Ca²⁺ signaling in the regulation of dendritic cell functions

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DENDRITIC CELLS (DCs), powerful antigen-presenting cells, were first described by R. Steinman and Z. Cohn in 1973 (92). There are two main subtypes of DCs, i.e., conventional DCs of myeloid origin and plasmacytoid DCs of lymphoid origin (4). Those main subtypes may be subdivided into different categories, like migratory and lymphoid tissue-resident cells or Langhans cells in stratified epithelia and interstitial DCs in all other tissues (4). DCs are endowed with a remarkable plasticity which allows them to undergo a complete genetic reprogramming in response to harmful non-self danger signals (4). Resting immature DCs are highly phagocytic and continuously internalize soluble and particulate antigens. After taking up an antigen from pathogens, tumors, or toxic stimuli, DCs undergo a complex maturation process. The major characteristics of this transformation from an immature to a mature DC are a decrease in endocytic activity, upregulation of antigen-presenting and costimulatory molecules, production of certain cytokines, and migration to draining lymph nodes (4). DCs migrate specifically into T cell areas of lymph nodes, where they secrete chemokines that permit the attraction of naïve T cells and induce the proliferation and differentiation of antigen-specific T cells (4). The intimate contact between T cells and DCs involves multiple adhesion receptor pairs as well as soluble signals and has been referred to as the "immunological synapse" (22). The outcome of interactions between T cells and DCs determines whether tolerance or immune activation will occur (32).

The present review addresses the role of cytosolic Ca²⁺ in the regulation of DC function. Ca²⁺ is a pervasive intracellular second messenger that initiates signaling cascades, leading to essential biologic processes such as secretion, cell proliferation, differentiation, and movement (99). In the following we describe how components of the Ca²⁺ signaling machinery influence different activities of myeloid DCs such as maturation, migration, and T cell stimulation and discuss how immunosuppressive hormones target the Ca²⁺ signaling to evoke their antiinflammatory effects in DCs.

Components of the Ca²⁺ Signalosome in DCs

Earlier studies that addressed the role of Ca²⁺ signaling for DC function utilized Ca²⁺ ionophores and chelators which cause long-lasting changes in the concentration of free intracellular Ca²⁺ ([Ca²⁺]ᵢ). As a result, addition of Ca²⁺ ionophores to immature DCs has been shown to stimulate the acquisition of many morphological and functional properties of activated mature DCs (3, 19, 47, 55). Accordingly, pretreatment of immature DCs with intracellular Ca²⁺ chelators prevents DC maturation induced by a variety of established maturation stimuli (47). Of note, although Ca²⁺ ionophores induce a more mature DC phenotype, this maturation is instant, bypassing the immature stage, and the cells are less viable than cytokine-induced mature DCs (84). Moreover, physiological
Ca$^{2+}$ signaling occurs through Ca$^{2+}$ oscillations and not through a long-lasting increase or decrease in [Ca$^{2+}$]. In both excitable and nonexcitable cells, generation of an intracellular Ca$^{2+}$ transient is due to the release of Ca$^{2+}$ from intracellular stores via inositol 1,4,5-trisphosphate (IP$_3$) or ryanodine receptors (RyRs) present on the endoplasmic (ER)/sarcoplasmic reticulum membranes and opening of Ca$^{2+}$-permeable channels in the plasma membrane (9). In general, in nonexcitable cells, activation of phospholipase C (PLC) triggers the cleavage of phosphatidylinositol-(4,5)-bisphosphate (PtdIns(4,5)P$_2$) into diacylglycerol and IP$_3$ (9). IP$_3$ binds to its receptors in the ER membrane, allowing Ca$^{2+}$ to flow out of the ER. In mouse bone marrow-derived DCs, fast Ca$^{2+}$ mobilization occurs upon tyrosine phosphorylation of PLC-$\gamma$2, leading to the release of Ca$^{2+}$ from intracellular stores (2, 109). Excitable cells, which need to respond to signals within milliseconds, are equipped with RyRs coupled with the L-type Ca$^{2+}$ channels present on the plasma membrane (9, 13). In skeletal muscle, depolarization of the plasma membrane is sensed by the L-type Ca$_{1.1}$ channel which acts as a voltage sensor activating through direct interaction the RyR1 to release Ca$^{2+}$ from intracellular stores (9). In heart cells, Ca$_{1.2}$ opens upon depolarization and the Ca$^{2+}$ entry activates then RyR2 through Ca$^{2+}$-induced Ca$^{2+}$ release (9). Notably, human and mouse DC express the “skeletal” RyR1 isoforms coupled to the “cardiac” Ca$_{1.2}$ (66, 101). Moreover, RyRs in different cell types including DCs are activated by adenine-based metabolites, cyclic AMP-ribosyl (cADPR), and nicotinic acid adenine dinucleotide phosphate (NAADP$^+$) (50, 71, 72) (Fig. 1).

Another mode of ligand-induced Ca$^{2+}$ signaling involves phosphorylation of the lipid sphingosine by sphingosine kinase. This yields the bioactive second messenger, sphingosine-1-phosphate (SIP) (15, 35, 54, 107). Activation of sphingosine kinase is mediated by phospholipase D (60), which cleaves phosphatidylcholine in choline and phosphatic acid. The latter recruits sphingosine kinase to the membrane. Intracellularly acting SIP induces the release of ER Ca$^{2+}$ via an IP$_3$-independent mechanism, the components of which remain to be defined. In addition, extracellularly, SIP activates a family of plasma membrane G protein-coupled receptors (SIP receptors), also present in DCs (65).

**Ion Channels and Transporters Modifying [Ca$^{2+}$], in DCs**

Cytosolic Ca$^{2+}$ activity is regulated by Ca$^{2+}$ entry through Ca$^{2+}$-permeable ion channels. The entry is sensitive to cell membrane potential and thus to the activity of K$^+$ and nonselective cation channels. The signal is terminated following Ca$^{2+}$ extrusion by the Na$^+/Ca^{2+}$ exchangers.

Ca$^{2+}$ release-activated Ca$^{2+}$ channels. Activation of IP$_3$ receptors in the ER and subsequent decline in the level of Ca$^{2+}$ in intracellular stores induces Ca$^{2+}$ entry via capacitative Ca$^{2+}$ release-activated Ca$^{2+}$ (CRAC) channels, an ubiquitous signaling mechanism in nonexcitable and excitable cells (70) (Fig.

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**Fig. 1.** Ca$^{2+}$ signaling inducing maturation of dendritic cells (DCs). Danger signals like ligands of Toll-like receptors (TLRs) stimulate in concert with modifying factors such as extracellular ATP the generation of Ca$^{2+}$ signals via Ca$^{2+}$ release from intracellular stores and subsequent capacitative Ca$^{2+}$ entry through Ca$^{2+}$ release-activated Ca$^{2+}$ (CRAC) channels. CRAC-generated Ca$^{2+}$ entry depolarizes the membrane potential, resulting in both the inactivation of the voltage-dependent CRAC channels and the decline of the inwardly directed driving force for Ca$^{2+}$. The Ca$^{2+}$ entry through CRAC is a function of cell membrane potential and thus modified by hyperpolarizing voltage-gated (K$_v$) K$^-$ and depolarizing transient receptor potential (TRP) melastatin channel TRPM4 nonselective cation channels. In addition, a rise in extracellular K$^+$ concentration by inflammatory necrotic cell death or by T cell K$^+$ channel activity at immunological synapses stimulates Ca$^{2+}$ entry through voltage-gated L-type (Ca$_{1.2}$) Ca$^{2+}$ channels. Finally, high extracellular ATP concentrations induce Ca$^{2+}$ entry via ionotropic P2X purinoceptors. The Ca$^{2+}$ signals, in turn, activate transcription factors such as nuclear factor of activated T cells (NFAT) or nuclear factor-κB (NF-κB). CaMKII, Ca$^{2+}$/calmodulin-dependent kinase II; IP$_3$, inositol triphosphate; IP$_{3}$R, inositol-trisphosphate receptor; MHC II, major histocompatibility complex class II; P2YR, metabotropic purinoceptor; RyR, ryanodine receptor. Components of Ca$^{2+}$ signalosome are shown in red, upstream signaling, modifying free intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) in turquoise, receptors in green and effectors in blue.
1. CRAC channels are highly Ca\(^{2+}\)-selective, low-conductance channels with a characteristic inwardly rectifying current-voltage relationship. Recently, the molecular composition of CRAC channels has been deciphered (68). High-throughput screens based on RNA-mediated interference have identified STIM (of which mammalian cells express the two isoforms STIM1 and STIM2), the sensor of calcium store depletion, and Orai (of which mammalian cells express the three isoforms Orai1–3), an essential transmembrane component of the CRAC channel (68). Functional CRAC channels are expressed in mouse DCs (38, 57). A recent study has demonstrated a predominant expression of Orai2 and STIM2 in mouse DCs (5).

Ca\(^{2+}\) entry through CRAC channels depolarizes the membrane which results in CRAC channel deactivation (70). Sustained CRAC channel activity thus requires concomitant K\(^{+}\) channel activity. Moreover, K\(^{+}\) channel-mediated stabilization of the membrane potential maintains the electrochemical gradient driving Ca\(^{2+}\) through CRAC into the cell (see below).

**L-type Ca\(^{2+}\) channels.** In contrast to CRAC channels, dihydropyridine (DHP)-sensitive L-type Ca\(^{2+}\) channels are active at depolarized membrane potential (9, 13). The expression of the Ca\(_{\text{a1.2}}\) cardiac isoform of DHP-sensitive L-type Ca\(^{2+}\) channel has been shown in the plasma membrane of human DCs where they are coupled to the release of intracellular Ca\(^{2+}\) via the RyR1 skeletal isoform (76, 101). Although DCs do not belong to the classic electrically excitable cells, the addition of necrotic cell extracts triggers plasma membrane depolarization and DHP-sensitive increase of [Ca\(^{2+}\)] (in DCs (101) (Fig. 1).

**Transient receptor potential channels.** Transient receptor potential (TRP) superfamily of cation channels are activated by a variety of different stimuli, including light, sound, chemicals, temperature, and touch (98). They sense changes in the local environment, such as alterations in osmolarity (98). Most TRP channels are Ca\(^{2+}\) permeable with the exception of TRPM4 and TRPM5, which are the only monovalent-selective ion channels of the TRP family (16).

Expression of TRPV1, a thermosensitive channel activated by high temperatures in sensory neurons, has been demonstrated in mouse DCs and the TRPV1 ligand capsaicin, which transmits the perception of pain in the nervous system, similarly engages TRPV1 on DCs, and transmits the inflammatory signals (7).

The TRPM2 channel, a nonselective channel permeable to monovalent cations and Ca\(^{2+}\), is activated by the nicotinamide adenine dinucleotide-positive (NAD\(^{+}\))-derived metabolite ADPR. TRPM2 transcripts are abundant in mouse bone marrow-derived DCs (56).

**Purinergic receptors.** At sites of tissue damage, such as inflammation or necrosis, injured cells could release ATP (21). ATP and other nucleotides bind to specific plasma membrane receptors called purinergic receptors (P2Rs), subdivided into P2X, a family of ligand-gated ion channels, and G protein-coupled P2Y receptors (21). DCs express both classes of P2Rs: P2YRs (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, and P2Y14) and P2XRs (P2X1, P2X4, and P2X7) and acute, transient stimulation with ATP or UTP causes many of the responses typically associated with P2Y or P2X activation, such as phosphatidyl-inositol breakdown, release of Ca\(^{2+}\) from intracellular stores, Ca\(^{2+}\) influx across the plasma membrane, and opening of the P2X7 large-conductance pore if a high ATP concentration is used (21, 25, 64, 86, 89) (Fig. 1).

**Voltage-gated K\(^{+}\) and TRPM4 channels.** Voltage-gated K\(^{+}\) channels (K\(_{\text{v}}\)) modulate Ca\(^{2+}\) entry by setting the membrane potential. Both mouse and human DCs express voltage-gated K\(_{\text{v.1.3}}\) and K\(_{\text{v.1.5}}\) channels (62, 91). K\(_{\text{v}}\) channels can sustain Ca\(^{2+}\) influx through CRAC channels by maintaining the negative membrane potential and providing the necessary electrical driving force (51). In human DCs, K\(_{\text{v}}\) channels might similarly contribute to Ca\(^{2+}\) influx through CRAC channels. If so, they would prevent activation of L-type Ca\(^{2+}\) channels, which are opened upon depolarization. However, in contrast to mouse DCs that exhibit K\(_{\text{v}}\) channel activity at both mature and immature stages (91), only mature human DCs have functional K\(_{\text{v}}\) channels (62) (Fig. 1).

The Ca\(^{2+}\)-activated nonspecific monovalent cation channel TRPM4 (transient receptor potential melastatin 4) has been detected in mouse DCs at both molecular and functional levels (6). Under physiological conditions, TRPM4 channels allow massive entry of Na\(^{+}\), thereby inducing membrane depolarization and decreasing the driving force for Ca\(^{2+}\) entry through CRAC channels. In mouse DCs, TRPM4 has been shown to act as “gatekeeper,” preventing Ca\(^{2+}\) overload (6) (Fig. 1).

**Na\(^{+}/Ca\(^{2+}\)** exchangers.** Physiological Ca\(^{2+}\) signaling depends on the activity of signal terminators, which transport Ca\(^{2+}\) back into intracellular compartments and extrude Ca\(^{2+}\) across the plasma membrane and thus modify duration, amplitude, and intracellular location of a particular Ca\(^{2+}\) signal (17). The transport into intracellular stores is mediated by a vesicular Ca\(^{2+}\) ATPase, the transport across the cell membrane by Ca\(^{2+}\) ATPases and/or Na\(^{+}/Ca\(^{2+}\) exchangers (17). Regulation of cytosolic Ca\(^{2+}\) in DCs is particularly sensitive to Ca\(^{2+}\) extrusion by the Na\(^{+}/Ca\(^{2+}\) ) exchangers. There are two families of Na\(^{+}/Ca\(^{2+}\) ) exchangers in mammalian cells, i.e., K\(^{+}\)-dependent (NCKX) and K\(^{+}\)-independent (NCX) exchangers (100). Both NCX and five NCKX isoforms have been identified by molecular cloning (100). Na\(^{+}/Ca\(^{2+}\) ) exchangers can move net Ca\(^{2+}\), either out of or into cells depending on the prevailing electrochemical driving forces (i.e., the Na\(^{+}\) and Ca\(^{2+}\) concentrations and the membrane potential) (10). NCX exchange three Na\(^{+}\) for one Ca\(^{2+}\), and, in the case of NCKX, K\(^{+}\) is transported in the same direction as Ca\(^{2+}\), with a coupling ratio of four Na\(^{+}\) to one Ca\(^{2+}\) plus one K\(^{+}\). Na\(^{+}/Ca\(^{2+}\) exchangers have a high maximal turnover rate (whereas ATP-driven Ca\(^{2+}\) pumps have relatively low turnover rates). The driving force and thus the net Ca\(^{2+}\) movement mediated by the exchangers can change in parallel with cell activity, when the membrane potential varies (e.g., during an action potential) and/or when the cytosolic Na\(^{+}\) or Ca\(^{2+}\) concentrations are altered (10). We have recently demonstrated that mouse DCs express functional K\(^{+}\)-independent (NCX1–3) and K\(^{+}\)-dependent (NCKX1, 3–5) Na\(^{+}/Ca\(^{2+}\) ) exchangers (90).

**Ca\(^{2+}\)-Sensitive DC Functions**

**DC activation and maturation.** The process of DC activation/maturation typically starts when DCs identify danger represented either as tissue damage or as a microbial invasion. Microbial products such as LPS, peptidoglycan (PGN), CpG DNA, or other macromolecules are sensed by DCs through their pattern recognition receptors such as Toll-like receptors.
(TLRs) [14, 59, 105, 106]. Bacterial LPS is a major molecule recognized by DCs. Complexes of LPS and soluble LPS-binding protein are first recognized by CD14, which, in turn, transfers LPS to the TLR4. One of the earliest events upon DC stimulation with LPS includes a fast increase in [Ca\(^{2+}\)]
. In mouse bone marrow-derived DCs, fast Ca\(^{2+}\) mobilization upon DC stimulation with LPS as well as with PGN critically depends on PLC-\(\gamma\)2 (2). LPS induces tyrosine phosphorylation of PLC-\(\gamma\)2, leading to the release of Ca\(^{2+}\) from intracellular stores (2) (Fig. 1). The molecular component of the LPS receptor responsible for Ca\(^{2+}\) mobilization is not TLR4 itself, but CD14, since Ca\(^{2+}\) transients are fully preserved in TLR4-deficient DCs but are completely abolished in CD14-deficient DCs (109). CD14 is a glycosylphosphatidylinositol-anchored receptor (GPI-AR). The GPI-ARs are located on the extracellular surface and are only anchored to the outer leaflet of the plasma membrane (94). Given that CD14 lacks any intracellular domain, activation of intracellular Ca\(^{2+}\) signaling must require an interaction of CD14 with some transmembrane-spanning plasma membrane protein. Although, such interaction has not been identified, it has been demonstrated that cross-linking of GPI-ARs (such as CD59) results in immobilization of GPI-anchored receptor clusters and recruitment of Src family kinases (such as Lyn), and G proteins (Gois) to the lipid rafts, which, in turn, results in Src kinase activation and triggering of the IP\(_3\)-Ca\(^{2+}\) pathway (94). In accordance, disruption of lipid rafts by cholesterol or Src kinase inhibition completely abolished LPS-induced Ca\(^{2+}\) mobilization (109).

We have demonstrated that stimulation of mouse DCs with LPS induces a biphasic [Ca\(^{2+}\)]\textsubscript{i} increase: Ca\(^{2+}\) release from the stores followed by CRAC channel activation (57) (Fig. 1). Similarly, an increase in [Ca\(^{2+}\)]\textsubscript{i}, upon DC stimulation with PGN, a TLR2 ligand, consists of Ca\(^{2+}\) release from the stores and Ca\(^{2+}\) entry and depends on the presence of TLR2, since in TLR2-deficient mice the PGN-induced increase in [Ca\(^{2+}\)]\textsubscript{i} is impaired (104).

LPS exposure further leads to activation of K\(_{\text{v}}\) channels (57, 91, 104), which sustains the Ca\(^{2+}\) influx through CRAC channels (see above). Accordingly, K\(_{\text{v}}\) channel blockers blunt the increase of [Ca\(^{2+}\)]\textsubscript{i}, following stimulation with LPS and PGN (57, 104).

However, LPS-induced transient increase in [Ca\(^{2+}\)]\textsubscript{i} seems to occur only in mouse bone marrow-derived DCs. Immature human monocyte-derived DCs display large rhythmic oscillations of [Ca\(^{2+}\)]\textsubscript{i}, and addition of LPS does not affect those oscillations or cause any immediate changes in [Ca\(^{2+}\)]\textsubscript{i} (102). The oscillations are due to IP\(_3\)-mediated release of Ca\(^{2+}\) from intracellular stores and influx of Ca\(^{2+}\) from the extracellular space. The high-frequency oscillations are a specific feature of immature human DCs, since they are lost upon maturation induced by TLR agonists (102).

Conflicting results have been obtained on the role of LPS as a Ca\(^{2+}\)-mobilizing signal in macrophages. Rat macrophages and Kupffer cells, resident macrophages in the liver, have been shown to respond with transient increase in [Ca\(^{2+}\)]\textsubscript{i} on acute LPS treatment (42, 43, 87, 110). In rat peritoneal macrophages the LPS-induced transient increase in [Ca\(^{2+}\)]\textsubscript{i} has been demonstrated to begin, similarly to mouse DC, with the release of Ca\(^{2+}\) from intracellular Ca\(^{2+}\) pools, and to be followed by Ca\(^{2+}\) influx from the extracellular space (110). In rat Kupffer cells, LPS-induced Ca\(^{2+}\) entry has been postulated to occur through L-type voltage-dependent Ca\(^{2+}\) channels, as evident from its sensitivity to DHP-type Ca\(^{2+}\) channel blockers, and thus depolarization of the cell membrane is required for the Ca\(^{2+}\) influx into these cells (42, 43, 87).

In contrast, bone marrow-derived mouse macrophages do not demonstrate Ca\(^{2+}\) transients upon LPS exposure, which might be due to different expression or distribution of particular IP\(_3\) receptors in different DC and macrophage models (108, 109).

DCs entering the maturation program lose the ability to capture antigen but increase the expression of antigen-presenting [major histocompatibility complex class II (MHC II)] and costimulatory (CD80, CD86, CD40) molecules, produce specific cytokines, and migrate to nearby lymph nodes where they present antigens to T cells (4). The early increase in [Ca\(^{2+}\)]\textsubscript{i} in mouse DCs seems to be important for all these maturation events. In our experiments, DC stimulation with LPS in the presence of a CRAC channel blocker SKF-96365 resulted in a less mature phenotype: LPS-induced MHC II expression, chemokine CCL21-dependent migration, TNF-\(\alpha\), and IL-6 production were decreased, whereas phagocytic capacity was increased in SKF-96365-treated cells (57). Similarly, PGN-induced DC maturation was affected by SKF-96365: PGN-induced production of TNF-\(\alpha\) and IL-10 was blunted, and phagocytosis was stimulated by SKF-96365 (104) (Fig. 1). Capsaicin, a ligand of a Ca\(^{2+}\)-permeable channel TRPV1, can similarly induce the maturation of mouse DCs, as obvious from the enhanced expression of MHC II and CD86 on the surface (7). LPS- and PGN-induced maturation of mouse DCs is inhibited by K\(_{\text{v}}\) channel blockers (57, 91, 104). K\(_{\text{v}}\) blockers blunt the stimulating effect of LPS on the expression of costimulatory molecules CD83, CD80, CD86, and CD40 and inhibit effect of LPS on IL-12 production (62).

Opposed to mouse bone marrow-derived DCs, immature human monocyte-derived DCs do not respond with a transient increase in [Ca\(^{2+}\)]\textsubscript{i}, upon stimulation with LPS, probably because they express very low levels of CD14 (102). Ca\(^{2+}\) oscillations exhibited by human immature DCs are lost during DC maturation and appear to be an endogenous characteristic of the immature phenotype (102). Moreover, human DCs are equipped with L-type Ca\(^{2+}\) channels Ca\(_{\text{v}}\)1.2 in the plasma membrane and RyR1 in the intracellular stores. According to the model proposed by Vukcevic et al. (101), at the very early stages of an infection, the DC membrane is depolarized either by K\(_{\text{v}}\) released from dead cells in the vicinity of DCs or from K\(^{+}\) influx from activated T cells at the sites of immunological synapses. The depolarization is sensed by Ca\(_{\text{v}}\)1.2 present on the DC membrane and RyR1 in the intracellular stores. The depolarization is sensed by Cav1.2 present on synapses. The depolarization is sensed by Cav1.2 present on synapses. The depolarization is sensed by Cav1.2 present on synapses.
However, it is worth noting that activation of RyRs can be achieved by Ca\(^{2+}\)-mobilizing metabolites, cADPR and NAADP\(^{+}\) (50), and not exclusively through the coupling with L-type Ca\(^{2+}\) channels. Moreover, even when human monocyte-derived DCs do not respond with increase in [Ca\(^{2+}\)]\(_i\), on LPS, they generate rapid Ca\(^{2+}\) fluxes in response to E. coli and E. coli supernatants (83).

DC maturation can be influenced by nucleotides released at sites of tissue damage (21). Released nucleotides are sensed by purinergic receptors on DCs. DC responses to nucleotides differ when they are exposed to chronic low (10–250 \(\mu\)M) or acute high (500 \(\mu\)M to millimolar) concentrations (21). Chronic stimulation triggers a process of DC maturation with expression of typical maturation markers (CD54, CD80, CD83, CD86), reduced endocytic activity, and enhanced capacity to promote proliferation of allogeneic lymphocytes (21, 48, 103). ATP alone, at variance with classical maturation factors, does not induce cytokine or chemokine secretion. However, in the presence of maturing factors (e.g., LPS or CD40L), ATP strongly distorts the pattern of cytokines produced and modifies the pattern of chemokine receptors expressed by DCs (21, 48, 85, 103). On the contrary, acute stimulation with high levels of ATP triggers secretion of proinflammatory cytokines (IL-1\(\beta\), TNF-\(\alpha\)) and may even be cytotoxic (25). In addition, ATP stimulation of mature and immature DCs leads via P2X7R-dependent mechanisms to the shedding of microvesicles, containing IL-1\(\beta\) (34, 75).

Not much is known about Ca\(^{2+}\)-sensing mechanisms in DCs. A recent study has demonstrated that LPS-induced Ca\(^{2+}\) mobilization in mouse DCs leads to calcineurin-dependent NFAT (nuclear factor of activated T cells) nuclear translocation and production of NFAT-dependent cytokines, such as IL-2 (109). In contrast, in human monocyte-derived immature DCs, NFAT nuclear translocation is possibly induced by spontaneous [Ca\(^{2+}\)]\(_i\) oscillations and may enhance the transcription of genes involved in maintaining the cells immature (102) (Fig. 1).

Maturation of DCs induced by Ca\(^{2+}\) ionophore is associated with the activation of NF-\(\kappa\)B (55), a transcriptional factor playing a central role in coordinating the expression of a wide variety of genes that control DC responses (52). Similar to what has been demonstrated in T cells (11), Ca\(^{2+}\) may activate calcium/calmodulin-dependent kinase II (CaMKII), which in turn phosphorylates and thus inactivates NF-\(\kappa\)B-inhibiting molecule 1\(\kappa\)B (Fig. 1). The role of CaMKII in DCs was extensively reviewed by Connolly and Kusner (18).

Synaptotagmin VII is a Ca\(^{2+}\)-sensing molecule that regulates lysosomal exocytosis in several cell types. In DCs, synaptotagmin VII appears to be involved in regulating the translocation of MHC II from late endosomes/lysosomes to the plasma membrane upon LPS-induced DC maturation (8). Accordingly, DCs deficient of synaptotagmin VII show a delayed translocation of MHC II upon LPS stimulation (8).

**DC migration.** DCs migrate throughout the body, attracted by molecules that are produced either endogenously (chemokines) or exogenously (microbiially derived chemotactants, e.g., N-formylmethionyl-leucyl-phenylalanine, fMLP). DCs sense chemotactic signals via G protein-coupled receptors (78, 88). Besides sensing of signal gradients, chemotaxis of eukaryotes requires the polar differentiation of the cells into a lamellipodium and a cell rear as the initial response of a cell to a migration stimulus (93). Protrusion of the lamellipodium forms the leading edge and generates in concert with the simultaneous retraction of the cell rear directed migration. Polarization and extension of protrusions then define the direction and velocity of cell locomotion toward or away from the stimulus source. It has been demonstrated for various cell types that transport processes across the plasma membrane establish and maintain this polar differentiation by generating gradients of Ca\(^{2+}\) and H\(^+\) (93). In addition, net uptake of NaCl into the lamellipodium and efflux of KCl from the cell rear paralleled by the respective osmotic H\(_2\)O fluxes contribute to the gain and loss of cell volume at the leading edge and the cell rear, respectively (Fig. 2).

The lamellipodium, which is devoid of mitochondria, metabolizes glucose to lactic acid (93). Proton export by cotransport with lactate or by antiport with Na\(^+\) counteracts cytosolic acidification of the lamellipodium (93). In addition, Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransport as well as Na\(^+\)/H\(^+\) antiport in concert with Cl\(^-\)/HCO\(_3\^-\) antiport mediate net uptake of NaCl and volume increase at the lamellipodium. Stimulation of DCs with LPS upregulates Na\(^+\)/H\(^+\) antiport via phosphoinositide 3-kinase (PI3K) and fosters DC migration (81). The stimulation of the Na\(^+\)/H\(^+\) antiport is pivotal for DC migration, as inhibition of the Na\(^+\)/H\(^+\) antiport abolishes LPS-induced DC migration (81) (Fig. 2).

The pronounced proton export results in acidification of the glyocalyx space at the lamellipodium surface (93). This extracellular acidification can elicit spatially and temporally distinct Ca\(^{2+}\) signals that are induced by activating intracellular and plasmalemal Ca\(^{2+}\) channels (39, 93). The chemotactic response of DCs to several chemokines, such as ligands of the mouse formyl peptide receptor 1, CXCR4, and CCR7, is dependent on Ca\(^{2+}\) influx (6, 47, 71, 82). Recent studies have shed light on ion channels associated with the chemokine-dependent migrations of DCs. Ligation of chemokine receptors results in PLC stimulation, production of IP\(_3\), release of Ca\(^{2+}\) from the stores, and subsequent activation of CRAC channels. Blocking of CRAC channels (or depolarizing cells by inhibition of K\(_c\) channels) impairs CCL21-dependent migration (57, 104). Moreover, inhibition of ADPR-gated TRPM2 channels by 8-bromo-ADPR blunted the stimulating effect of the chemokine receptor (CXCR4 and CCR7) ligands CXCL12 or CCL21 in both immature and mature DCs (71). Ligation of the chemokine receptors stimulates the exoenzyme CD38 which catalyzes the transformation of NAD\(^+\) into cADPR and ADPR (71, 72) (Fig. 2). Once imported into the DC, cADPR and ADPR stimulate Ca\(^{2+}\) release and Ca\(^{2+}\) entry through RyR and TRPM2, respectively (71, 72). Notably, at acidic pH, CD38 catalyzes the transformation of NADP\(^+\) and nicotinic acid to NAADP, which induces Ca\(^{2+}\) release from acidic Ca\(^{2+}\) stores (72). It is tempting to speculate that the pH dependency of these CD38 reactions might contribute to the polar differentiation of the migrating cell operated by an extracellular pH gradient. Ca\(^{2+}\) entry seems to be sufficient for the induction of DC migration. Thus, administration of TRPV1 activator capsain induced the migration of DCs to draining lymph nodes in vivo, whereas TRPV1-deficient mouse DCs were unresponsive (7).

Similar to other leukocytes (58, 63, 69), DCs migrate toward nucleotides, ligands of P2R receptors (41, 63). In immature DCs, ligation of G\(_s\) protein-coupled P2Y receptors represents...
The Ca²⁺ signaling regulating DC functions

Fig. 2. Transport processes contributing to the polar differentiation of a migrating DC. The DCs polarize, forming a protruding lamellipodium and a retracting cell rear. Extracellular gradients of chemokines are detected by an unequal ligation of chemokine receptors (CXCR4 and CCR7) distributed around the DC. Ligation of CXCR4 or CCR7 induces IP₃-mediated Ca²⁺ release from the stores and subsequent Ca²⁺ entry through store-operated CRAC channels. CRAC-generated Ca²⁺ entry is enhanced by activation of voltage-gated Kᵥ₁.x K⁺ channels and suppressed by activation of Ca²⁺-activated TRPM4 nonselective cation channels. The Ca²⁺ sensitivity of the latter provides a negative feedback loop preventing Ca²⁺ overload. In addition, ligation of CXCR4 and CCR7 induces Ca²⁺ entry and release via stimulation of the exoenzyme CD38 and the transformation of nicotinamide adenine dinucleotide (NAD⁺) into cyclic adenosine diphosphate ribose (cyclicADPribose) and ADPribose. Local extracellular acidification is induced by Na⁺/H⁺ antiport at the lamellipodium. In DCs, LPS stimulates the Na⁺/H⁺ antiport via phosphoinositide 3-kinase (PI3K). Ion channels and transporters are shown in red, upstream signaling, modifying [Ca²⁺], in turquoise, receptors in green, and effectors in blue.

Potent chemotactic stimuli leading to Ca²⁺ mobilization, actin polymerization, and finally migration toward ATP which is released following cell injury or acute cell death in inflammatory sites (41, 63) (Fig. 2). Upon DC maturation, the chemotactic P2YR responsiveness is downregulated, which might support the migration of mature DCs to secondary lymphoid organs (41).

Though Ca²⁺ influx is required for DC migration, Ca²⁺ overload, however, considerably impairs chemokine-dependent DC migration. The Ca²⁺-impermeable nonselective cation channel TRPM4 is activated by Ca²⁺ and allows massive influx of Na⁺, resulting in membrane depolarization. The latter limits both the activity of CRAC channels and the inwardly directed driving force for Ca²⁺ (6). TRPM4 acts hence as gatekeeper, preventing Ca²⁺ overload, and disruption of TRPM4 function profoundly impairs migration capacity of DCs (6) (Fig. 2). Chronic Ca²⁺ overload in DCs isolated from trpm4⁻/⁻ mice impairs release of Ca²⁺ from internal stores by downregulation of PLC-β2 (6). Therefore Ca²⁺ entry must be tightly regulated to avoid DC unresponsiveness to chemokines.

When E. coli or E. coli supernatants are released from a pipette tip in close vicinity to the cells, human monocyte-derived DCs respond with a Ca²⁺ influx and fast cytoskeletal rearrangements as evident from the extension of lamellipodia in the direction of the pipette tip (83). Which Ca²⁺-dependent proteins induce cytoskeletal rearrangements in DCs is largely unknown. In mouse DCs, inhibition of calpain, a Ca²⁺-dependent cysteine protease, fosters the formation of actin filaments and other proteins in podosomes (12). This accumulation of cytoskeletal components is associated with stabilization of podosome turnover, overall reduction in velocity of cell locomotion, and impaired transmigration across an endothelial monolayer (12). Finally, annexins, Ca²⁺-regulated phospholipid-binding proteins, are supposed to integrate Ca²⁺ signaling with actin dynamics at membrane contact sites (33). Annexins I, III, IV, V, and VI are expressed by DCs (49), and in annexin I-deficient mice, DCs show decreased migratory activity in vivo (40).

Interaction with T cells. The immunological synapse exhibits many similarities with classical neuronal synapses, including requirement for cell-to-cell adhesion and close membrane apposition (22). Upon antigen-specific contact with T cells, DCs secrete several cytokines, such as IL-1β and IL-18 by exocytosis (29, 30). Exocytosis of secretory vesicles is modulated by Ca²⁺: in human monocyte-derived DCs, Ca²⁺-ionophore ionomycin induces the secretion of pro-IL-1β and pro-IL-18 (30). The DHP derivate BayK 8644, an L-type Ca²⁺ channel agonist, potentiates, whereas the L-type Ca²⁺ channel blocker nifedipine prevents the secretion of pro-IL-1β induced by alloreactive T cells (29, 30). Ca²⁺ transients occur in DCs during interactions with T cells (29, 30, 61). Formation of a contact zone between DCs and alloreactive CD8⁺ T cells is followed by Ca²⁺ rises in DCs (29), whereas triggering of DCs by allospecific CD4⁺ T cells results in a Ca²⁺ response in T cells, but not in DCs (20, 29). Furthermore, the components of CRAC channel, STIM1 and Orai1 in T cells and STIM2 and Orai2 in DCs, are recruited to the immunological synapse (5, 53).

Remarkably, not only cytokines but also the neurotransmitters serotonin (5-hydroxytryptamine, 5-HT) and glutamate are released into the synaptic cleft between DCs and T cells via Ca²⁺-dependent processes (1, 67). Immature DCs sequester 5-HT released by activated platelets, degranulating mast cells or sympathetic nerves at sites of injury and inflammation.
through the 5-HT transports (SERTs) (67). At the sites of immunological synapses, DCs release 5-HT via Ca$^{2+}$-dependent exocytosis (67). Extracellular 5-HT, in turn, can reduce T cell levels of cAMP, a modulator of T cell activation (67). By this shuttling of 5-HT from the sites of inflammation to naïve T cells, DCs thereby modulate T cell proliferation and differentiation. Moreover, activated T cells also synthesize 5-HT, and since DCs sequentially interact with many T cells, they could effectively sequester 5-HT from an initial contact with an activated T cell and subsequently release 5-HT to naïve T cells, thereby amplifying adaptive immune responses (67).

Glutamate is also released by DCs through a Ca$^{2+}$-dependent mechanism and seems to play a role for the negative selection of T cells in the thymus (1). In the contact zone between DCs and T cells, glutamate release is sensed by T cells via an array of glutamate receptors (1). Thymocytes respond to glutamate release with an immediate Ca$^{2+}$ signal, a sustained plateau of which is mediated by glutamate (NMDA) receptors. Sustained Ca$^{2+}$ signaling occurring in the context of immunological synapse triggers caspase-3 activation and thymocyte apoptosis and probably mediates the negative selection of T cells in the thymus (1).

**Immunosuppression.** DCs are a primary target of 1,25-dihydroxyvitamin D3 [1,25(OH)$_2$D$_3$], a secosteroid hormone, that, in addition to its well-established action on Ca$^{2+}$ homeostasis, possesses immunomodulatory properties. 1,25(OH)$_2$D$_3$ inhibits the differentiation and maturation of DCs as well as their capacity to secrete the Th1-polarizing cytokine IL-12 (31, 55, 73, 96). Exposure of myeloid DCs to 1,25(OH)$_2$D$_3$ impairs DC migration in response to inflammatory and lymph node-homing chemokines (31), upregulates production of CCL22, a chemokine attracting regulatory T cells while decreasing the chemokine attracting CD86-producing cytotoxic T cells (44). Since 1,25(OH)$_2$D$_3$ is a potent regulator of cytosolic Ca$^{2+}$ concentration, we assumed that some immunomodulatory effects of 1,25(OH)$_2$D$_3$ in DCs could be mediated through Ca$^{2+}$-ion channels and transporters. We could demonstrate that in mouse DCs the K$^+$/Ca$^{2+}$ exchanger NCKX1 is profoundly upregulated by 1,25(OH)$_2$D$_3$ (90). By increasing the activity of the Na$^+$/Ca$^{2+}$ exchanger the hormone accelerates the extrusion of Ca$^{2+}$ and thus blunts the increase of [Ca$^{2+}$], following stimulation with LPS (90). We have further shown that downregulation of the costimulatory molecule CD86 expression by 1,25(OH)$_2$D$_3$ is NCKX dependent in DCs, and, accordingly, a NCKX blocker 3',4'-dichlorobenzamyl reversed the inhibiting effect of 1,25(OH)$_2$D$_3$ on CD86 expression (90) (Fig. 3).

Moreover, glucocorticoids, such as dexamethasone, are powerful suppressors of the immune response (23, 26, 45, 77, 79). They are partially effective by inhibiting DC maturation and function (24, 26, 36, 46, 80, 95, 97). After exposure to glucocorticoids, DCs decrease their capacity to secrete the Th1-polarizing cytokine IL-12 (31, 24). The endocytic activity of DCs is enhanced by glucocorticoids (26). Moreover, glucocorticoids impair the production of several cytokines by DCs, including IL-6, IL-12 and TNF-α (23, 24). The endoactive activity of DCs is enhanced by glucocorticoids (74), which is in line with the immature phenotype of glucocorticoid-treated DCs with high antigen presentation molecule expression. Glucocorticoids prevent the upregulation of MHC class II and costimulatory molecules (26). Moreover, glucocorticoids, such as dexamethasone, are powerful suppressors of the immune response (23, 26, 45, 77, 79). They are partially effective by inhibiting DC maturation and function (24, 26, 36, 46, 80, 95, 97). After exposure to glucocorticoids, DCs decrease their ability to present antigens and elicit a T cell response, primarily because glucocorticoids prevent the upregulation of MHC class II and costimulatory molecules (26). Moreover, glucocorticoids impair the production of several cytokines by DCs, including IL-6, IL-12 and TNF-α (23, 24). The endoactive activity of DCs is enhanced by glucocorticoids (74), which is in line with the immature phenotype of glucocorticoid-treated DCs with high antigen capture, low antigen presentation, and decreased ability to stimulate T cells. We could show that, similarly to 1,25(OH)$_2$D$_3$, glucocorticoid dexamethasone blunts the increase of cytosolic Ca$^{2+}$ concentrations following LPS treatment in mouse DCs (37). Dexamethasone upregulates the Na$^+$/Ca$^{2+}$ exchanger NCKX3 by increasing transcription and subsequently the membrane abundance of the carrier. The enhanced Na$^+$/Ca$^{2+}$ exchanger...
activity therefore accelerates the extrusion of Ca\textsuperscript{2+} and blunts the increase of cytosolic Ca\textsuperscript{2+} concentration following stimulation of DCs with LPS. Moreover, the influence of dexamethasone on the Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger contributes to the inhibitory effect of glucocorticoids on CD86 expression and TNF-\textalpha production, which in part contribute to the immunosuppressive effects of glucocorticoids (37) (Fig. 3).

An endogenous negative feedback serving to limit excessive innate immune responses in DCs includes PI3K. PI3K activation suppresses proinflammatory cytokine production in DCs, which limits excessive Th1 polarization (27). Thus, IL-12 synthesis by DCs is markedly augmented following genetic knockout of the p85\textalpha, the regulatory subunit of PI3K, or by pharmacological inhibition of PI3K activity (28, 91). PI3K inhibits K\texttextsubscript{\textalpha} channels in DCs, because K\textsubscript{\textalpha} currents are dramatically upregulated upon PI3K inhibition (91). The PI3K-induced inhibition of K\textalpha channels might play a role in preventing excessive DC responses by decreasing the Ca\textsuperscript{2+} entry through CRAC channels (Fig. 3).

**Concluding Remarks**

Intracellular Ca\textsuperscript{2+} signaling plays a pivotal role throughout DC life. Ca\textsuperscript{2+} signals act as key regulators of the DC immune response including DC activation, maturation, migration, and formation of immunological synapses with T cells. Moreover, immune suppression or switching off DC activity is similarly transduced by modification of the cytosolic free Ca\textsuperscript{2+} concentration.

Ca\textsuperscript{2+} signals are generated by the orchestration of Ca\textsuperscript{2+} transport processes across plasma, ER, and inner mitochondrial membrane. These processes include active pumping of Ca\textsuperscript{2+}, Ca\textsuperscript{2+}/Na\textsuperscript{+} antiport, and electrodiffusion through Ca\textsuperscript{2+}-permeable channels or uniporters. Ca\textsuperscript{2+} channels in the plasma membrane such as CRAC or L-type Ca\textsuperscript{2+} channels are tightly regulated by the membrane potential which in turn depends on the activity of voltage-gated K\textsuperscript{+} or Ca\textsuperscript{2+}-activated nonselective cation channels. The emerging knowledge about the fine-tuning of these membrane transport proteins by upstream signaling processes increases more and more our understanding of how Ca\textsuperscript{2+} signals are induced in DCs. Harnessing Ca\textsuperscript{2+} signaling in DCs could provide therapeutic tools to target the immune system thus favorably influencing infectious disease, tumor growth, or autoimmunity.

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**DISCLOSURES**

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