol Chem* 1999;274:14838–14849.
 30. Wulf A, Schwab A. Regulation of a calcium-sensitive K⁺ channel (CIK1) by protein kinase c. *J Membr Biol* 2002;187:71–79.
 31. Gao YD, Hanley PJ, Rinne S, Zuzarte M, Daut J. Calcium-activated K⁺ channel (K(Ca)3.1) activity during Ca²⁺ store depletion and store-operated Ca²⁺ entry in human macrophages. *Cell Calcium* 2010;48:19–27.
 32. Wonderlin WF, Strobl JS. Potassium channels, proliferation and G1 progression. *J Membr Biol* 1996;154:91–107.
 33. Barbiero G, Munaron L, Antoniotti S, Baccino FM, Bonelli G, Lovisolo D. Role of mitogen-induced calcium influx in the control of the cell cycle in Balb-c 3T3 fibroblasts. *Cell Calcium* 1995;18:542–556.
 34. Schwab A, Wojnowski L, Gabriel K, Oberleithner H. Oscillating activity of a Ca²⁺-sensitive K⁺ channel: a prerequisite for migration of transformed madin-darby canine kidney focus cells. *J Clin Invest* 1994;93:1631–1636.
 35. Hoffmann EK, Lambert IH, Pedersen SF. Physiology of cell volume regulation in vertebrates. *Physiol Rev* 2009;89:193–277.
 36. Stutzin A, Hoffmann EK. Swelling-activated ion channels: functional regulation in cell-swelling, proliferation and apoptosis. *Acta Physiol (Oxf)* 2006;187:27–42.