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Voltage-Gated Sodium Channel Nav1.7 Maintains the Membrane Potential and Regulates the Activation and Chemokine-Induced Migration of a Monocyte-Derived Dendritic Cell Subset

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Expression of CD1a protein defines a human dendritic cell (DC) subset with unique functional activities. We aimed to study the expression of the Nav1.7 sodium channel and the functional consequences of its activity in CD1a− and CD1a+ DC. Single-cell electrophysiology (patch-clamp) and quantitative PCR experiments performed on sorted CD1a+ and CD1a− immature DC (IDC) showed that the frequency of cells expressing Na+ current, current density, and the relative expression of the SCN9A gene encoding Nav1.7 were significantly higher in CD1a+ cells than in their CD1a− counterparts. The activity of Nav1.7 results in a depolarized resting membrane potential (−8.7 ± 1.5 mV) in CD1a+ IDC as compared with CD1a− cells lacking Nav1.7 (−47 ± 6.2 mV). Stimulation of DC by inflammatory signals or by increased intracellular Ca2+ levels resulted in reduced Nav1.7 expression. Silencing of the SCN9A gene shifted the membrane potential to a hyperpolarizing direction in CD1a+ IDC, resulting in decreased cell migration, whereas pharmacological inhibition of Nav1.7 by tetrodotoxin sensitized the cells for activation signals. Fine-tuning of IDC functions by a voltage-gated sodium channel emerges as a new regulatory mechanism modulating the migration and cytokine responses of these DC subsets. The Journal of Immunology, 2011, 187: 1273–1280.

Dendritic cells (DC) act as conductors of the immune system through connecting the innate and adaptive arms of various immune responses (1). Conventional DC differentiate from a common progenitor of macrophages via two independent pathways into CD11c+ blood precursors with or without the expression of membrane CD1 molecules (2, 3). CD1a, widely used as a cell surface marker of human DC, is a type 1 membrane protein (4) that is stabilized by captured self- or pathogen-derived modified lipids (5) to activate CD1a-restricted T lymphocytes (6). CD11c+CD1a+ DC differentiate to Langerhans cells in epithelial surfaces, whereas the CD11c−CD1a− subtype replenishes various tissues with interstitial DC (7). In peripheral tissues, DC also differentiate from blood monocytes driven by microenvironmental factors generated by pathogens, inflammation, Ags, or metabolites (8). In vitro differentiation of monocytes to CD1a− and CD1a+ DC requires GM-CSF to support cell survival and IL-4 driving cell differentiation (9). These DC subsets resemble immature tissue DC (IDC) that can be further activated by stimulatory signals. Previously, we have shown (10) that the CD1a− and CD1a+ DC subsets exhibit different functional properties and are able to skew T cell polarization toward tolerogenic or immunogenic directions, respectively.

Upon Ag encounter and concomitant danger signals, IDC undergo drastic phenotypic and functional changes and the transition toward mature DC (MDC). Activated MDC migrate to draining lymph nodes where they act as potent professional APC that are able to prime and polarize naive Ag-specific T lymphocytes to inflammatory or tolerogenic directions (11).

The presence of voltage-gated ion channels was first described in excitable cells, where they are traditionally involved in the electrogensis of action potential (12). Later, several studies reported that ion channels are also expressed in nonexcitable cells, including immune cells (lymphocytes, macrophages, monocytes, etc.) (13–15). The voltage-gated potassium channels (VGPC) are key elements of sustaining a negative membrane potential required for efficient Ca2+ signaling in these cells. Changes in the expression of VGPCs and other ion channels accompany immune cell differentiation, and thus may have an impact on cellular functions. In a recent study, Black et al. (16) described that microglia cells, which exclusively reside to the CNS, express multiple voltage-gated sodium channels (VGSC), playing a role in cell migration and phagocytosis. The role of sodium channels in the activation and phagocytosis of microglia and macrophages in experimental autoimmune encephalomyelitis and multiple sclerosis was described, and the anti-inflammatory role of sodium channel blockers was suggested (17).
It has also been reported that various VGSC antagonists are able to influence the activation of lymphocytes; modify volume regulation, migration, and apoptosis in Jurkat cells; and impair phagocytosis and inflammatory responses of macrophages (18). However, the function of VGSC in immune cells is still elusive.

In our previous study, we characterized a novel VGSC, the Nav1.7 channel in monocyte-derived DC, and demonstrated a developmental switch of Nav1.7 to VGPC Kv1.3 expression in the course of in vitro DC maturation (19). The α-subunit of Nav1.7 was shown to amplify small depolarization events to generate threshold currents close to the resting potential of neuronal cells (20). The SCN9A gene encoding for the Nav1.7 protein is expressed preferentially in nociceptive dorsal root ganglion neurons and in sympathetic ganglia (21, 22). Nav1.7 expression is upregulated in pathophysiological conditions such as metastatic prostate cancer, where its activity potentiates numerous cellular activities of the metastatic cascade (23). Other studies also indicated that Nav1.7, via mutation or altered transcription, plays a key role in pain induction. Upregulation of Nav1.7 expression and activity results in increased pain sensation, and therapies based on blocking Nav1.7 activity or silencing gene expression emerge as means for effective pain relief (24). Consistent with this scenario, mice lacking the SCN9A gene through specific deletion in nociceptive sensory neurons exhibited reduced responses to inflammatory, mechanical, and thermal pain (25). Furthermore, mutations of the SCN9A gene that cause excessive channel activities revealed the inherited pain syndrome erythermalgia and paroxysmal extreme pain disorder (26). Despite the potential therapeutic importance of Nav1.7 as a pain modulator, the major factors, which regulate the expression of SCN9A, have not been identified. Moreover, the role of VGSCs and in particular that of Nav1.7 in the regulation of Na⁺ flux to immune cells and their contribution to cellular functions are still poorly understood.

In this study, we describe that the expression and function of the Nav1.7 channel are restricted to a subset of human monocyte-derived DC specified by the expression of CD1a. We also identified the role of Nav1.7 in setting the membrane potential of CD1a⁺ IDC and thus modulating the threshold of DC activation. Our results show that the presence of active Nav1.7 channels in the cell membrane of CD1a⁺ IDC keeps the membrane potential at a depolarized state, thus protecting the cell from unnecessary or harmful activation below an actively set threshold. Because the transition of IDC to MDC is a crucial decision step in triggering both innate and adaptive immunity, these findings provide a new regulatory mechanism to control DC functions. Moreover, differences in the expression level of the Nav1.7 channel may shed light on the distinct responses of CD1a⁻ and CD1a⁺ cells to various stimuli.

Materials and Methods

DC and cell cultures

Human monocyte-derived DC were generated from CD14⁺ blood monocytes, as described previously (10). Activation of IDC was induced on day 5 by inflammatory cytokine mixture containing 10 ng/ml TNF-α, 5 ng/ml IL-1β, 20 ng/ml IL-6, 75 ng/ml GM-CSF (PeproTech EC), and 1 μg/ml PGE2 (Sigma-Aldrich, St. Louis, MO) for 24 h. When indicated, IDC were treated on day 5 with 180 ng/ml Ca²⁺ ionophore (ionomycin; Sigma-Aldrich) or with 250 nM thapsigargin (Calbiochem, La Jolla, CA) for 24 h in the absence or presence of EGTA that was added to the cultures at 5 mM together with 10 mM MgCl₂ 1 h before ionomycin, thapsigargin, or tetrodotoxin (TTX) treatments. For the specific inhibition of Nav1.2 channel activity, TTX (Alomone Laboratories) was used at 10–40 μM concentrations for 24 h.

Flow cytometry and cell sorting

Phenotypic characterization of DC was performed by flow cytometry using fluorochrome-conjugated Abs, as follows: anti-CD1a PE for subtype specification, CD80 FITC (Biologend, San Diego CA), CD83 FITC (ImmunoTech, Marseille, France), and CD86 PE (R&D Systems, Minneapolis, MN) for DC maturation as compared with isotype-matched control Abs (BD Pharmingen, San Diego, CA). Fluorescence intensities were measured and analyzed by a FACS Calibur flow cytometer (BD Biosciences Immunocytometry Systems, Franklin Lakes, NJ). CD1a⁺ and CD1a⁻ DC for quantitative PCR (Q-PCR) experiments were separated using a FACS DiVa high-speed cell sorter (BD Biosciences Immunocytometry Systems).

Q-PCR

Q-PCR was performed, as described previously (27). Briefly, total RNA was isolated from sorted CD1a⁺ and CD1a⁻ DC by TRIzol reagent (Invitrogen, Carlsbad, CA). Reverse transcription was performed at 37 °C for 120 min from 100 ng total RNA using the High Capacity CDNA Archive Kit (Applied Biosystems, Foster City, CA). Q-PCR for Nav1.7, IKCa1, and MMP12 genes was performed with ABI PRISM 7000 (Applied Biosystems) with 40 cycles at 94°C for 12 s and at 60°C for 60 s using TaqMan assays (Applied Biosystems). All Q-PCR were run in triplicates with a control reaction containing no reverse-transcriptase enzyme. The comparative cycle threshold method was used to quantify transcripts relative to the endogenous housekeeping control gene 36B4.

Transfection of small interfering RNA

The mix of three different constructs of Nav1.7-specific and control small interfering RNA (siRNA; Applied Biosystems) was transfected into differentiating IDC on day 3 at a final concentration of 1 pmol using the GenePulser X Cell electroporator and 0.4-cm cuvettes (Bio-Rad Laboratories, Hercules, CA). After 2 d of transfection, the level of Nav1.7 mRNA expression was tested by Q-PCR. Viability of the transfected cells was controlled by 7-aminoactinomycin D and annexin V staining (BD Pharmingen).

Electrophysiology

Patch clamping. Standard whole-cell patch-clamp techniques were used in voltage-clamp (current detection) or current-clamp (membrane potential measurement) configuration, as described previously (28). Whole-cell measurements were carried out using Multiclamp 700B and Axopatch-200A amplifiers connected to personal computers using software (Axon Instruments, Foster City, CA). For data acquisition and analysis, the pClamp9 and 10 software packages (Molecular Devices, Sunnyvale, CA) were used. Pipettes were pulled from GC 150 F-15 borosilicate glass capillaries (Clark Biomedical Instruments, Pangbourne, U.K.) in five stages and fire polished to gain electrodes of 2-MΩ resistance to gain electrodes of 2-MΩ resistance. Series resistance compensation up to 85% was used to minimize voltage errors and achieve good voltage-clamp conditions (Vleak < 5 mV). CD1a⁺ and CD1a⁻ cells were identified and selected for patch-clamp recording by anti-CD1a FITC labeling in a Nikon TE2000 fluorescence microscope. When the SCN9 gene in differentiating DC was silenced by specific siRNA on day 3 of differentiation, the cells were labeled with anti-CD1a FITC Ab on day 5 and used for the electrophysiological measurements. The same procedure was applied for the transfection of the control siRNA.

Solutions. The normal bath or extracellular (EC) solution given in mM was as follows: 145 NaCl, 5 KCl, 1 MgCl₂, 2.5 CaCl₂, 5.5 glucose, and 10 HEPES (pH 7.35, 305 mM). The Na⁺⁻free bath solution contained (in mM) the following: 150 KCl, 5 HEPES, 11 EGTA, 8.7 CaCl₂, and 2 glucose (pH 7.2), were used in the pipette (free Ca²⁺ concentration is 360 μM). The high K⁺ bath solution consisted of (in mM) the following: 150 KCl, 1 MgCl₂, 2.5 CaCl₂, 5.5 glucose, and 10 HEPES (pH 7.35, 305 mM). The pipette solution for membrane potential recording in perforated patch mode contained (in mM) the following: 145 KCl, 5 HEPES, 11 EGTA, 1 CaCl₂, 2.5 MgCl₂, and 10 HEPES (pH 7.20, ~295 mM). The pipette filling solution for membrane potential recording in perforated mode contained (in mM) the following: 150 KCl, 2 MgCl₂, 1 CaCl₂, 5 HEPES, 10 EGTA (pH 7.2), and 0.3 g/l Nystatin. For IKCa1 current measurements Digitida 1440 or 1230 data acquisition boards (Axon Instruments, Foster City, CA). For data acquisition and analysis, the pClamp9 and 10 software packages (Molecular Devices, Sunnyvale, CA) were used. Pipettes were pulled from GC 150 F-15 borosilicate glass capillaries (Clark Biomedical Instruments, Pangbourne, U.K.) in five stages and fire polished to gain electrodes of 2-MΩ resistance to gain electrodes of 2-MΩ resistance. Series resistance compensation up to 85% was used to minimize voltage errors and achieve good voltage-clamp conditions (Vleak < 5 mV). CD1a⁺ and CD1a⁻ cells were identified and selected for patch-clamp recording by anti-CD1a FITC labeling in a Nikon TE2000 fluorescence microscope. When the SCN9 gene in differentiating DC was silenced by specific siRNA on day 3 of differentiation, the cells were labeled with anti-CD1a FITC Ab on day 5 and used for the electrophysiological measurements. The same procedure was applied for the transfection of the control siRNA.

Determination of IKCa1 channel numbers. The whole-cell current was evoked by voltage ramps, and the magnitude of the current was measured at ~85 mV (I−85) and at −120 mV (I−120). Because the reversal potential for K⁺ is approximately −85 mV (EK), calculated from the composition of the pipette and the normal bath solution using the Nernst equation, the current at this voltage is purely the leak current. In contrast, the current measured at −120 mV consists of the IKCa1 current and the leak. Because the leak current reverses at 0 mV and has linear current-voltage relationship, its 2 value can be calculated at −120 mV using the following formula: Ileak(−120 mV) = −I−85 × 1−120 mV, and can be subtracted from the whole-

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cell current measured at $-120 \text{ mV}$ to give the pure IKCa1 current at that voltage $[I_{\text{IKCa1}}(@ -120 \text{ mV})]$. Furthermore, the IKCa1 current does not show time- and voltage-dependent activation (current-voltage relationship is linear); hence, the whole-cell conductance for IDC expressing only IKCa1 channels can be calculated as follows: $G = I_{\text{IKCa1}}(@ -120 \text{ mV})/(-120 \text{ mV} - E_k)$, where $I_{\text{IKCa1}}(@ -120 \text{ mV})$ is the current of IKCa1 channels at $-120 \text{ mV}$ and $E_k$ is the reversal for K$. The single channel conductance of IKCa1 was determined earlier ($\gamma = 11 \text{ pS}$) (29); thus, the number of channels in a cell is as follows: $n = G/\gamma$.

**Test substances.** Toxins (TTX, charybdotoxin [ChTx]; Alomone Laboratories) were dissolved in bath solution supplemented with 0.1 mg/ml BSA (Sigma-Aldrich) to prevent nonspecific binding of the toxins to the plastic wall. TRAM-34 (Sigma-Aldrich) was dissolved in DMSO. Bath perfusion around the measured cell with different test solutions was achieved using a gravity-flow perfusion setup with six input lines and PE10 polyethylene tube output tip having flanged aperture to reduce the turbulence of the flow. Excess fluid was removed continuously.

**Cell migration assay**

IDC were suspended in migration medium (0.5% BSA in RPMI 1640) at $10^6$ cells/ml. Matrigel-coated or noncoated transmigration inserts (diameter 6.5 mm; pore size 3 $\mu$m) were obtained from BD Biosciences. MIP-1α chemokine (PeproTech) was diluted in migration medium to 200 ng/ml and added to the lower chambers in a final volume of 600 $\mu$L. A total of 40 $\mu$M TTX or equal volume of DMSO was added to the lower and the equivalent upper chambers. When spontaneous trans-endothelial migration assays were performed, the migration medium in the lower chamber was RPMI 1640 plus 0.5% BSA in the absence of chemokines. IDC were added to the upper chamber in a final volume of 250 $\mu$L, and 24-h chemotaxis assays were performed in 5% CO$_2$ at 37°C. At the end of the assay, the inserts were discarded and cells migrated to the lower chamber were collected. Migrated cell numbers were counted by using polystyrene standard beads (Sigma-Aldrich) by flow cytometry. Specific and control siRNA-transfected IDC were prepared, as described above, and tested in the cell migration assay.

**Statistical analysis**

Statistical analysis and sample comparisons were made by unpaired $t$ test, and the level of significance was set to 0.05. The mean and SEM of at least four or more independent experiments are shown. $\chi^2$ test with Yates correction was used for correlation analysis.

**Results**

**Nav1.7 is expressed predominantly in the CD1a$^+$ subpopulation of monocyte-derived IDC**

We have previously shown that functionally active Nav1.7 channels are expressed by monocyte-derived IDC and their expression is downregulated upon activation (19). In this study, we compared Nav1.7 expression in the CD1a$^-$ and CD1a$^+$ IDC subsets previously characterized by distinct functional activities. A robust inward, rapidly activating and inactivating Na$^+$ current was previously characterized by distinct functional activities. A robust inward, rapidly activating and inactivating Na$^+$ current was detected in CD1a$^+$ IDC evoked by a 15-ms–long depolarization to $0 \text{ mV}$ from a holding of $-120 \text{ mV}$ upon perfusion with normal EC solution (Fig. 1A). The incidence of Na$^+$ current detection in CD1a$^-$ IDC was significantly less frequent than in CD1a$^+$ cells ($\chi^2$ test with Yates correction: CD1a$, n = 25$ and CD1a$^+$, $n = 19$, $p = 0.021$). The Na$^+$ current density of CD1a$^-$ and CD1a$^+$ IDC (CD, peak current at $0 \text{ mV}$ divided by the capacitance of the cell, a quantity proportional to the number of channels/unit membrane area) also showed substantial differences, being significantly higher in the CD1a$^+$ IDC subset ($I_{\text{CD1a}^+} = -83.7 \pm 6.6 \text{ pA/}pF$, $I_{\text{CD1a}^-} = -43.4 \pm 13.4 \text{ pA/}pF$ [$n = 9$, $p = 0.015$]), as shown in Fig. 1B. However, the capacitance of the two subtypes was found to be the same (11.9 ± 5.5 pF and 13.5 ± 1.5 pF for CD1a$^+$ and CD1a$^-$ cells, respectively [$n = 9$, $p = 0.457$]). Moreover, the relative expression of Nav1.7 mRNA measured by Q-PCR was also higher in the immature CD1a$^-$ than in the CD1a$^+$ subset (fold change CD1a$^+$ versus CD1a$^-$ = 3.5; $p = 0.05$) (Fig. 1C). The expression of Nav1.7 was dramatically downregulated upon DC maturation (Fig. 1C). Kinetic studies revealed the rapid upregulation (Fig. 2A) and downregulation (Fig. 2B) of Nav1.7 mRNA expression in the course of monocyte to IDC and IDC to MDC differentiation, respectively. As early as 3 h after the addition of GM-CSF and IL-4, the expression of Nav1.7 mRNA was upregulated and was increasing continuously during the entire process of IDC differentiation (Fig. 2A), whereas DC maturation triggered by an inflammatory cytokine mixture was able to downregulate the expression of the Nav1.7 channels (Fig. 2B). Other stimuli, known to induce DC activation [LPS + IFN-γ, poly(I:C), CD40L] also led to the rapid reduction of Nav1.7 transcription (data not shown). These results indicate that the role of the Nav1.7 channels is closely connected to the immature state of DC and acts primarily in the CD1a$^+$ subset.

**The expression of Nav1.7 is downregulated via the elevation of intracellular Ca$^{2+}$ concentration**

Although the full complexity of the molecular mechanism(s) that regulates the activation of DC remains to be clarified, several studies support the role of cytosolic Ca$^{2+}$ signaling (reviewed in Ref. 30). Thus, next we investigated whether sustained high intracellular Ca$^{2+}$ concentration by itself would be able to decrease the expression of the Nav1.7 channel. We found that elevation of intracellular Ca$^{2+}$ concentration, induced either by the Ca$^{2+}$ ionophore ionomycin or by the sarco(endo)plasmic reticulum Ca$^{2+}$-ATPase antagonist thapsigargin, was a potent inhibitor of Nav1.7 gene expression and had a comparable inhibitory capacity to that of the inflammatory cytokine mixture (Fig. 3A). A total of 5 mM extracellular EGTA partially or completely reverses the expression of Nav1.7 mRNA expression, respectively. However, EGTA had marginal effect on the inhibition of Nav1.7 mRNA expression induced by the inflammatory mixture.

The ability of increased intracellular Ca$^{2+}$ level to downregulate Nav1.7 channel expression was further verified by the decreased number of CD1a$^+$ IDC expressing the Nav1.7 channel after 24-h thapsigargin treatment (Fig. 3B). Similarly to the inhibition of mRNA expression, EGTA partially reversed the downregulation of Nav1.7 expression in the plasma membrane. When stimulated IDC by the inflammatory mixture, ionomycin or thapsigargin, and compared the cell surface expression of the activation molecules CD83, CD80, and CD86, we found that induction of CD83 and CD80 by ionomycin or thapsigargin, but not by the inflammatory mixture could also be partially reversed by EGTA (Supplemental Fig. 1). These signals also resulted in cytokine secretion in DC and production of TNF-α could be inhibited, whereas IL-6 secretion was augmented by EGTA (Supplemental Fig. 1). These results suggest that changes in calcium levels may have complex effects on DC activation and cytokine production. Hence, not only various maturation stimuli of IDC, but environmental factors that increase intracellular Ca$^{2+}$ levels may trigger Nav1.7 channel downregulation.

**Nav1.7 dependence of the membrane potential in CD1a$^+$ IDC**

The role of VGSC in immune cells is unknown, but they are thought to contribute to the maintenance of the resting membrane potential. Hence, we analyzed the membrane potential of Nav1.7-expressing CD1a$^+$ IDC, their CD1a$^-$ counterparts, and the corresponding MDC. Whole-cell current-clamp measurements demonstrated that the resting membrane potential of CD1a$^+$ IDC is significantly depolarized ($-8.7 \pm 1.5 \text{ mV}$, $n = 14$) as compared with CD1a$^-$ IDC or MDC (between $-40$ and $-60 \text{ mV}$, with an average of $-47 \pm 6.2$, $n = 7$, Fig. 4A). We postulated that this difference in the membrane potential may originate from the presence of func-
tional Na⁺ channels in CD1a⁺ IDC. To test this hypothesis, we de-
termined the extracellular Na⁺ sensitivity of the membrane po-
tential in IDC. Fig. 4 demonstrates that the membrane potential
of CD1a⁺ IDC hyperpolarizes during transient exposure to a Na⁺-
free extracellular solution to values close to those recorded in
CD1a⁻ IDC and MDC of both CD1a⁻ and CD1a⁺ subtypes. This
solution completely abolishes the inward Na⁺ current in voltage-
clamp experiments (Fig. 1A). On the contrary, the membrane
potential of CD1a⁻ IDC remained unchanged during the exposure
to a Na⁺-free extracellular solution (Fig. 4C). The Nav1.7 de-
pendence of the membrane potential was further studied by si-
Priming the Nav1.7 gene by using specific siRNA. Transfection of
the Nav1.7-specific siRNA into IDC decreased the expression of
Nav1.7 mRNA by ∼60% (data not shown) and the number of
Nav1.7 current-positive CD1a⁺ IDC to 13% (87% inhibition),
whereas 96% of CD1a⁺ cells treated by control siRNA remained
positive for Na⁺ current expression (n = 20 for control and
SCN9A-silenced cells). Current-clamp recordings showed that the
membrane potential of Nav1.7-specific siRNA-transfected CD1a⁺
IDC was significantly more negative (−22.3 ± 1.5 mV; n = 8)
than in nontransfected CD1a⁺ IDC (p < 0.001, Fig. 4A). Fur-
thermore, the membrane potential of SCN9A siRNA-transfected
cells did not change upon exposure to a Na⁺-free solution (Fig.
4D) similarly to the behavior of CD1a⁻ IDC lacking Nav1.7 channels (Fig. 4C).

Fig. 4B–D shows that the membrane potential of IDC is sensitive
to the extracellular K⁺ concentration: transient exposure of IDC to
a high K⁺ solution (150 mM K⁺ concentration) depolarized the
membrane potential to ∼0 mV. This and the relatively negative
membrane potential of CD1a⁻ IDC (Fig. 4A,4C) suggested the
presence of K⁺ channels in the membrane. As our previous study
showed IDC lacking voltage-gated K⁺ channels (19), the expression
of a nonvoltage-gated K⁺ channel was postulated in the membrane
of IDC. Fig. 5A shows current traces recorded in CD1a⁻ IDC upon
a voltage-ramp protocol using a pipette solution containing 1
mM free Ca²⁺. The biophysical and pharmacological properties of the
current indicated the expression of IKCa1 Ca²⁺-activated K⁺

**FIGURE 1.** Expression levels of Nav1.7 in
CD1a⁻ and CD1a⁺ IDC. A, Whole-cell Na⁺
current in a CD1a⁺ IDC recorded upon a 15-
ms-long depolarization to 0 mV from a holding
of −120 mV. Arrows indicate the perfusion with
normal and Na⁺-free extracellular solutions. B, Current density of Nav1.7 channels in
IDC. The peak Na⁺ currents were determined at
0 mV and divided by the capacitance of the cell
(measured in pF). This ratio was averaged for
n > 6 CD1a⁺ and CD1a⁻ cells; error bars indi-
cate SEM. C, Gene expression of Nav1.7 in
monocytes (Mon.), IDC (open bars), and MDC
(hatched bars). Triplicates of a typical experi-
ment of three are shown. Error bars indicate SD. *p < 0.05.

**FIGURE 2.** Transcriptional changes of Nav1.7 mRNA upon monocyte-
derived DC differentiation. A, Time course of Nav1.7 transcript levels in
IDC upon addition of IL-4 and GM-CSF to monocytes. B, Nav1.7 mRNA
levels during IDC to MDC transition induced by inflammatory cytokine
mixture given at the indicated time points. Triplicates of a typical exper-
iment of three are shown. Error bars indicate SD.

**FIGURE 3.** Effects of increased intracellular Ca²⁺ concentration on
Nav1.7 gene expression in IDC. A, Nav1.7 gene expression in IDC (con-
trol) and in IDC triggered by 250 nM thapsigargin, 180 ng/ml ionomycin,
or inflammatory mixture was measured, as described in Materials and
Methods, for 24 h in the absence (open bars) or presence (hatched bars) of
5 mM EGTA and 10 mM MgCl₂. The expression level measured in MDC
is shown as a positive control. B, Percentage of Nav1.7 expressing CD1a⁺
IDC in control and in thapsigargin-treated cells in the absence (open bars)
and presence (hatched bars) of EGTA. Cells were considered to express
Nav1.7 channels if a characteristic inward current was recorded at 0 mV
test potential and the peak current exceeded 100 pA. Statistical calculation
was based on the electrophysiological records from n > 13 cells in each
group; error bars show SEM.
channels: the current reversed at \( \sim -80 \) mV and could be blocked by 25 nM ChTx (\( K_d \) value for IKCa1 is \( \sim 5 \) nM) or with 125 nM TRAM-34, a selective antagonist of IKCa1 channels (\( K_d \) \( \sim 20-30 \) nM). The number of IKCa1 channels/cell was estimated for both the CD1a\(^{-} \) and CD1a\(^{+} \) IDC, as detailed in Materials and Methods. No significant difference between the two DC subsets (\( p = 0.269 \)) could be detected (Fig. 5B). The gene expression levels of IKCa1 in the two IDC subtypes were also comparable (Fig. 5C). As both IDC subtypes express similar levels of IKCa1, we concluded that the lack of IKCa1 channels in CD1a\(^{+} \) cells cannot be responsible for the depolarized resting membrane potential.

**Nav1.7 regulates cytokine secretion and migration of CD1a\(^{+} \) IDC**

As the inflammatory nature of monocyte-derived MDC was attributed to the CD1a\(^{-} \) subset (10), and Nav1.7 channels were predominantly expressed in this subpopulation of IDC, we proposed that the functional role of Nav1.7 in DC may be connected to the maintenance of the immature state of the cells. We hypothesized that CD1a\(^{-} \) IDC can get activated more readily than CD1a\(^{+} \) cells and this sensitized state is connected to ion channel activity. To test the potential role of Nav1.7 in fine-tuning CD1a\(^{-} \) IDC activation, 10–40 \( \mu M \) TTX was used to inhibit Nav1.7 channel function. Inhibition of Nav1.7 activity by TTX neither affected the monocyte to IDC and the IDC to MDC differentiation pathway nor modulated the viability or the internalizing capacity of IDC (data not shown). However, activation by optimal and suboptimal (10-fold dilution) concentration of the inflammatory mixture for a short (6-h) activation period slightly increased the level of CD83 expression (24 \( \pm 7\% \) and 34 \( \pm 23\% \) at optimal and suboptimal concentrations, respectively; \( n = 5 \)), but this effect was statistically not significant (Fig. 6A). A similar sensitizing effect of TTX treatment on DC could be shown when the production of various DC-derived cytokines was tested after activation by the

**FIGURE 4.** Membrane potential of CD1a\(^{-} \) and CD1a\(^{+} \) IDC. Membrane potential of the cells was recorded in current-clamp configuration using the perforated patch method. The holding current was 0 pA. CD1a\(^{-} \) cells were identified in the patch-clamp microscope based on anti-CD1a FITC fluorescence staining. A, Membrane potential of CD1a\(^{-} \) and CD1a\(^{+} \) IDC (open bars), the corresponding MDC (right-hatched bars), and Nav1.7 siRNA-transfected CD1a\(^{+} \) IDC (filled bar) measured in normal extracellular solution. Errors indicate SEM for \( n > 7 \) cells measured in each group. *\( p < 0.05. \) Membrane potential of a CD1a\(^{-} \) (B), a CD1a\(^{-} \) IDC (C), and a Nav1.7 siRNA-transfected CD1a\(^{+} \) IDC (D). Unless indicated otherwise, cells were perfused with normal extracellular solution. The switch of the bath perfusion to a high K\(^{+} \) bath solution (right-hatched bar, 150 mM K\(^{+} \) concentration) or to a Na\(^{-} \)-free bath solution (left-hatched bar) is indicated by the horizontal bars. The dashed lines indicate the zero potential.

**FIGURE 5.** Expression and activity of the IKCa1 channel in DC. A, Whole-cell current recorded upon a voltage-ramp protocol in a CD1a\(^{-} \) IDC in normal EC solution and in the presence of ChTx and TRAM-34. Dashed line represents zero current level. B, Number of IKCa1 channels on CD1a\(^{-} \) and CD1a\(^{+} \) IDC was calculated, as described in Materials and Methods (\( n > 10 \)); error bars indicate SEM. C, mRNA expression of IKCa1 channels in CD1a\(^{-} \) and CD1a\(^{+} \) IDC. Triplicates of a typical experiment of three are shown. Error bars indicate SD.
cytokine mixture. In these experiments, IDC were treated with 40 μM TTX on day 2 and in combination with the cytokine mixture on day 5. After 6-h activation, the excess of cytokines was removed by washing, and the activated MDC were cultured in fresh medium for another 16 h. The activation status of MDC was monitored by measuring the expression of CD83 and by determining the concentration of TNF-α (B), IL-10 (C), IL-1β (D), and IL-6 (E) cytokines by ELISA. Results obtained from DC of a selected donor (88% of CD1a+ cells) of four are documented.

**FIGURE 6.** Effect of TTX-induced Nav1.7 functional blockade on the activation of DC. IDC were treated with 40 μM TTX on days 2 and 5 of differentiation. Activation of IDC was induced by addition of the inflammatory mixture used at optimal or in 10-fold diluted concentration on day 5, 1 h after TTX treatment. After 6 h, the excess of the mixture was removed by washing, and the activated MDC were cultured in fresh medium for another 16 h. A. The activation status of MDC was monitored by measuring the expression of CD83 and by determining the concentration of TNF-α (B), IL-10 (C), IL-1β (D), and IL-6 (E) cytokines by ELISA. Results obtained from DC of a selected donor (88% of CD1a+ cells) of four are documented.

**FIGURE 7.** Effects of the Nav1.7 channel blocker TTX and specific siRNA transfection on DC migration. Cell migration of IDC induced by MIP-1α was measured in Boyden chamber in IDC treated by 200 ng/ml TTX (A) or in Nav1.7-specific siRNA-transfected IDC (B). Mean ± SD of three independent experiments is shown. Expression of MMP12 mRNA in control and TTX-treated (C) and control siRNA- and Nav1.7 siRNA-transfected IDC (D) was measured by Q-PCR. *p < 0.05.
centrally been described in mice (33). Furthermore, Ca\textsuperscript{2+} takes part in the elevation of Ca\textsuperscript{2+} concentration, depending on the type and higher TNF-related events, and the importance, complexity, and impact of Ca\textsuperscript{2+}-mediated signaling in various cell types is one of the most rapid signal transduction processes. Our previous electrophysiological and pharmacological analysis showed a characteristic change in the expression of ion channels associated with the maturation of monocyte-derived DC. IDC were shown to express TTX-sensitive, rapidly inactivating voltage-gated Nav1.7 Na\textsuperscript{+} channels, whereas the dominant ion channel of MDC was the Kv1.3 K\textsuperscript{+} channel (19).

The current study extends these findings by characterizing the expression pattern and functional activity of Nav1.7 in distinct DC subsets generated from a common precursor. To our knowledge, our results show for the first time that the frequency of cells expressing Nav1.7 current and current density in Nav1.7-expressing cells is significantly higher in the CD1a\textsuperscript{+} DC population than in CD1a\textsuperscript{−} cells. This conclusion is based on ion current measurement performed on individual cells identified by their CD1a expression in the cell membrane, the analysis of relative Nav1.7 mRNA expression measured in CD1a-sorted DC subpopulations, and time dependence of Nav1.7 mRNA upregulation and downregulation.

Our data on the rapid activation-driven upregulation and downregulation of the channel suggested direct transcriptional control of Nav1.7 expression. Transcriptional regulation of ion channel expression has been reported in naïve T cells, where the expression of the Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel IKCa1 is increased upon activation (32). Elevation of intracellular Ca\textsuperscript{2+} concentration in various cell types is one of the most rapid signal transduction events, and the importance, complexity, and impact of Ca\textsuperscript{2+}-mediated signaling on the cell cycle of LPS-induced DC have recently been described in mice (33). Furthermore, Ca\textsuperscript{2+} takes part in cell motility, which is one of the most characteristic features of DC. Various agents raising the intracellular concentration of Ca\textsuperscript{2+} have been investigated for their ability to alter the functions of sodium channels in the plasma membrane and at the level of mRNA expression (34–36). We report in this study the Ca\textsuperscript{2+}-dependent downregulation of Nav1.7 both at the mRNA and protein levels in a subpopulation of human monocyte-derived DC, which may result in increased sensitivity to activation signals, as documented by slightly increased CD83 expression and significantly higher TNF-α and IL-10 secretion in the presence of TTX. Thus, the elevation of Ca\textsuperscript{2+} concentration, depending on the type and strength of the activating stimulus (37), is able to modulate the expression and function of Nav1.7 channels that lead to changes of the membrane potential. We propose that this process opens up a DC state ready to respond to activation signals.

To reveal the functional role of Nav1.7 channel expression in IDC, we demonstrated that expression of the Nav1.7 channel in CD1a\textsuperscript{+} IDC results in a depolarized resting membrane potential. This conclusion is supported by the results showing the following: 1) removal of extracellular Na\textsuperscript{+} hyperpolarizes the membrane of Nav1.7-expressing CD1a\textsuperscript{+} IDC, but does not affect CD1a\textsuperscript{−} IDC lacking Nav1.7 channels; 2) silencing of Nav1.7 gene expression in CD1a\textsuperscript{+} IDC results in a more negative and extracellular Na\textsuperscript{+}-independent resting membrane potential than in Nav1.7 expressing CD1a\textsuperscript{+} IDC; and 3) the IKCa1/KCa3.1 channel is expressed in both CD1a\textsuperscript{−} and CD1a\textsuperscript{+} IDC at similar levels.

Contribution of VGSC to set the resting membrane potential has been demonstrated in astrocytes (38, 39). Furthermore, they are also implicated as key regulators of intracellular pH and cell migration (40, 41). Our current-clamp experiments revealed that Nav1.7 channels are functional even at depolarized membrane potential; their presence is responsible for the maintenance of this depolarized state, but they act exclusively in CD1a\textsuperscript{+} IDC. Although depolarized membrane potential may induce inactivation of Na\textsuperscript{+} channels, others reported that even at ~0 mV an ample fraction of Nav1.7 channels is in available state (42, 43), and thus, the inward (depolarizing) Na\textsuperscript{+} current may contribute to the regulation of membrane potential of CD1a\textsuperscript{+} IDC. Indeed, the lack of extracellular Na\textsuperscript{+}, which abolishes the depolarizing Na\textsuperscript{+} influx through Nav1.7, hyperpolarizes the membrane potential in CD1a\textsuperscript{+} IDC. Alternatively, the lack of extracellular Na\textsuperscript{+} may interfere with Na\textsuperscript{+}-dependent electrogenic transports, and thus, indirectly hyperpolarize the cells independent of Nav1.7 channels. In principle, the net inward current produced by the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger run in forward mode could be responsible for extracellular Na\textsuperscript{+}-dependent membrane potential changes. We eliminated this possibility by our siRNA experiment showing that knocking down Nav1.7 expression resulted in a hyperpolarizing shift in CD1a\textsuperscript{+} cells and the membrane potential became insensitive to extracellular Na\textsuperscript{+}, similar to CD1a\textsuperscript{−} IDC with low Nav1.7 expression. These findings cannot be explained by the sensitivity of the membrane potential to Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger currents; rather, it can be interpreted as Nav1.7 dependence of the membrane potential. These, in combination with the similar number of IKCa1 channels in the IDC subpopulations, support our conclusion that Nav1.7 channel is an active regulator of the membrane potential in CD1a\textsuperscript{+} IDC.

As it was shown earlier, the existence of a negative membrane potential in various immune cells is necessary for the proper activation of Ca\textsuperscript{2+}-dependent signaling (44). We propose that the presence and activity of IKCa1 channels in both IDC subtypes may be responsible for maintaining the negative membrane potential necessary for a sustained Ca\textsuperscript{2+} signaling during differentiation. The expression level of IKCa1 channels in CD1a\textsuperscript{+} and CD1a\textsuperscript{−} IDC is much higher than in other immune cells, suggesting its exclusive role in the early phase of Ca\textsuperscript{2+}-dependent DC maturation (45).

The critical dependence of the membrane potential in Nav1.7 channels suggested that several physiological responses of CD1a\textsuperscript{+} IDC may be impaired if Nav1.7 is inhibited. Although we performed multiple functional studies to demonstrate the effect of TTX on IDC functions, we failed to detect inhibition of DC differentiation, phagocytosis, pinocytosis, or activation by the toxin (19). However, by using TTX and the siRNA strategy, we showed that Nav1.7 takes part in regulating the secretion of certain cytokines and spontaneous as well as chemokine-driven migration of IDC through or together with upregulating the MMP12 enzyme, known to be involved in DC mobilization from tissues.

In summary, we showed that Nav1.7 channels in IDC are expressed in the cell membrane in contrast to microglia, where they are targeted to endosomes (16), and play an inevitable role in the maintenance of the resting membrane potential, setting the
threshold and fine-tuning of DC activation and regulating the migration of the human CD1a+ DC subset. We propose that the depolarized membrane potential (∼−10 mV) inhibits the activation of highly inflammatory CD1a+ DC under a critical threshold, and thus, may protect these cells against unnecessary activation. As blocking of channel activity by TTX or silencing Nav1.7 expression inhibited DC maturation, we also suggest that these sodium channels support the migration of DC to specific chemotactic attractants, keeping these cells in tissues, where they exert their sentinel function. When sensing danger signals strong enough to activate DC maturation, the expression of Nav1.7 channel is rapidly downregulated, allowing DC to mature and transport their cargo to draining lymph nodes. Identification and functional characterization of the Nav1.7 channel in a human DC subset offer novel means of immune modulation and support targeted drug design, whereas the novel, voltage-gated ion channel–dependent regulation of DC activation may have an impact on the targeted manipulation of DC functions in various clinical settings.

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Disclosures
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References
Supplemental Information

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Figures A

Figures B

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Supplementary Figure 1. Effects of ionomycin and thapsigargin treatment on the expression of activation molecules and the secretion of cytokines by dendritic cells.

DC were activated on day 5 by inflammatory cocktail, 180 ng/ml ionomycin and 250 nM thapsigargin with or without 5 mM EGTA+10 mM MgCl₂ for 24h. (A) Cell surface expression of CD83, CD80 and CD86 molecules was measured by flow cytometry. (B) TNF-α and IL-6 cytokine secretion was measured by ELISA. A typical experiment out of 4 is documented. The percentage of CD1a⁺ cells in the DC population of the presented donor was 70.1 %.