Fundamental Ca\textsuperscript{2+} Signaling Mechanisms in Mouse Dendritic Cells: CRAC Is the Major Ca\textsuperscript{2+} Entry Pathway


Although Ca\textsuperscript{2+}-signaling processes are thought to underlie many dendritic cell (DC) functions, the Ca\textsuperscript{2+} entry pathways are unknown. Therefore, we investigated Ca\textsuperscript{2+}-signaling in mouse myeloid DC using Ca\textsuperscript{2+} imaging and electrophysiologic techniques. Neither Ca\textsuperscript{2+} currents nor changes in intracellular Ca\textsuperscript{2+} were detected following membrane depolarization, ruling out the presence of functional voltage-dependent Ca\textsuperscript{2+} channels. ATP, a purinergic receptor ligand, and 1–4 dihydropyridines, previously suggested to activate a plasma membrane Ca\textsuperscript{2+} channel in human myeloid DC, both elicited Ca\textsuperscript{2+} rises in murine DC. However, in this study these responses were found to be due to mobilization from intracellular stores rather than by Ca\textsuperscript{2+} entry. In contrast, Ca\textsuperscript{2+} influx was activated by depletion of intracellular Ca\textsuperscript{2+} stores with thapsigargin, or inositol trisphosphate. This Ca\textsuperscript{2+} influx was enhanced by membrane hyperpolarization, inhibited by SKF 96365, and exhibited a cation permeability similar to the Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} channel (CRAC) found in T lymphocytes. Furthermore, ATP, a putative DC chemotactic and maturation factor, induced a delayed Ca\textsuperscript{2+} entry with a voltage dependence similar to CRAC. Moreover, the level of phenotypic DC maturation was correlated with the extracellular Ca\textsuperscript{2+} concentration and enhanced by thapsigargin treatment. These results suggest that CRAC is a major pathway for Ca\textsuperscript{2+} entry in mouse myeloid DC and support the proposal that CRAC participates in DC maturation and migration. The Journal of Immunology, 2001, 166: 6126–6133.

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lthough dendritic cells (DC)\textsuperscript{3} are recognized to play key roles in both initiating and modulating immune responses (1, 2) many fundamental aspects of their biology remain unknown. Ca\textsuperscript{2+} signaling in DC represents one such area, despite the fact that alterations in intracellular Ca\textsuperscript{2+} are known to underlie many immune responses. Indeed, a sustained increase in intracellular Ca\textsuperscript{2+} accompanies T and B cell receptor signaling and is necessary for gene activation, cellular proliferation, and Ab secretion (3, 4).

Similarly, many critical functions in DC appear to involve Ca\textsuperscript{2+} signaling. Apoptotic body engulfment and processing are accompanied by a rise in intracellular Ca\textsuperscript{2+} and are dependent on external Ca\textsuperscript{2+} (5). Chemoattractant molecules uniformly produce Ca\textsuperscript{2+} increases in DC (6–9), suggesting that Ca\textsuperscript{2+} transients regulate DC migration. DC maturation, including the enhanced expression of MHC class II and costimulatory molecules, is inhibited by chelation of external Ca\textsuperscript{2+} (10). Conversely, agents that mobilize intracellular Ca\textsuperscript{2+} can promote DC maturation in the absence of normal cytokine stimulation (10, 11).

However, the Ca\textsuperscript{2+}-signaling pathways involved in these DC functions are not well defined. Chemokine-induced Ca\textsuperscript{2+} mobilization likely occurs via intracellular inositol trisphosphate (IP\textsubscript{3}) receptors, because in many cell types the G protein-coupled chemokine receptors are known to activate phospholipase C \( \beta_2 \), and in turn, generate IP\textsubscript{3}. Less is known about Ca\textsuperscript{2+} entry pathways. Previous studies have suggested the presence of dihydropyridine (DHP)-sensitive Ca\textsuperscript{2+} channels and ATP-gated channels, although these have not been functionally characterized. Here we have examined Ca\textsuperscript{2+} entry in DC using electrophysiological and calcium imaging techniques. We show that mouse, myeloid DC express neither functional voltage- nor DHP-gated channels; instead, DHPs mobilize Ca\textsuperscript{2+} from internal stores. Similarly, ATP signaling leads predominantly to Ca\textsuperscript{2+} mobilization rather than entry via plasma membrane channels. We show that the major Ca\textsuperscript{2+} entry pathway in DC is through the Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} channel (CRAC) (12–14), a plasma membrane channel expressed in many cell types and activated by the depletion of intracellular Ca\textsuperscript{2+} stores. Furthermore, we show that CRAC is activated during physiologic DC signaling and that activation of CRAC promotes DC maturation.

Materials and Methods

Animals

C57BL/10J (C57) mice, 6–12 wk old, were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in the specific pathogen-free facility of the University of Pittsburgh Medical Center.

Reagents

Recombinant (r) GM-CSF and IL-4 were gifts of S. K. Narula (Schering-Plough, Kenilworth, NJ). Bay K8644, nifedipine, and Na\textsubscript{a}-ATP were obtained from Sigma (St. Louis, MO). Thapsigargin, SKF 96365, and IP\textsubscript{3} were obtained from Calbiochem (San Diego, CA).

DC culture and purification

DC were cultured using the method initially reported by Inaba et al. (15) with the following modifications. Bone marrow cells were prepared from

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3 Abbreviations used in this paper: DC, dendritic cells; CRAC, Ca\textsuperscript{2+} release-activated Ca\textsuperscript{2+} channel; SOC, store-operated channel; DHP, dihydropyridine; IP\textsubscript{3}, inositol trisphosphate; NMDG, N-methyl-D-glucamine; BAPTA, bis(2-aminophenoxy)ethane-N,N',N"-tetraacetate.
the femurs and tibias of normal C57 mice and cultured at a density of 3 × 10^7 cells/ml in RPMI 1640 (Life Technologies, Gaithersburg, MD) supplemented with 10% FCS (Nalgene, Miami, FL), nonessential amino acids, L-glutamine, sodium pyruvate, penicillin-streptomycin, and 2-ME (all obtained from Life Technologies). Cultures were supplemented with GM-CSF and IL-4 (at 4 ng/ml and 1000 U/ml, respectively). DC were harvested after a total of 3–4 days of culture for immature cells or 5–6 days of culture for mature cells and purified by metrizamide density (16.5 or 14.5%, respectively) centrifugation. Free [Ca^{2+}] in the medium was varied by the addition of a pH-buffered EGTA stock (final concentration 0.4–0.5 mM) or CaCl_2. The actual free [Ca^{2+}] with EGTA was verified with a Ca^{2+}-selective electrode. The free [Ca^{2+}] in normal RPMI 1640 medium (0.44 mM total Ca^{2+}) and supplemented medium (5.44 mM total Ca^{2+}) was estimated to be ~0.36 and 4.6 mM, respectively, using the software Bound and Determined (16) (available online at http://superior.carleton.ca/~kbstorey).

Fluorescence measurements

DC were plated on coverslips in culture medium and loaded with Fluo-3AM (3–5 μM) for 20 min at 25 or 37°C degree. Cells were then washed with several volumes of bathing solution and left for another 20 min before recording. Standard bathing solution was (in mM): 130 NaCl, 4 KCl, 10 glucose, 2 CaCl_2, 2 MgCl_2, 5 HEPES, and pH 7.3. Fluorescence measurements were made with either a Zeiss Axiosvert 100 TV confocal microscope, or a Deltascan fluorometer (Photon Technology International, South Brunswick, NJ) coupled to a Diastar microscope (Leica, Deerfield, IL). Fluo-3 was excited at 488 nm, and emitted fluorescence was filtered with a 535 nm bandpass filter. The fluorescence signal was calibrated in ATP experiments by measuring the maximal fluorescence after treatment with ionomycin (10–50 μM). Absolute estimates of [Ca^{2+}] were then obtained by the expression [Ca^{2+}] = K_d (F - F_{min})/(F_{max} - F), where K_d is the Ca^{2+} dissociation constant for Fluo3, and F_{max} and F_{min} are the maximal and minimal fluorescence, respectively. F_{min} was assumed to be negligible.

Electrophysiology

Whole cell patch clamp recordings were made using an EPC-7 amplifier interfaced to a Macintosh Power PC running IgorPro software (WaveMetrics, Lake Oswego, OR). Patch pipettes with resistances between 2 and 4 MΩ were prepared from aluminosilicate glass (Garner Glass, Claremont, CA). Series resistance compensation was routinely set at ~50%. Data were filtered at 1 kHz and sampled at 5 kHz. For I_{V_{HAC}} recording the bathing solution was (in mM): 145 NaCl, 2 KCl, 2 MgCl_2, 5 Glu, 5 HEPES, and 10 either Ca^{2+}/Ba^{2+}/Mg^{2+}/Sr^{2+}. The pipette solution contained (in mM):

\[ \text{Ca}^{2+}\text{Cl}_{2}, \text{ATP} (20 \mu M), \text{MgCl}_{2}, \text{EGTA}, \text{and NaCl as needed.} \]

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Results

Absence of voltage-, ATP-, or DHP-activated Ca\textsuperscript{2+} entry

To explore immature and mature DC (Fig. 1) for Ca\textsuperscript{2+} entry pathways, we first tested for functional voltage-gated channels. Voltage-clamped cells were depolarized with pulses from \textasciitilde90 to 0 mV. In some experiments we also made simultaneous Ca\textsuperscript{2+} fluorescence recordings. Fig. 2A shows that a 5-s depolarization failed to activate an inward Ca\textsuperscript{2+} current or produce any change in intracellular [Ca\textsuperscript{2+}]. In summary, no detectable Ca\textsuperscript{2+} currents were observed in either immature or mature DC (<0.1 pA/pF, n = 25).

Next we tested for ATP-gated ion channels (19, 20), which are expressed in many leukocytes including macrophages (21) and T lymphocytes (22–24). We found that ATP (10–500 μM) produced similar large Ca\textsuperscript{2+} transients in the majority of immature and mature DC tested (109/121). From calibration experiments with the calcium ionophore, ionomycin (see Materials and Methods), we estimated that 100 μM ATP increased free Ca\textsuperscript{2+} to 2.6 ± 0.9 μM (n = 4). The ATP-evoked responses desensitized both during ATP application (Fig. 2C) and with repeated applications. In addition, ADP evoked a similar response to ATP (data not shown). Dual ATP/ADP sensitivity is characteristic of the metabotropic P2Y class of receptors (20). Indeed, simultaneous voltage clamping and Ca\textsuperscript{2+} imaging in NMDG\textsuperscript{1+} based medium confirmed that the ATP-
evoked Ca\(^{2+}\) rise was largely independent of inward Ca\(^{2+}\) current (Fig. 2B). In some experiments small inward currents were observed (20 ± 4 pA, n = 5), but these currents occurred at variable times following ATP application and did not always correlate with the Ca\(^{2+}\) transient. Furthermore, we found that the magnitude of ATP-induced Ca\(^{2+}\) rises was unaffected when Ca\(^{2+}\) was removed from the external medium (Fig. 2C); control 277 ± 20%, n = 5, zero Ca\(^{2+}\), 275 ± 28%, n = 18), although the duration was considerably shortened (τ = 89 ± 20 s vs τ = 22 ± 3 s, p < 0.01) due to elimination of a late Ca\(^{2+}\) plateau or hump. This Ca\(^{2+}\) hump may be the result of store-operated Ca\(^{2+}\) entry as described below. These results suggest that mouse myeloid DC predominantly express metabotropic purinoceptors, which mobilize Ca\(^{2+}\) by formation of IP\(_3\) and not via plasma membrane ATP-gated ion channels. This is consistent with the recent report of functional P2Y type receptors in human myeloid DC (25).

Next, we examined whether a presumed voltage-insensitive L-type Ca\(^{2+}\) channel, recently identified in human myeloid DC (26), was present in mouse myeloid DC. Similarly we found that 10–25 μM Bay K8644 (an L-type channel agonist) evoked large Ca\(^{2+}\) rises (Fig. 3A, n = 20). However, inward currents did not accompany these Ca\(^{2+}\) rises. In addition, we found that nifedipine (an L-type channel antagonist) also elicited Ca\(^{2+}\) rises, and these Ca\(^{2+}\) responses persisted in Ca\(^{2+}\)-free medium (Fig. 3B). In contrast, pretreatment with thapsigargin to deplete Ca\(^{2+}\) stores occluded the Bay K8644-evoked response (Fig. 3C). These results indicate that 1,4 DHPs do not induce Ca\(^{2+}\) entry through a plasmalemmal channel, but rather mobilize Ca\(^{2+}\) from internal stores.

**Store-operated Ca\(^{2+}\) entry**

The presence of store-operated channels (SOCs) in DC was investigated by treatment of Fluo-3-loaded cells with the microsomal Ca\(^{2+}\)-ATPase inhibitor, thapsigargin. This is a commonly used method for activating SOCs in other cell types (4, 27). In Fig. 4A, the upper trace shows the Ca\(^{2+}\) fluorescence of a single cell, whereas the lower trace shows the mean fluorescence of 10 cells from the same experiment. Application of thapsigargin in 10 nM bathing Ca\(^{2+}\) (0.2 mM EGTA and no added Ca\(^{2+}\)) produced a small increase in cytosolic Ca\(^{2+}\) due to depletion of internal stores, and this slowly declined over 3 min. Reapplication of 2 mM external Ca\(^{2+}\) induced a large and sustained increase in intracellular Ca\(^{2+}\). Similar responses to thapsigargin treatment were observed in the majority of immature (38/39) and mature (25/26) DC tested. No responses were seen when cells were incubated in zero Ca\(^{2+}\) without thapsigargin. This dependence of Ca\(^{2+}\) entry on prior Ca\(^{2+}\) mobilization suggests that SOCs mediate the entry. We tested several common pharmacological blockers of SOCs. SKF 96365 (10 μM) completely inhibited the response (n = 20), as did...
similar to values reported for T lymphocytes of 

When Mg\(^{2+}\) was replaced with Na\(^{+}\)-containing or Na\(^{+}\)-free (Na\(^{+}\) replaced with NMDG) bathing solutions. Cell capacitance was 18 pF. I\(_{\text{CRAC}}\) recorded from a different cell (C = 30 pF) was reversibly inhibited by 1 µM SKF 96365.

To investigate the properties of these SOCs further, and to determine whether these channels carried a nonspecific cation current or alternatively a Ca\(^{2+}\) selective current (I\(_{\text{CRAC}}\)), we conducted whole-cell voltage clamp experiments. In these experiments Ca\(^{2+}\) stores were depleted by the inclusion of IP\(_3\) together with the Ca\(^{2+}\)-chelator BAPTA in the patch pipette solution (Fig. 5A). The whole cell currents elicited by voltage ramps from −120 to +100 mV in the presence of external solutions containing different divalent cations. In Fig. 5B (from the same experiment as in Fig. 5A) the I\(_{\text{CRAC}}\) develops after break-in. The current is robust and reproducibly altered with different divalent cations. Inward currents were activated at hyperpolarized potentials with either 10 mM Ca\(^{2+}\), Ba\(^{2+}\), or Sr\(^{2+}\). Most of the inward current was inhibited when Mg\(^{2+}\) replaced Ca\(^{2+}\). The remaining inward current and the outward current in Mg\(^{2+}\) most likely represents leak current. Subtracting this leak current from the other currents revealed the pure I\(_{\text{CRAC}}\). Consistent with I\(_{\text{CRAC}}\) in other cells (12, 28) this current exhibits a characteristic inward rectification (inset). The relative conductivity was Ca\(^{2+}\) > Ba\(^{2+}\) > Sr\(^{2+}\) with Ca\(^{2+}\) conductivity roughly 2-fold greater than Ba\(^{2+}\) and Sr\(^{2+}\). A similar permeability sequence was reported for I\(_{\text{CRAC}}\) in T lymphocytes (27). The Ca\(^{2+}\) current density at −80 mV was ~0.7 pA/pF (n = 3) and again is similar to values reported for T lymphocytes of ~1 pA/pF (12, 29). I\(_{\text{CRAC}}\) is known to be highly selective for divalent over monovalent cations. In agreement with this, we found that I\(_{\text{CRAC}}\) in DC was essentially independent of the external [Na\(^{+}\)] (Fig. 6A). In addition, the current was reversibly inhibited by 1 µM SKF 96365 (Fig. 6B). This inhibition by SKF 96365 is consistent with the block of Ca\(^{2+}\) entry observed in our imaging experiments. These results indicate that SOC in DC are Ca\(^{2+}\)-selective channels and similar to the channels that mediate I\(_{\text{CRAC}}\).

**FIGURE 6.** I\(_{\text{CRAC}}\) in DC is Na\(^{+}\) independent and blocked by SKF 96365. Currents were recorded in response to 200-ms voltage ramps from −110 to +50 mV. A. I\(_{\text{CRAC}}\) was similar in Na\(^{+}\)-containing or Na\(^{+}\)-free (Na\(^{+}\) replaced with NMDG) bathing solutions. Cell capacitance was 18 pF. B. I\(_{\text{CRAC}}\) recorded from a different cell (C = 30 pF) was reversibly inhibited by 1 µM SKF 96365.

**CRAC is activated during ATP signaling**

We next considered whether CRAC is activated under physiological conditions. ATP is a putative DC chemotactic factor (25) and may attract DC to sites of cell injury and inflammation and stimulate DC maturation (30, 31). ATP-evoked Ca\(^{2+}\) responses exhibited two components: a fast Ca\(^{2+}\) transient that was independent of external Ca\(^{2+}\) and a slower Ca\(^{2+}\)-dependent plateau (Fig. 2C). This slower ATP-evoked Ca\(^{2+}\) response suggests that a SOC may be activated in DC during purinergic receptor signaling. To explore this further we studied the dependence of this delayed ATP-induced Ca\(^{2+}\) entry on both external Ca\(^{2+}\) and voltage. In these experiments DC were either perfused with the standard saline solution (4 mM K\(^{+}\)) to generate a normal resting potential of −50 to −60 mV, or a solution containing 140 mM K\(^{+}\) to clamp the membrane potential close to 0 mV. In separate experiments where DC were held under current-clamp, we confirmed that high K\(^{+}\) does
delayed ATP-evoked Ca\(^{2+}\) vate CRAC, and then double immunostained as described in Materials and Methods. Untreated DC cultures were heterogeneous in their expression of MHC class II and costimulatory molecules. In contrast, thapsigargin treatment induced phenotypic DC maturation as shown by the homogenous expression of high levels of MHC class II and costimulatory molecules. A representative isotype-matched control is shown. The results are representative of two separate experiments.

FIGURE 8. Activation of CRAC with thapsigargin induces DC maturation. Flow cytometric analysis of mouse DC cultures after treatment with 50 nM thapsigargin (18 h). Cells were double-immunolabeled with CD11c-FITC vs MHC class II (IA\(^{\alpha}\)), CD80, or CD86-PE. Histograms, gated for CD11c\(^{+}\) DC depict thapsigargin-treated (bold curves) or control (normal) cultures. Untreated DC cultures were heterogeneous in their expression of MHC class II and costimulatory molecules. Thapsigargin-treated cells are shown for comparison. The percentage of CD86-positive cells increased [Ca\(^{2+}\)] entry pathway, then varying the transmembrane Ca\(^{2+}\) gradient should mainly affect current via CRAC. Fig. 9 shows that the percentage of CD86\(^{+}\) DC (right-hand peak) increased in direct proportion to [Ca\(^{2+}\)]. Thus, this result suggests that modulating the passive Ca\(^{2+}\) entry via CRAC is capable of influencing the spontaneous, in vitro maturation of DC.

FIGURE 9. DC maturation is modulated by extracellular Ca\(^{2+}\) levels. DC were cultured for 18 h in medium containing low Ca\(^{2+}\) (1 \(\mu\)M free), normal Ca\(^{2+}\) (~0.36 mM free), or high Ca\(^{2+}\) (~4.6 mM free), double-immunolabeled with CD11c-FITC and CD86-PE, and analyzed by flow cytometry. Histograms, gated for CD11c\(^{+}\) DC, depict CD86 expression under varying [Ca\(^{2+}\)]. The percentage of CD86-positive cells increased with [Ca\(^{2+}\)]. Thapsigargin-treated cells are shown for comparison. The data are representative of two experiments.

Discussion

This study constitutes the first in-depth investigation of Ca\(^{2+}\) signaling in DC. Our results demonstrate that mouse myeloid DC (both immature and mature) express SOCs with the properties of CRAC, but express neither functional voltage-dependent Ca\(^{2+}\) channels nor DHP-gated channels and few if any ATP-gated ion channels. Importantly, this suggests that CRAC is likely to be the major Ca\(^{2+}\) entry pathway in DC and thus an intrinsic component of the Ca\(^{2+}\) signaling processes that drive DC maturation and migration. The absence of voltage-dependent channels is not surprising given that myeloid DC, like other leukocytes, are essentially nonexcitable, and voltage-gated Ca\(^{2+}\) channels have not been clearly demonstrated in leukocytes (4). In contrast, ATP-gated ion channels are expressed in macrophages (closely related myeloid-lineage cells) (21), mast cells (33), and T cells (22–24). ATP-gated channels are Ca\(^{2+}\) permeable. In macrophages they are important in lipopolysaccharide-activated inflammatory responses (34, 35), in mast cells they modulate histamine secretion (33), and in T lymphocytes they may play a role in cell differentiation (24) or death (22, 23). Our results indicate that ATP signaling in DC predominantly occurs via the metabotropic (P2Y) class of receptors. In support of this, ATP responses were recorded without accompanying membrane current and in zero external Ca\(^{2+}\), and similar responses were seen with ADP. These results agree with a previous patch-clamp study using human myeloid DC (25). Several types of P2Y receptor have been identified in human DC (30, 36). The P2Y receptor is structurally similar to the chemokine receptor family; both are seven-transmembrane G protein-coupled depolarize cells to \(~-0\text{ mV}\). This dependence of membrane potential on external [K\(^{+}\)] may arise from the presence of leak or voltage-activated K\(^{+}\) currents (32). Fig. 7 shows that application of 100 \(\mu\)M ATP in Ca\(^{2+}\)-free medium evoked a rapid Ca\(^{2+}\) transient in a DC. The readdition of Ca\(^{2+}\) induced a smaller, long lasting Ca\(^{2+}\) rise but only when the DC was held at a negative resting potential; no Ca\(^{2+}\) rise was seen when the cell was depolarized to 0 mV, and no Ca\(^{2+}\) rise was seen when cells were incubated in zero Ca\(^{2+}\) (up to 3 min) without ATP (F/F\(_{0}\) = 0.98 \(\pm\) 0.04, n = 20), ruling out the possibility that CRAC was activated by a passive loss of Ca\(^{2+}\). In a total of 10 cells, ATP evoked a normalized (F/F\(_{0}\)) Ca\(^{2+}\) plateau of 1.3 \(\pm\) 0.2. The voltage dependence of the delayed ATP-evoked Ca\(^{2+}\) entry is consistent with it being mediated by CRAC.

Activation of CRAC induces phenotypic maturation of DC

Next we assessed whether CRAC participates in DC maturation. A heterogeneous culture of immature and mature mouse myeloid DC were incubated overnight (18 h) with 50 nM thapsigargin to activate CRAC, and then double immunostained as described in Materials and Methods to detect their surface expression of MHC class II and costimulatory molecules. Fig. 8 shows that untreated controls were a heterogenous mix of immature (MHC class II\(^{-dim}\), CD80\(^{-dim}\), CD86\(^{-dim}\)) and mature (MHC class II\(^{high}\), CD80\(^{high}\), CD86\(^{high}\)) DC. In contrast, DC exposed to thapsigargin overnight were homogenously mature, and expressed high levels of MHC class II, CD80, and CD86. These results complement those previously described for thapsigargin in myeloid leukocytes, including transformed cell lines, monocytes, and cultured bone marrow cells (10). Thapsigargin also mobilizes Ca\(^{2+}\) from intracellular stores. To test that stimulation by thapsigargin depended on Ca\(^{2+}\) entry via CRAC, we repeated the experiment in a low Ca\(^{2+}\) medium (1 \(\mu\)M free); however, under these conditions DC viability was reduced by \(~50\%). As an additional test of the involvement of CRAC in DC maturation, we cultured DC overnight in medium containing different free Ca\(^{2+}\) concentrations (0.001, 0.36, and 4.6 mM) without any other stimuli. Because our results above indicate that CRAC is the major Ca\(^{2+}\) entry pathway, then varying the transmembrane Ca\(^{2+}\) gradient should mainly affect current via CRAC. Fig. 9 shows that the percentage of CD86\(^{+}\) DC (right-hand peak) increased in direct proportion to [Ca\(^{2+}\)]. Thus, this result suggests that modulating the passive Ca\(^{2+}\) entry via CRAC is capable of influencing the spontaneous, in vitro maturation of DC.

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receptors and they share identical intracellular signal transduction cascades (37). Therefore, P2Y-mediated signaling may contribute to chemotaxis, attracting DC to sites of inflammation. Consistent with this idea, ATP has been shown to alter DC shape and dendrite orientation (25). Although mRNA for the P2X channels (P2X₁,4,5,7) have been identified in DC by RT-PCR (30, 36, 38) functional evidence for channel expression is weak. For example, although Berchtold et al. observed that ATP (100 μM) evoked rapid Ca²⁺ rises, these were not affected by the buffering of external Ca²⁺. In contrast, there are reports that high ATP concentrations (0.75–5 mM) can permeabilize DC to low molecular mass dyes (36, 38, 39), indicating functional P2X7 channels. The high concentrations required may explain why no P2X7 currents were seen here with 10–500 μM ATP. Whether millimolar extracellular ATP levels play a biological role in DC function is unclear. Moreover, the fact that large Ca²⁺ transients can be evoked by low ATP (≈10 μM) via P2Y receptors suggests strongly that this latter pathway is more physiological. It is also significant that DC express high levels of membrane ATPase activity (30, 40) and that extracellular ATP is very rapidly hydrolyzed by DC (41). Thus, this would limit the activation of P2X7 channels and serve to protect cellular ATP is very rapidly hydrolyzed by DC (41). Thus, this way is more physiological. It is also significant that DC express functional DHP-gated channels. Poggi et al. (22, 23, 41) reported that human myeloid DC express the β subunit of L-type Ca²⁺ channels and that the DHP, Bay K8644, but not membrane depolarization, induced increases in intracellular [Ca²⁺]. These data were interpreted as evidence for the presence of non-voltage-gated L-type Ca²⁺ channels. In this study we found that Bay K8644 could indeed produce Ca²⁺ increases in mouse myeloid DC. However, we further found that these Ca²⁺ increases were independent of external Ca²⁺ and changes in membrane conductance. These data are not inconsistent with Poggi et al. because they did not report whether Bay K8644 responses were dependent on external Ca²⁺. Our data clearly indicate that DHP-induced Ca²⁺ rises are not due to Ca²⁺ entry but rather the result of Ca²⁺ mobilization from internal stores. Accordingly, we found that responses to DHPs were occluded by emptying intracellular Ca²⁺ stores with thapsigargin. Moreover, we found that Ca²⁺ mobilization was also induced by nifedipine, an L-type channel antagonist. Thus, this strongly suggests that DHPs do not act via L-type channels. Just how DHPs mobilize Ca²⁺ is unclear. Nevertheless, the signaling pathway deserves further attention because nifedipine has been found to modulate numerous DC functions, including inhibition of Ag processing (42), apoptotic body engulfment, and IL-12 secretion (26). The biophysical properties of CRAC in DC are similar to those reported in mast cells and Jurkat T cells (12, 29, 39). The relative conductance sequence is Ca²⁺ > Ba²⁺ > Sr²⁺ > Mg²⁺, with Ca²⁺ permeability approximately twice that of Ba²⁺ and Sr²⁺. The Ca²⁺ current density at −80 mV is ≈0.7 pA/pF, similar to that in Jurkat T cells (≈1 pA/pF). The current exhibits inward rectification such that the current at −60 mV is 3- to 4-fold larger than at 0 mV (Fig. 5A), more than what would be expected from the difference in driving force. Again this is similar to that reported in T cells (44). Thus, modulation of resting membrane potential is likely to have marked effects on the degree of Ca²⁺ entry. Interestingly, the resting membrane potential of human myeloid DC becomes more hyperpolarized following activation with TNF-α (P.J.O. and G.P.A., unpublished observations), and this may serve to augment Ca²⁺ entry through SOCs. It is notable that store-operated Ca²⁺ entry was observed in the majority of both immature and mature DC, indicating its importance in a range of DC functions. Ca²⁺ signaling is involved in DC maturation, chemotaxis, and migration to secondary lymphoid tissue (6–9), thus, it is likely that CRAC plays an important role in all these processes. Our data are consistent with this proposal. First, ATP, a putative physiological activator of DC, induced store-operated Ca²⁺ entry with properties similar to CRAC (Figs. 2C and 7). Second, activation of CRAC with thapsigargin induced marked DC maturation (Fig. 8). This finding in mouse myeloid DC is consistent with the previously reported observations for thapsigargin using human myeloid cell lines, monocytes, and DC (10, 11). Third, spontaneous DC maturation was directly proportional to the extracellular Ca²⁺ concentration (Fig. 9). Thus, a CRAC signaling pathway is likely to be involved in DC maturation. On a practical note, these results suggest that it may be useful to supplement DC culture medium (such as RPMI 1640), containing only 0.4 mM total Ca²⁺, with extra Ca²⁺ to optimize maturation.

In summary, this study has shown that a SOC with properties similar to CRAC plays a dominant role in DC Ca²⁺ signaling, with little contribution from the other Ca²⁺ entry pathways that are common in leukocytes.

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References


