Developmental Switch of the Expression of Ion Channels in Human Dendritic Cells

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Modulation of the expression and activity of plasma membrane ion channels is one of the mechanisms by which immune cells can regulate their intracellular Ca\(^{2+}\) signaling pathways required for proliferation and/or differentiation. Voltage-gated K\(^{+}\) channels, inwardly rectifying K\(^{+}\) channels, and Ca\(^{2+}\)-activated K\(^{+}\) channels have been described to play a major role in controlling the membrane potential in lymphocytes and professional APCs, such as monocytes, macrophages, and dendritic cells (DCs). Our study aimed at the characterization and identification of ion channels expressed in the course of human DC differentiation from monocytes. We report in this study for the first time that immature monocyte-derived DCs express voltage-gated Na\(^{+}\) channels in their plasma membrane. The analysis of the biophysical and pharmacological properties of the current and PCR-based cloning revealed the presence of Nav1.7 channels in immature DCs. Transition from the immature to a mature differentiation state, however, was accompanied by the down-regulation of Nav1.7 expression concomitant with the up-regulation of voltage-gated Kv1.3 K\(^{+}\) channel expression. The presence of Kv1.3 channels seems to be common for immune cells; hence, selective Kv1.3 blockers may emerge as candidates for inhibiting various functions of mature DCs that involve their migratory, cytokine-secreting, and T cell-activating potential. The Journal of Immunology, 2009, 183: 4483–4492.

Characterization of ion channels of immune cells other than dendritic cells (DCs) revealed the essential role of transmembrane ion fluxes in various functions of the immune system (1). These electrically nonexcitable cells have been shown to possess both voltage-dependent and second messenger-gated ion channels that are crucial for their activation, proliferation, and migration (1). Voltage-gated K\(^{+}\) channels (VGPCs) have been described as the predominant ion channels controlling the resting membrane potential and tuning intracellular Ca\(^{2+}\) signaling in lymphocytes, monocytes, macrophages, and mouse DCs (1–4). The dynamic changes in the expression of ion channels, including that of VGPCs upon various extracellular (EC) stimuli, are essential for the activation and terminal differentiation of immune cells, and thus may modulate specific immune responses (5). The biophysical characteristics of VGPCs are modified by the presence of auxiliary subunits, which provide with an additional mechanism for fine-tuning K\(^{+}\) currents to the ever changing conditions (6). Among VGPCs, a member of the Shaker family, the Kv1.3 channel is extensively studied for its potential role in lymphocyte and macrophage activation. Kv1.3 channels are almost exclusively expressed in the immune system, and the blockade of these channels is associated with selective inhibition of T cell activation and proliferation (for review, see Refs. 4 and 7). Inwardly rectifying K\(^{+}\) channels (K\(_{IR}\)) and Ca\(^{2+}\)-activated K\(^{+}\) channels (IKCa1 or KCa3.1), and Kv1.5/Kv1.1 heterotetramer channels have also been described in T cells and in cells of the mononuclear phagocytic system (3, 4, 7–9). These channels, along with Kv1.3, maintain a hyperpolarized membrane potential, which enhances and sustains the Ca\(^{2+}\) signal upon activation. The intracellular Ca\(^{2+}\) signal is also influenced by the presence of Ca\(^{2+}\)-release-activated Ca\(^{2+}\) channels in lymphocytes, macrophages, and DCs. These channels are responsible for the sustained Ca\(^{2+}\) signal required for the expression of numerous functionally important genes, such as the IL-2 gene (10).

Changes in the expression of ion channels accompanying terminal differentiation of myeloid cells directed our attention toward DCs, the most potent professional APCs. DCs localized to peripheral tissues act as molecular sensors of microenvironmental changes such as stress, inflammation, or pathogenic invasion. They possess a high capacity to acquire exogenous soluble or particulate substances, which involve self and foreign proteins, various metabolites, apoptotic and damaged cells, or other particles. Tissue DCs are poor APCs, and require danger signals to get activated and acquire high T cell priming and activating potential. Danger signals for DCs include mediators exposed by inflamed or damaged tissues, the feedback signals released by the activated DCs themselves or by other constitutive or induced signals (11). The phenotypic characteristics and physiological functions of these cells change dramatically during the maturation and activation process, making DCs capable of inducing either T cell immunity or tolerance.
Human monocyte-derived DCs provide a good tool for studying the phenotypic and functional properties of immature, tissue-resident and mature, T cell stimulatory myeloid DCs. The objective of this study was to compare the expression and functional activity of voltage-gated ion channels in DCs generated in vitro from human peripheral blood monocytes. We studied the biophysical and pharmacological characteristics of the currents expressed by immature and mature monocyte-derived DCs, and by the means of PCR-based cloning we identified the ion channels being responsible for the measured currents. We report in this study for the first time that immature DCs (IDCs) express voltage-gated sodium channels (VGSCs) of type Nav1.7. The transition from the immature to the mature DCs (MDCs) induced by an inflammatory mixture results in dramatic changes in the expression of voltage-gated ion channels. The expression of Nav1.7 is downregulated within 24 h after stimulation, whereas morphological and phenotypic changes associated with the development into MDCs are accompanied by the up-regulation of Kv1.3 K+ channels, consistent with previously reported studies on other immune cells (1–4). We propose that the switch of ion channel expression during maturation states.

### Materials and Methods

#### Dendritic cells

Human myeloid DCs were generated from blood monocytes isolated from buffy coats by Ficoll-Paque (Amsher Biosciences) gradient centrifugation (12). Monocytes were separated by positive selection with anti-CD14-coated magnetic beads (Miltenyi Biotec) from PBMC. Purified monocytes (>95%) were plated at 2 × 10⁷ cell/ml concentration and cultured in serum-free AIMV medium (Invitrogen) in the presence of 100 ng/ml IL-4, 20 ng/ml IL-12, 20 ng/ml IL-6, 75 ng/ml GM-CSF (Peprotech) and 1 μg/ml PGE2 (Sigma-Aldrich). MDCs were identified by flow cytometry using anti-CD83 mAb (Immunotech), as described elsewhere (9).

#### Protein extracts and Western blotting

Cells were washed twice in cold PBS and lysed on ice with lysis solution (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 (pH 7.4)) supplemented with 2 μM peptatin A, 2 μM leupeptin, 2 μg/ml aprotinin, and 1 mM PMSF as protease inhibitors. Cell lysates were centrifuged at 10,000 g for 10 min at 4°C, and the protein content of the supernatant was determined using Bradford protein assay. Samples were separated into aliquots and stored at −80°C. Protein samples (50 μg) were boiled in Laemmli SDS loading buffer and separated on 10% SDS-PAGE. Series resistance compensation up to 85% was used to minimize capacitance artifacts. The peak currents were determined, plotted as a function of test potential, and fitted using the following equation:

\[
I(t) = I_0 \times e^{-\frac{t}{\tau_{in}}} + I_{SS}
\]

where \(I_0\) is the amplitude of the depolarizing current, \(\tau_{in}\) is the inactivation time constant for either the Na\(^+\) (\(\tau_{in,a}\)) or the K\(^+\) (\(\tau_{in,k}\)) current, and \(I_{SS}\) is the steady-state value of the current at the end of depolarizing pulse.

#### Electrophysiological protocols and data analysis

##### Inactivation kinetics of the Na\(^+\) and the K\(^+\) current

Cells were depolarized to evoke whole-cell currents, and the decaying parts of current traces were fitted using a single-exponential function, as follows:

\[
I(V) = G \times V \times \left( \frac{1 - e^{-(V - E_{rev})/25 mV}}{1 + e^{-(V - V_m)/25 mV}} \right)
\]

where \(G\) is the whole-cell peak Na\(^+\) conductance and \(E_{rev}\) is the reversal potential for Na\(^+\) ions. The free parameters of the last term of the equation, \(V_m\) and \(s_a\), are the midpoint and the slope factor of the voltage dependence of steady-state activation, respectively. Current-voltage relationships were individually fitted for each cell, and the free parameters (\(G, E_{rev}, V_m, s_a\) and \(s_i\)) were averaged to obtain the characteristic values.

##### Steady-state inactivation of Na\(^+\) current

To elucidate the voltage dependence of steady-state inactivation of the Na\(^+\) current in IDCs, 5-s-long prepulse potentials \(V_p\) ranging between −120 and 0 mV were applied, followed by a test potential to 0 mV for 20 ms. The inward peak currents were normalized to the maximal peak currents recorded at \(V_p = −120\text{ mV}\) to express the fraction of channels that were not inactivated by the prepulse \(I_{norm}(V_p) = I_{ss}(V_p)/I_{ss}(−120\text{ mV})\). The current-voltage plot was fitted as a function of the prepulse potential for each cell separately, and the Boltzmann function was fit to each data series, resulting in \(V_{0.5}\) and \(s_i\) corresponding to the midpoints and the slope factors of the voltage dependence of steady-state inactivation, respectively, as follows:

\[
I_{norm}(V_p) = \frac{1}{1 + e^{-(V_p - V_{0.5})/s_y}}
\]

\(V_{0.5}\) and \(s_i\) obtained for individual cells were averaged to report the characteristic values.

#### Recovery from inactivation of Na\(^+\) currents

A conventional two-pulse protocol was used to determine the kinetics of recovery from inactivation of Na\(^+\) channels at a hyperpolarized potential. A pair of depolarizing pulses from −120 to 0 mV was separated by the interpulse interval (IP). IP was varied between 1 and 20 ms, and the membrane potential was held at −120 mV during IP. The degree of recovery from inactivated state at a given IP was characterized by the recovery fraction (RF) of the Na\(^+\) current: RF = \(I_{ss}(P_1 - I_{ss}(P_2))/I_{ss}(P_1)\), where \(P_1\) and \(P_2\) denote the peak currents obtained during the first and the second depolarizing pulses, respectively, and \(I_{ss}(P_1)\) represents the current at the end of the first depolarizing pulse. RF was plotted as a function of IP, and a single exponential rising function was fitted to the data, resulting in the time constant for recovery from inactivation (\(\tau_r\)), as follows:

\[
RF(t) = 1 - e^{-\frac{t}{\tau_r}}
\]

\(\tau_r\) was determined separately for each cell and averaged to give the characteristic value reported.
Voltage dependence of steady-state activation of K⁺ current in MDCs. MDCs were held at −120 mV and depolarized to the test potentials ranging from −70 mV to +60 mV for 800 ms every 90 s. Peak, whole-cell conductance (G(V)) at each test potential was calculated from the peak current (Iₚ) at test potential V and the K⁺ reversal potential (E_R) using G(V) = Iₚ/(V - E_R). G(V) values were normalized to the maximal K⁺ conductance of the cell; the resulting G(V)_norm data were plotted as a function of test potential. Data points were fitted using the Boltzmann function to obtain the midpoint (V_1/2) and the slope (k) of the voltage dependence of steady-state activation, as follows:

\[
G(V)_{\text{norm}} = \frac{1}{1 + e^{-(V - V_{1/2})/k}}
\]  

(5)

V_1/2 and k were determined separately for each cell and averaged to give the characteristic values reported.

Dose-response curves. The extent of current inhibition by various inhibitors was defined by the remaining current fraction (RCF), which is calculated as I/I₀, where I and I₀ are the peak currents in the presence and absence of a blocker (e.g., TTX), respectively. RCF values obtained at different toxin concentrations were plotted, and the Hill equation assuming 1:1 channel-toxin stoichiometry was fitted to the data points to obtain the equilibrium dissociation constant (K_d), as follows:

\[
\text{RCF} = \frac{I}{I_0} = \frac{K_d}{K_d + [T]}
\]  

(6)

where [T] is the concentration of the toxin applied. Where indicated, K_d was estimated from RCF obtained at a single toxin concentration using Equation 6.

Cloning and sequencing

To prepare cDNA for cloning of Na⁺ channels, monocyte-derived IDCs or MDCs were collected on days 5 and 6, respectively, and total RNA was isolated with TRI Reagent (Sigma-Aldrich). Reverse transcription was performed at 42°C for 60 min from 500 ng of total RNA using Superscript II

MDCs were collected on days 5 and 6, respectively, and total RNA was isolated with TRI Reagent (Sigma-Aldrich). Reverse transcription was performed at 42°C for 60 min from 500 ng of total RNA using Superscript II

The representative whole-cell current trace was recorded in an IDC bathed in normal EC solution. A 150-ms-long voltage-ramp protocol ranging from −100 mV to +50 mV was delivered. The horizontal dashed line indicates the zero current level on each panel in this study, where applicable. B, The inward current disappears upon perfusing the cells with Na⁺-free choline EC solution. The cell was whole cell clamped and depolarized to 0 mV from −120 mV for 50 ms every 15 s (solid line, control; dashed line, washout; trace recorded in choline bath is indicated by the arrow). PS online leak subtraction was applied. Inset, The wash-in and washout time course of the choline EC solution. Peak currents were determined from a series of records shown in B and plotted as a function of time. The right-hatched bar indicates the perfusion with choline EC solution. C, Reversible inhibition of the inward current by TTX. Representative traces show the inward current before the application of the toxin (solid line, control), after the equilibration of the block in the presence of 100 nM TTX (as indicated by the arrow) and after full recovery from block during the perfusion of the bath with toxin-free solution (dashed line, washout). Currents were evoked by the same pulse protocol as described in B. Inset, Time course of the development and the removal of the current block. Peak currents were determined from a series of records shown in C and plotted as a function of time. Right-hatched bar indicates the duration of perfusion with 100 nM TTX. D, Dose response of the inhibition of the inward current by TTX. The RCF was defined by the ratio of I and I₀, where I and I₀ are the peak currents measured at 0 mV depolarization in the presence and absence of TTX. The Hill equation (Equation 6) assuming 1:1 channel-toxin stoichiometry was fitted to the data points, resulting in a K_d value of 55 nM (n = 3). Error bars indicate SEM.

Results

TTX-sensitive Na⁺ current in IDCs

Fig. 1A shows whole-cell currents measured in a single IDC in response to a voltage ramp from −100 mV to +50 mV. The record shows the activation of a marked inward current at membrane potentials more depolarized than −30 mV. This inward current is voltage gated and potentially can be attributed to the influx of either Na⁺ or Ca²⁺ ions or even both. To clarify the nature of the charge carrier, ion substitution experiments were performed by changing the normal EC bath solution for Na⁺-free choline EC solution. As shown in Fig. 1B, the robust inward current recorded at 0 mV test potential in the Na⁺-containing solution completely disappears in the Na⁺-free choline-based solution. The loss of the Na⁺ current in choline-EC solution was quickly reversed by perfusing the bath with normal EC bath solution (Fig. 1B, dashed line). The inset shows that the peak current was reduced to −0 pA between two consecutive pulses upon changing to choline-EC and recovered to the control value upon changing to normal EC bath with a similar rate, i.e., between two consecutive pulses. These experiments demonstrated that the decrease of the inward current is due to the replacement of Na⁺ with the impermeable monovalent choline ion, and thus, strongly argue for the presence of Na⁺ channels in the membrane of IDCs. The peak Na⁺ current density

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at 0 mV test potential was quite variable, ranging between $-15.1 \text{ pA/pF}$ and $-110 \text{ pA/pF}$ with median and mean current densities of $-56.8 \text{ pA/pF}$ and $-61.9 \pm 7.3 \text{ pA/pF}$ ($n = 20$), respectively.

To characterize the Na$^+$ current in IDCs, we used TTX, a ubiquitous inhibitor of voltage-gated Na$^+$ channels. Fig. 1C shows typical current traces recorded in the absence and in the presence of 100 nM TTX. The effect of the toxin was rapid and reversible; the peak current was reduced to $\sim 37\%$ of the control upon perfusion with TTX-containing normal EC bath, and recovered to the control value within a few pulses following a switch to the toxin-free normal EC bath (see inset). The effect of TTX was concentration dependent, and the dose-response data were fit with the Hill equation (Equation 6), resulting in a $K_t$ value of 55 nM (Fig. 1D). These results revealed that the inward current detected in IDCs is mediated by TTX-sensitive voltage-gated Na$^+$ channels.

**Biophysical characterization of the Na$^+$ current in IDCs**

To further characterize the Na$^+$ channel expressed in IDCs, we determined the biophysical characteristics (equilibrium and kinetic properties) of the whole-cell Na$^+$ current gating. In general, VGSCs are closed at hyperpolarized potentials, open quickly upon depolarization, and then get inactivated. Recovery from the inactivated state into the closed state takes place at negative membrane potentials and is necessary for repopulating the closed, activable channel pool. Thus, we used the simplest model that was proposed and worked out by Hodgkin and Huxley in 1952 (17) as a working hypothesis of our additional experiments. Fig. 2A shows a family of currents recorded by depolarizing an IDC from the holding potential of $-120 \text{ mV}$ to various test potentials (for additional details, see the figure legend and Materials and Methods). The peak current at each test potential was determined and plotted as a function of the test potential in Fig. 2B. The peak current-voltage relationship shows that the activation threshold of the current is approximately $-50 \text{ mV}$, and the current reverses close to $+60 \text{ mV}$. The current-voltage relationship is nonlinear at depolarized test potentials; thus, Equation 2 was used to characterize the voltage dependence of steady-state activation of the Na$^+$ channels. The superimposed solid line in Fig. 2B displays the best fit of Equation 2 to the data points. This cell is characterized by a peak whole-cell Na$^+$ conductance (G) of $3.5 \text{ nS}$, and a reversal potential ($E_{rev}$) of $+62.6 \text{ mV}$ for the Na$^+$ current. Parameters describing the voltage dependence of steady-state activation, i.e., the test potential at which 50% of the Na$^+$ channels are activated (midpoint, $V_{ma}$) and the slope of voltage-dependent gating ($s$), were $-24.8$ and $9.1 \text{ mV}$, respectively. Characteristic values for the parameters of Equation 2 were obtained by fitting individual I-V relationships cell by cell and averaging the obtained values. This resulted in $G = 4.1 \pm 1.2 \text{ nS}$, $E_{rev} = +64.4 \pm 4.2 \text{ mV}$, $V_{ma} = -19.8 \pm 3.0 \text{ mV}$, and $s = 10.0 \pm 0.7 \text{ mV}$ ($n = 5$).

The voltage dependence of steady-state inactivation was studied using the pulse protocol shown in Fig. 2C. The cell was held at different prepulse potentials ($V_p$) for 5 s and then depolarized to $0 \text{ mV}$ to evoke whole-cell Na$^+$ currents with magnitudes proportional to the fraction of Na$^+$ channels not inactivated by the preceding prepulse. The resulting family of current records is shown in Fig. 2C. The peak currents normalized to the maximal values were plotted vs $V_p$ cell by cell, and the Boltzmann function (Equation 3) was fitted to the data points. The characteristic values of the voltage dependence of steady-state inactivation, i.e., the membrane potential at which 50% of the channels are inactivated was $V_{ma} = -87.6 \pm 3.3 \text{ mV}$ ($n = 3$), and the slope was $s = -5.1 \pm 0.1 \text{ mV}$ ($n = 3$). The voltage dependence of steady-state inactivation is represented in Fig. 2D as the average of the fraction of noninactivated channels at different prepulse potentials for $n = 3$ independent experiments along with the best fit Boltzmann function.

**FIGURE 2.** Steady-state parameters of activation and inactivation for Na$^+$ current in IDCs. A, Voltage dependence of Na$^+$ current activation. The 50-ms-long depolarizing step pulses ranging from $-50 \text{ mV}$ to $+100 \text{ mV}$ were applied to the cells every 15 s in 10-mV increments from the holding potential of $-120 \text{ mV}$ (pulse protocol is shown on the top; P/5 online leak subtraction was applied). Twenty-ms-long segments of the current traces evoked by depolarizations between $-50 \text{ mV}$ and $+50 \text{ mV}$ and to $+100 \text{ mV}$ are shown for clarity of the figure and for the emphasis of the fast kinetics. B, Voltage dependence of Na$^+$ current activation. Peak whole-cell current (●) at each test potential was determined from the currents shown in A. The superimposed solid line shows the best fit of Equation 2 to the data points with $G = 3.5 \text{ nS}$, $E_{rev} = 62.6 \text{ mV}$, $V_{ma} = -24.8 \text{ mV}$, and $s = 9.1 \text{ mV}$. C, Voltage dependence of steady-state inactivation of the Na$^+$ current. The cell was held for 5 s at various prepulse potentials ($V_p$) ranging between $-120$ and $0 \text{ mV}$, and then depolarized to $0 \text{ mV}$ (Test pulse) for 20 ms (pulse protocol is shown on the top). Prepulse/Test pulse sequences were separated by 15 s at the holding potential of $-120 \text{ mV}$. The gradual loss of the current with depolarizing prepulses (indicated with arrows) demonstrates steady-state inactivation of Na$^+$ channels. D, The inward peak currents recorded following prepulse potential $V_{p}$ were normalized to the maximal peak current recorded following a $-120 \text{ mV}$ prepulse potential to express the fraction of channels that were not inactivated by the prepulse ($I_{norm}(V_p)$). The fraction of noninactivated channels was averaged and plotted as a function of the prepulse potential ($n = 3$). The superimposed solid line is the best fit of the Boltzmann function (Equation 3) to the data points with $V_{ma} = -88.0 \text{ mV}$ and $s = -5.9 \text{ mV}$. Error bars indicate SEM.
Following a full inactivation of the current, the membrane has to be hyperpolarized to allow recovery of the channels from inactivation. The kinetics of recovery from inactivation was studied using a conventional two-pulse protocol (see Materials and Methods) in which the time between two consecutive depolarizing pulses (IPI) was gradually increased, thereby resulting in larger and larger currents during the second pulse (Fig. 3C). The RF of the current as a function IPI was plotted for individual cells, and a single exponential function was fitted to the individual datasets (Equation 4; see Materials and Methods), resulting in the time constant for recovery from inactivation of $\tau_r = 4.9 \pm 0.9$ ms ($n = 3$). The kinetics of recovery from inactivation is represented in Fig. 3D as the average of the RF at different IPIs for $n = 3$ independent experiments along with the best fit of Equation 4 to the data points.

These results show that IDCs express TTX-sensitive rapidly inactivating Na$^+$ channels. Depolarizations up to $+100$ mV and 50 ms in duration failed to elicit any other type of inward or outward current under our experimental conditions ($n > 20$).

**Outward K$^+$ current in MDCs**

The activation of IDCs by an inflammatory cytokine mixture (see Materials and Methods) induced a dramatic change in the electrophysiological properties of the cells. The voltage-ramp experiment in Fig. 4A indicates that the inward current characteristic of IDCs disappeared in MDCs; furthermore, a voltage-gated outward current is activated at membrane potentials more depolarized than $-30$ mV.

A similar approach, used for the biophysical characterization of the Na$^+$ channels, was applied to specify the type(s) of channels generating the outward current, including the determination of the
current-voltage relationship, the voltage dependence of steady-state activation, the inactivation kinetics, and the pharmacological properties of the current.

Fig. 4B illustrates a series of current traces evoked by 800-ms-long step depolarization pulses to different test potentials from a holding potential of −120 mV in every 90 s. The records show that the activation threshold of the current is between −40 and −30 mV; the current quickly activates and almost completely inactivates with a relatively slow kinetics. The leak-corrected peak current at each test potential was determined and plotted as a function of the test potential in the inset of Fig. 4B. This $I_p-V$ relationship shows that extrapolated reversal potential of the current is more negative than −60 mV, and that the voltage dependence of the activation of the channels is very steep above −40 mV. Based on these characteristics, the expression of voltage-gated $K^+$ channels is predicted in MDCs. The peak $K^+$ current density at +50 mV test potential varied between 6.7 pA/pF and 111.9 pA/pF with median and mean current densities of 38.6 pA/pF and 48.1 ± 9.4 pA/pF ($n = 13$), respectively.

The $I_p-V$ relationships were used to calculate the $K^+$ conductance of the membrane at each test potential, and the normalized conductance-test potential relationship ($G_{norm}-V$) was generated for each cell individually, as detailed in Materials and Methods, and the Boltzmann function (Equation 5) was fitted to the data points. The fits resulted in $−25.0 ± 0.8$ mV for the midpoint and 5.2 ± 0.4 mV for the slope of the voltage dependence of steady-state activation ($n = 4$). The $G_{norm}$-V relationships are illustrated in Fig. 4C as the average of the $G_{norm}$ at different test potentials for $n = 4$ independent experiments along with the best fit of the Boltzmann function (Equation 5) to the data points.

Fig. 4B shows that the whole-cell $K^+$ current in MDCs displays inactivation. The superimposed dashed lines in Fig. 4B indicate the best fit single exponential functions (Equation 1) to the decaying part of the current traces. The resulting time constants ($\tau_{ik}$) were used to characterize inactivation kinetics of the $K^+$ current. $\tau_{ik}$ at +50 mV was 127.4 ± 9 ms ($n = 3$); the inactivation kinetics showed negligible voltage dependence at membrane potentials more positive than −10 mV (Fig. 4D).

The biophysical characteristics of the current, especially the inactivation kinetics, narrow significantly the range of ion channels suitable to produce the whole-cell currents in MDCs. Based on these and the predominant expression of Kv1.3 channels in various cell types of the immune system, we hypothesized that Kv1.3 channels may be expressed in MDCs. To test this hypothesis, we applied peptide and nonpeptide ion channel blockers.

**Pharmacological characterization of the outward $K^+$ current**

Kv1.3 channels inactivate by the slow (PC-type) inactivation mechanism that is characterized by the ability of extracellularly applied TEA to inhibit the current and to slow the inactivation process simultaneously by the foot-in-the-door mechanism (18). This feature is demonstrated in Fig. 5 for the whole-cell $K^+$ current recorded in MDCs. The figure shows that the amplitude of the whole-cell $K^+$ current is reduced to ∼55% of the control in the presence of 10 mM TEA; the single-point estimate of the equilibrium dissociation constant from Equation 6 is 12.4 ± 0.7 mM ($n = 3$; see Materials and Methods). The figure also demonstrates that the inactivation kinetics of the current is slower in the presence of TEA; the time constants were 189 ms for control and 282 ms in the presence of 10 mM TEA.

Two peptide toxins with different affinities and selectivities for Kv1.3, ChTx, and MgTx were used in subsequent pharmacological experiments. Fig. 6A shows typical current traces recorded in the absence and in the presence of 2 nM ChTx. The effect of the toxin was reversible; the peak current was reduced to ∼50% of the control upon perfusion with ChTx-containing normal EC bath and recovered to the control value following a switch to the toxin-free normal EC bath (washout). ChTx reduced the $K^+$ current in a dose-dependent manner (Fig. 6B). The Hill equation (Equation 6) was fit to the dose-response data, resulting in an equilibrium dissociation constant of 3.4 nM (Fig. 6B). The inhibition of the whole-cell $K^+$ current by MgTx, a Kv1.3-specific toxin, is shown in Fig. 6C. The trace recorded 300 s after the start of the perfusion with toxin-containing (50 pM) EC solution indicates the equilibrium block of the current by MgTx. The dose-response of the current inhibition by MgTx is shown in Fig. 6D. Fitting the Hill equation to the data points resulted in an equilibrium dissociation constant of 39.8 pM, which is characteristic of the inhibition of Kv1.3 channels. The washout of the toxin was extremely slow, requiring 20–30 min for significant recovery from block (data not shown).

Based on the biophysical and pharmacological data, we propose that the channel responsible for the outward $K^+$ currents in MDCs is Kv1.3.

**Identification of the electrophysically characterized voltage-gated $Na^+$ channel of IDCs**

A PCR-based strategy was used to identify the TTX-sensitive VGSC responsible for the $Na^+$ current detected in the membrane of IDCs. Before this study, no $Na^+$ channel-related DNA sequences of DCs were known; thus, we probed a cDNA library using degenerate primers based on a conserved sequence common to all VGSCs. Using these degenerate primers, a 387-bp portion of the gene was amplified, cloned, and sequenced, as described in Materials and Methods. The nucleotide sequence of 5 of 20 cloned constructs fitted perfectly to the human gene SCN9A (GenBank accession number NM_002977; http://www.ncbi.nlm.nih.gov/GenBank/), which encodes for the voltage-gated $Na^+$ channel 1.7 (Nav1.7) subunit. Sequences corresponding to Nav channels other than Nav1.7 were not found in either of the clones. These findings indicate that the molecular identity of the channels responsible for the inward $Na^+$ currents in of IDCs is Nav1.7.

**Expression of mRNAs for voltage-gated ion channels in differentiating monocyte-derived DCs**

To quantify the relative expression of the identified voltage-gated ion channels, we measured mRNA expression levels by real-time
significant change in the expression during differentiation. The
from both IDCs and MDCs, however, at a lower level, with no
Reversible inhibition of the whole-cell K⁺ channels in differentiating monocyte-derived DCs.

stoichiometry was fitted to the data points to give a
indicate SEM. The Hill equation (Equation 6) assuming 1:1 channel-toxin
potential of −120 mV. B, ChTx inhibits the outward K⁺ current in a dose-
dependent manner. The RCF was calculated as I/I₀, where I and I₀ are the peak currents in the presence and absence of ChTx. The voltage protocol and toxin application procedure were the same as in A. RCF at each toxin concentration was calculated from n ≥ 4 independent experiments and plotted as a function of ChTx concentration. Error bars indicate SEM. The Hill equation (Equation 6) assuming 1:1 channel-toxin stoichiometry was fitted to the data points to give a K₅₀ of 3.4 nM. C, MgTx inhibits the K⁺ current with high affinity. Currents were evoked by the same protocol as in A. Representative traces show the K⁺ current before the application of the toxin (control), after the equilibration of the block in the presence of 50 pM MgTx (as indicated by the arrow). The toxin in 50 pM concentration reduced the peak current to ~40% of the control. D, MgTx inhibits the outward K⁺ current in a dose-dependent manner. RCF was calculated as in B; voltage protocol and toxin application procedure were the same as in A. RCF at each toxin concentration was calculated from n ≥ 4 independent experiments and plotted as a function of MgTx concentration. Error bars indicate SEM. The Hill equation (Equation 6) assuming 1:1 channel-toxin stoichiometry was fitted to the data points to give a K₅₀ of 39.8 nM.

FIGURE 6. Inhibition of the K⁺ current in MDCs by ChTx and MgTx. A, Reversible inhibition of the whole-cell K⁺ current by ChTx in a MDC. Representative traces show the K⁺ current before the application of the toxin (control), after the equilibration of the block in the presence of 2 nM ChTx (as indicated by the arrow), and after full recovery from block during the perfusion of the bath with toxin-free solution (washout). The peak current was reduced by ChTx to 50% of the control. Currents were evoked by depolarizations to +50 mV for 15 ms every 30 s from a holding potential of −120 mV. B, ChTx inhibits the outward K⁺ current in a dose-dependent manner. The RCF was calculated as I/I₀, where I and I₀ are the peak currents in the presence and absence of ChTx. The voltage protocol and toxin application procedure were the same as in A. RCF at each toxin concentration was calculated from n ≥ 4 independent experiments and plotted as a function of ChTx concentration. Error bars indicate SEM. The Hill equation (Equation 6) assuming 1:1 channel-toxin stoichiometry was fitted to the data points to give a K₅₀ of 3.4 nM. C, MgTx inhibits the K⁺ current with high affinity. Currents were evoked by the same protocol as in A. Representative traces show the K⁺ current before the application of the toxin (control), after the equilibration of the block in the presence of 50 pM MgTx (as indicated by the arrow). The toxin in 50 pM concentration reduced the peak current to ~40% of the control. D, MgTx inhibits the outward K⁺ current in a dose-dependent manner. RCF was calculated as in B; voltage protocol and toxin application procedure were the same as in A. RCF at each toxin concentration was calculated from n ≥ 4 independent experiments and plotted as a function of MgTx concentration. Error bars indicate SEM. The Hill equation (Equation 6) assuming 1:1 channel-toxin stoichiometry was fitted to the data points to give a K₅₀ of 39.8 nM.

RT-PCR in IDCs and MDCs activated by an inflammatory mixture. Because several cell types of myeloid origin also express Kv1.5 channel subunits, the mRNA expression level for Kv1.5 was also determined along with that of the Nav1.7 and the Kv1.3 channel. To compare the relative expression of these mRNA transcripts in IDCs and MDCs, the data were normalized to the expression of the housekeeping gene 36B4. In line with the results of the biophysical characterization, the expression of Nav1.7 mRNA was the highest in IDCs, whereas the MDCs expressed Kv1.3 transcripts (Fig. 7, A and B). As compared with IDCs, the changes of the relative expression of Nav1.7 and Kv1.3 in MDCs were 118-fold decrease and 38-fold increase, respectively, calculated using the comparative method (2⁻ΔΔCt). These data demonstrate the coordinated, but opposing regulation of well-defined Nav1.7 and Kv1.3 channels in differentiating monocyte-derived DCs.

Fig. 7C shows that Kv1.5 mRNA transcripts were also isolated from both IDCs and MDCs, however, at a lower level, with no significant change in the expression during differentiation. The presence of Kv1.5 mRNA and the functional absence of Kv1.5 subunits in the current records indicated Western blot experiments to assess the expression of this subunit in DCs of various differentiation states. Fig. 7D shows that the expression of the Kv1.5 subunit relative to that of β-actin is similar in both IDCs and MDCs. HEK-293 cells transfected with Kv1.5 and Raw 264.7 macrophages, endogenously expressing the Kv1.5 subunit (14), were used to demonstrate the sensitivity of the assay. In addition, similar to Kv1.5 gene expression, the relative β-actin abundance and the fact that the same amount of protein was evaluated in each lane (group) indicate that the protein expression of Kv1.5 is much lower in either IDCs or MDCs than in Raw 264.7 macrophages.

Discussion

Differentiation and maturation of DCs from monocyes are linked to marked phenotypic, gene expression, and functional changes (19). The major function of IDCs is to internalize exogenous soluble and particulate tissue components and collect stimuli associated with environmental changes for delivering this molecular information to lymph node T lymphocytes. Mobilization and maturation of DCs to highly efficient APCs with the unique capability to prime Ag-specific T lymphocytes in peripheral lymphoid organs require inflammatory and danger signals. Activation-induced changes of DCs include the down-regulation of some endocytic/phagocytic receptors; up-regulation of specific sets of chemokine receptors, adhesion, and costimulatory molecules; changes in morphology, cytoskeleton, and mobility; and reorganization of the endolysosomal and MHC class II-rich intracellular compartments (20). In this study, we describe a dramatic change in the plasma membrane expression of two specific ion channels, i.e., Nav1.7 and Kv1.3, in the course of monocyte-derived DC maturation.

FIGURE 7. Expression of specific voltage-gated channels in IDCs and MDCs. The relative mRNA expression of Nav1.7 (A), Kv1.3 (B), and Kv1.5 (C) voltage-gated channels was measured by real-time RT-PCR in IDCs and MDCs stimulated by inflammatory mixture. Values are expressed relative to the expression of the 36B4 housekeeping gene transcript (see details in Materials and Methods). Mean ± SEM of triplicate measurements of three independent experiments are shown. ***p < 0.001, D, Kv1.5 Western blot analysis. Lines loaded as indicated: HEK-293, HEK-293 cells expressing Kv1.5; HEK-293Δ, control HEK-293 without transfection of Kv1.5; Raw 264.7 macrophages; IDC 1–3, IDCs from three independent isolations; and MDC 1–3, MDCs from the same three independent isolations after maturation. Anti-β-actin was used as loading and transfer control.
For the first time in the literature, we demonstrated the presence and activity of voltage-gated Na\(^+\) channels in the plasma membrane of IDCs. The identification of the channel being responsible for the whole-cell current required both electrophysiological and molecular biological approach. Using PCR-based cloning, we identified the Na\(^+\) channels expressed in IDCs as Nav1.7 encoded by the SCN9A gene, and the cloned sequence did not match the sequence of any other VGSC. The Nav1.7 channel is classified as TTX-sensitive; it is inhibited by nanomolar concentrations of TTX (21). Our results are consistent with this; however, the affinity of the channels for TTX is somewhat lower (\(K_d = 55\) nM) than reported by Klugbauer et al. (21) for human Nav1.7 expressed in oocytes (\(K_d = 25\) nM) and much lower than for rat Nav1.7 (\(K_d = 4\) nM) (22). Although we do not know the explanation for these differences, it must be noted that the affinities of toxins for ion channels obtained in different expression systems vary significantly (23).

Regarding the biophysical parameters of gating, the previously published values for the voltage dependence of steady-state activation of Nav1.7 are between −20 and −25 mV for the \(V_{m,a}\) regardless of the expression system used (21, 22, 24–26), and our results in IDCs are compatible with those (\(V_{m,a} = −19.8 \pm 3.0\) mV). Fast inactivation kinetics of Nav1.7 is characterized by time constants in the order of 1 ms at voltages in which the current is maximal (−10 to 0 mV) (25); however, biphasic current decay has also been reported (26). This fast inactivation kinetics has only been achieved by coexpressing the Nav1.7 subunits with the auxiliary \(\beta_1\) subunit, which accelerates the inactivation kinetics as much as 10-fold (24). The fast inactivation kinetics reported in this study \((0.70 \pm 0.09\) ms at −10 mV at maximal inward current; Figs. 2 and 3) indicate that Nav1.7 might be in complex with a \(\beta_1\) subunit in IDCs; furthermore, the small size of IDCs allows better voltage-clamp conditions as compared with oocytes, thereby resulting in apparently faster kinetics. The midpoint for the voltage dependence of steady-state inactivation (\(V_{m,i}\)) obtained in this study is slightly more negative than reported by Cummins et al. (25) (−73.6 mV), but significantly more negative than −60.5 mV reported by Klugbauer et al. (21) for the human Nav1.7 expressed in HEK cells in both studies. In the latter study, KCl was used in the pipette-filling solution, whereas in our study and in Cummins et al. (25) F\(^-\) was used as the major anion and F\(^-\) is known to shift the voltage dependence of steady-state inactivation of Nav1.9 channels in the hyperpolarizing direction (27). Other factors explaining the discrepancies might be the difference in \(V_{m,i}\) obtained in various expression system (21, 24) and native cells (26), and the difference in the pulse protocols and holding potentials applied in various studies. In summary, apart from slight differences, the biophysical and pharmacological properties of Nav1.7 currents recorded in IDCs agree well with those reported for Nav1.7 in other preparations.

The \(\alpha\) subunits of the Nav1.7 channel are expressed in the dorsal root ganglion neurons, sympathetic neurons, Schwann cells, and neuroendocrine cells (28). Their physiological functions include action potential initiation and transmission in peripheral neurons in response to nociceptive stimuli. These channels are mainly responsible for mechanosensation and for the development of inflammatory pain (29). IDCs reside primarily within or beneath of epithelial surfaces such as the skin, bronchial, and gastrointestinal mucosal layers (30), and are also concentrated at peripheral nerve endings and endoneurium, which may suggest a possible relationship between nociceptive stimuli and DC functions (31, 32).

The presence of voltage-gated Na\(^+\) channels has been previously confirmed in cells derived from precursors of the myeloid lineage. TTX-sensitive Nav1.6 channels were studied in brain macrophages (microglia), where the inhibition of these channels with TTX significantly reduced the phagocytic activity of these cells and led to the improvement of neuroinflammatory diseases (33). In another study, intracellular localization of Nav1.5 (TTX-insensitive) and Nav1.6 channels was described in human monocyte-derived macrophages, with no detectable cell surface expression of either of these channels (34). Nav1.5 was shown to localize in the late endosome, and TTX inhibited the phagocytosis and certain endosomal functions in this cell type. Although monocyte-derived DCs, microglia, and macrophages all originate from a common myeloid precursor, these data suggest that different VGSC and specific localization of these channels are required for their dedicated functions.

We also demonstrated that IDCs challenged by inflammatory stimuli changed the cell surface expression of ion channels, and thus, the MDCs are characterized by another set of specific channels. Real-time RT-PCR demonstrated the expression of transcripts for Kv1.3 and Kv1.5 in MDCs. This raises the possibility that a mixture of Kv1.3 and Kv1.5 homotetramers and Kv1.3/ Kv1.5 heterotetramers might be responsible for the whole-cell currents observed in MDCs, similarly to bone marrow-derived macrophages (14, 35) and MDCs of the CNS (36). Based on the following considerations, we argue that Kv1.3 determines the properties of the whole-cell currents in our study: 1) The whole-cell current displays single-exponential inactivation kinetics with a time constant of ~127 ms at +50 mV (Fig. 4, B and D), which is characteristic of Kv1.3 (37). Kv1.5 homotetrameric channels, on the contrary, have very slow and biphasic inactivation kinetics with time constants of ~450 ms and 5 s (38). Kv1.3/Kv1.5 heterotetramers should have intermediate inactivation kinetics depending on the subunit stoichiometry and calculated from the cooperative interaction between subunits (37). Thus, the whole-cell current carried by five different species of channels (homotetrameric Kv1.3 and Kv1.5 and three types of heterotetramers) should have complex decay kinetics with slow components in it (37), which was clearly not the case for whole-cell currents in MDCs. 2) Homotetrameric Kv1.5 channels are ~30-fold less sensitive to TEA than Kv1.3 homotetramers (39). As all four subunits contribute equally to the TEA binding site (40) Kv1.3/Kv1.5 heterotetramers should have lower affinity for TEA than Kv1.3 homotetramers. Again, our results show the contrary, i.e., ~50% of the whole-cell current is inhibited by 10 mM TEA (Fig. 5), which is characteristic of Kv1.3 channels. 3) Kv1.5 channels are resistant to inhibition by scorpion toxins used in this study (ChTx, MgTx) (38). Kv1.3/Kv1.5 heterotetramers are less sensitive to MgTx inhibition, depending on the subunit stoichiometry 3 to 100-fold decrease in the affinity was reported by Felipe and colleagues (14, 35). Thus, a significant proportion of Kv1.5 homo- or heterotetramers would result in a residual toxin-insensitive current even at a high toxin concentration, which otherwise blocks almost completely Kv1.3 channels. In contrast, we measured higher than 90% blockage of the whole-cell current at 50 nM ChTX concentration (Fig. 6B) and a full block at 500 pM MgTx concentration (Fig. 6D). 4) The presence of the mixture of homo- and heterotetrameric channels would result in a significant deviation of the dose-response relationship from a model (Equation 6), in which a single species of channels interacts with a single inhibitory peptide. On the contrary, our dose-response relationships showed \(K_d\) values of 3.4 and 39.8 nM, for ChTX and MgTx, respectively, which values are characteristic for inhibition of Kv1.3 (39), and Equation 6 fits perfectly to the dose-response relationships (Fig. 6, B and D), thereby arguing against the existence of Kv1.5 homotetramers and Kv1.3/Kv1.5 heterotetramers in MDCs. In conclusion, although mRNA specific for Kv1.5 can be found in both IDCs and MDCs
and Western blot analysis confirmed the expression of Kv1.5 subunits in these cells, the protein expression level is too small to affect the properties of whole-cell current.

We can exclude the contribution of IKCa1 channels (the Ca\(^{2+}\)-activated K\(^{+}\) channel, which is expressed in many lymphoid cells) to the whole-cell current by the lack of the elevated cytosolic free Ca\(^{2+}\) concentration required for the activation of these channels (i.e., the pipette-filling solution contained 11 mM EGTA and 140 mM F12). (9)

Based on these arguments, we conclude that Kv1.3 channels are responsible for the whole-cell outward current in MDCs, and the properties of the current agree with those reported in the literature for Kv1.3 in human T cells (41). Kv1.3 is involved in the regulation of membrane potential and Ca\(^{2+}\) signaling in lymphocytes, macrophages, and monocyes (1, 42, 43). The expression of Kv1.3 channels is important in the activation of macrophages, where their blockade inhibits the secretion of TNF-\(\alpha\) and IL-8 cytokines (43). Brain macrophages express another type of voltage-gated channel, the Kv1.5, which was proven not to be functional in bone marrow-derived macrophages (3). A recent study, however, proposed that the native currents seen in bone marrow-derived macrophages are due to the expression of three voltage-gated channels: Kv1.3, Kv1.5, and Kv1.3/Kv1.5 heteromeric channels (14, 35). The oligomeric composition of functional VGPCs could have a crucial effect on intracellular signaling events, determining the macrophage-specific response upon different physiological stimuli (14). The presence of an outward rectifying K\(^{+}\) conductance was described in DCs isolated from murine spleen (2), but the ion channel responsible for that current was not identified. The expression of Ca\(^{2+}\)-activated K\(^{+}\) channels of IKCa1 type were described in the KG-1 erythroleukemia cell line that possesses numerous characteristics of human DCs (44). A recent study showed the existence of Kv1.3-like and Kv1.5 currents in MDCs of the CNS (36). The lack of a Kv1.5 current in monocyte-derived MDCs and its presence in MDCs in the CNS raise the question as to whether Kv1.5 expression could be linked to a special function and distribution of these latter cells.

Although significant efforts have been made to demonstrate the inhibition of different functions of IDCs (pinocytosis and phagocytosis) and MDCs (cytokine release and T cell proliferation) by TTX and MgTx, respectively, we failed to inhibit any of these functions by the toxins (data not shown). These negative results, however, do not exclude the biological role of the channels described in this study. For example, currently unknown factors in the culture medium might mask the biological effects, but not the inhibition of the currents. A similar phenomenon was described earlier, in which the inhibition of proliferation of T lymphocytes by ChTx could only be demonstrated in serum-free medium, but not in a culture medium containing human AB serum (45). A detailed analysis of the components revealed that the presence of IL-2 in the culture medium can overcome the antiproliferative effects of ChTx in cultured T cells.

In this study, we demonstrate that the inflammation-induced maturation of monocyte-derived DCs is accompanied by a substantial change in the cell surface expression of VGSCs. Such alterations are common in the course of the terminal differentiation of immune cells, and the identification of two DC-related ion channels with restricted expression and linked, but opposing regulation in MDCs may have an impact on the targeted modulation of the cross-talk of immune cells (46). The Nav1.7 channels described in this study are known to be abundantly expressed in the peripheral nervous system, but their presence in the plasma membrane of IDCs is unique in the immune system. Because VGSCs of noncitable immune cells are considered to play a role in phagocytosis and migration, which are principal functions of IDCs, the selective blockade of VGSCs can be a useful tool to modulate MDC functions. The presence of Kv1.3 channels in MDCs is not surprising because Kv1.3 is probably responsible for adjusting membrane potential, and through this may control Ca\(^{2+}\) signaling during the activation of these cells. Further investigation of the specific function of these channels, as well as the regulation of intracellular Ca\(^{2+}\) signaling during the maturation process, may open up new avenues for targeting DC-directed T lymphocyte activation, polarization, and differentiation for immunomodulation.

Our detailed biophysical approach and expression profiling also revealed that these channels exhibit distinct functional activities and are regulated concomitantly during monocyte-derived DC maturation.

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Disclosures

The authors have no financial conflict of interest.

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