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Review Article

How dendritic cells sense and respond to viral infections

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The ability of dendritic cells (DCs) to sense viral pathogens and orchestrate a proper immune response makes them one of the key players in antiviral immunity. Different DC subsets have complementing functions during viral infections, some specialize in antigen presentation and cross-presentation and others in the production of cytokines with antiviral activity, such as type I interferons. In this review, we summarize the latest updates concerning the role of DCs in viral infections, with particular focus on the complex interplay between DC subsets and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Despite being initiated by a vast array of immune receptors, DC-mediated antiviral responses often converge towards the same endpoint, that is the production of proinflammatory cytokines and the activation of an adaptive immune response. Nonetheless, the inherent migratory properties of DCs make them a double-edged sword and often viral recognition by DCs results in further viral dissemination. Here we illustrate these various aspects of the antiviral functions of DCs and also provide a brief overview of novel antiviral vaccination strategies based on DCs targeting.

Introduction

Dendritic cells (DCs) are a heterogeneous population of innate immune cells with the unique ability to prime T lymphocytes and to orchestrate antigen-specific adaptive immunity. The first description of these cells in mouse lymphoid organs was reported by Ralph Steinman and Zanvil Cohn in 1973. After years of investigation, it was established that DCs represent the most potent professional antigen-presenting cells (APCs), distinct from macrophages, with the strongest ability to initiate and regulate T-cell responses. Following the discovery of this first type of DCs, subsequently called conventional DCs (cDCs), other cells with very similar features have been identified and generally called non-cDCs. cDCs express a large repertoire of Pattern Recognition Receptors (PRRs) that recognize pathogen- or damage-associated molecular patterns (PAMPs and DAMPs, respectively) [1]. In peripheral tissues, the binding of PAMPs or DAMPs to PRRs triggers transduction signals that lead to DC maturation, and, specifically, to the up-regulation of co-stimulatory molecules and C—C chemokine receptor (CCR) 7 (CCR7), a key chemokine receptor whose expression allows DCs to migrate to draining lymph nodes (LNs) through afferent lymphatic vessels [2]. During maturation, DCs process intracellular proteins or exogenous antigens for their presentation in association with molecules of the major histocompatibility complex (MHC), either class I or class II, for $CD8^+$ or $CD4^+$ T-cell activation, respectively. Moreover, exogenous antigens can also be presented on MHC-I through a cross-presentation process that allows the activation of $CD8^+$ cytotoxic T lymphocytes (CTLs) to protect the host against cancer cells and viruses. In the LN, the interaction of mature DCs with naive T cells allows the establishment of an adaptive immune response [3]. Many differences can be observed between human and mouse DCs in terms of taxonomy and functions, however, as they have been extensively described in other recent reviews [3,4], they will not be discussed here. In this review, we will report the recent advances on DC biology in the context of viral infections, with particular attention on

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the role of human DCs in coronavirus disease 2019 (COVID-19). In addition, DC responses against influenza A virus (IAV), human immunodeficiency virus (HIV) and human herpes viruses (HHVs) will also be discussed.

Subtypes of human DCs: know yourself

It has long been known that human cDCs could be subdivided into different subsets classified according to phenotype, location and function [5]. However, in the latest years much effort has been put into the characterization of cDC subpopulations by using new, advanced approaches. The two main populations of cDCs are cDC1s and cDC2s, which in spite of originating from the same pre-DC precursor ($CD123^+$) in the bone marrow, acquire a different specialization. Pre-DC precursors leave the bone marrow and differentiate in the blood into early pre-DCs that give rise to cell adhesion molecule 1 ($CADM1^+$) pre-cDC1s and $CD1c^+$ pre-cDC2s. Therefore, pre-cDC1s and pre-cDC2s represent the closest uncommitted cells related to cDC1s and cDC2s, respectively [6]. cDC1 represents a rare population of cDCs found in both lymphoid and non-lymphoid tissues as well as in the blood. cDC1 development depends on the transcription factors interferon response factor (IRF) 8 [7] and basic leucine zipper ATF-like transcription factor 3 (BATF3) [8]. These cells are characterized by the surface expression of X−C motif chemokine receptor 1 (XCR1), CADM1 and C-type lectin domain family (CLEC)9A, efficiently activate Th1 and natural killer (NK) cells, and, being specialized in antigen cross-presentation, also efficiently activate $CD8^+$ T cells. Moreover, cDC1s are able to produce type I and type III interferons (IFN-I and IFN-III, respectively) and IL-12. cDC2s are more abundant than cDC1s in peripheral tissues and blood and exhibit a certain level of heterogeneity. cDC2s are characterized by the surface expression of CD1c and FcεRIα, express a large repertoire of PRRs, and produce a variety of pro- and anti-inflammatory cytokines, including a large amount of IL-12. Depending on the context, cDC2s are able to activate Th1, Th2, Th17 and $CD8^+$ T cells, eliciting the activation of a broad range of immune responses [9]. The recent high-throughput single cell approaches have revealed the complexity of cDC2s driving their subdivision into additional subsets: $CD5^+$ DC2s and $CD5^-$ DC3s [10–12]. It is worth noting that DC3s have been described as quite heterogeneous. Indeed, they form a continuum of cells spanning from the less inflammatory $CD163^{low}$ $CD5^-$ $CD14^{low}$, to the most inflammatory $CD163^+$ $CD5^-$ $CD14^+$ [11]. Interestingly, the concept that cDCs could express CD14 was highly debated, as DCs were historically defined as $CD14^-$ cells and were distinguished from monocytes that, on the contrary, express a high amount of CD14. However, the recent advances in molecular approaches have greatly improved our knowledge of DC and monocyte taxonomy, with a number of studies defining a combination of unique cell markers which allow us to clearly distinguish monocytes from CD14-expressing DC3s [10–12]. In the context of inflammation, the expression of CD14 proved to be of fundamental importance. Studies performed in murine and human DCs have shown that CD14 triggers crucial inflammatory pathways [13–15]. So far, the role of DC3s in inflammation remains to be clarified, as both a pro- and anti- inflammatory role has been described for these cells depending on the disease context [11,12]. However, it is reasonable to speculate that human DC3s may have a more inflammatory role thanks to the expression of additional PRRs such as CD14.

The function of cDCs is complemented by other cells with DC-like functions, which share some phenotypic and morphological characteristics with cDCs, but have distinct origins and transcriptomic profiles. These differences are maintained either in resting, but also in activated conditions, which reflects their distinct intrinsic functional properties. Among these cellular populations, plasmacytoid DCs (pDCs) are non-cDCs that play a fundamental role during viral infections. Both pDCs and cDCs originate from a common DC progenitor (CDP) in the bone marrow but their differentiation process diverges with the appearance of pre-DC and pDC precursors [6]. One of the essential properties of pDCs is their capacity to produce a massive amount of IFN-I in response to viral pathogens. Indeed, although pDCs have a limited set of PRRs, such as Toll-like receptor (TLR) 7 and 9 which recognize single-strand RNA and unmethylated CpG motif-containing DNA, respectively, they express them at high levels in the endosomal compartment. In the resting phase, pDCs show low levels of MHC-II and co-stimulatory molecules that can be efficiently up-regulated upon activation [16]. Moreover, pDCs are also able to secrete type III interferons (IFN-III) and inflammatory cytokines, such as tumor necrosis factor α (TNFα), and chemokines [17]. Most of the signaling pathways involved in pDC activation and IFN-I production start from the endosomal compartment (where TLR7 and TLR9 reside) and involve myeloid differentiation factor 88 (MyD88) adaptor protein which enables the activation of IRF7, the master regulator of IFN-I production in pDCs [18,19]. Furthermore, the activation of TLRs-MyD88 axis also guarantees the efficient activation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) ensuring the production of cytokines and chemokines as well as the expression of co-stimulatory molecules. In addition, non-endosomal pathways also contribute to the activation of pDCs. Among these, the plasma membrane TLR2-MyD88-dependent pathway is associated with a functional response of pDCs to some stimuli [20]. Moreover, it has been demonstrated that both cyclic GMP-AMP synthase (cGAS)/stimulator of interferon genes (STING) and

retinoic acid-inducible gene I (RIG-I) pathways may be involved in pDCs intracellular sensing of replicating DNA virus and RNA virus, respectively [21,22]. An intriguing aspect of pDCs' activation is still an open question: why are they so efficient in IFN-I production? The expression of molecules necessary for IFN-I production, such as TLRs and IRF7, are not exclusive of pDCs. So far, it has been proposed that mechanisms of cell–cell interactions and pDC density are essential for a robust production of IFN-I. Indeed, two different studies correlate the IFN-I concentration and pDC density [23,24] and two additional studies demonstrate a role for Lymphocyte function-associated antigen 1 (LFA-1) integrin in the contexts of influenza virus and Murine Cytomegalovirus (MCMV) infection [25,26]. Taken together these studies highlighted the fact that IFN-I production from pDCs is a unique feature of these cells that cannot be solely ascribed to the activation of PRRs, but should be better investigated considering the integration of molecular and cellular processes.

Another type of non-cDCs is represented by Langerhans cells (LCs) which are located in the epidermis, where a deep immune surveillance is required. These cells are phenotypically characterized by a high amount of surface expression of 'langerin', a C-type-lectin receptor also known as CD207 [27]. LCs are ontogenically distinct from cDCs as they originate from a fetal liver monocyte precursor that appears in the skin at approximately embryonic day 14.5 and differentiates into mature LCs after birth. LC differentiation is strictly dependent on colony stimulating factor 1 (CSF-1) receptor signaling whose agonists are IL-34 and CSF-1 [28]. IL-34 is a cytokine highly present in the skin from embryonic day 17.5 and induces LC development after birth and maintains LC homeostasis during the entire life. Therefore, in physiological conditions repopulation of LCs in the tissue occurs independently of circulating precursors. During inflammation LCs that migrate to skin LNs are replaced by new cells that are generated from blood monocytic progenitors, in a way dependent on CSF-1, which is highly present in inflamed tissues [29,30]. In inflamed tissue an additional source of DCs can be those deriving from circulating monocytes that extravasate from activated endothelium and differentiate in response to local stimuli into the so-called monocyte-derived DCs (mo-DCs). Of note, from an ontogenic point of view, all DCs that developed from monocytes are considered non-cDCs, including the tumor necrosis factor/inducible nitric oxide synthase-producing DCs (Tip-DCs) [31].

Viral detection by PRRs: a game of PAMPs

The detection of viruses involves a plethora of PRRs that can detect viral genomes and replication intermediates, but also structural and non-structural proteins. The infection dynamics, which involve the injection of the viral genome into the host cell and the hijacking of cellular machinery for replication, require a differential localization of the virus-detecting receptors. The first contact between virions and host cells takes place at the plasma membrane, where viral structural and glycoprotein-sensing PRRs such as TLR1/2/6, TLR4 are located [32]. Subsequently, the release of viral nucleic acids (NAs) that follows endocytosis allows for the activation of endosomal PRRs such as TLR3, TLR7/TLR8 and TLR9 (Figure 1A). These receptors recognize viral double-stranded (ds)RNA, single-stranded (ss)RNA and unmethylated CpG DNA, respectively [32]. Ultimately, successful infection causes the activation of intracellular PRRs which span across various families and are generally dedicated to the sensing of viral NAs. The RIG-I like receptor (RLR) family consists of three members: RIG-I, melanoma differentiation-associated protein 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2). RIG-I recognizes uncapped, tri- or di-phosphorylated RNAs that have a peculiar panhandle structure [33], while MDA5 recognizes long dsRNAs [34]. Unlike the previous two RLRs, LGP2 lacks the caspase recruitment domains (CARDs) necessary for downstream signaling. Instead, recent data suggest that it has a synergistic effect with MDA5 [35,36] (Figure 1A). Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) make up another family of cytoplasmic PRRs involved in viral recognition, with one of their most well-known roles being inflammasome activation. NLR family pyrin domain containing 3 (NLRP3) can sense a broad range of viruses, though the precise mechanism through which activation occurs remains elusive [37] (Figure 1A). Other NLRs such as NOD2 (or NLRC2) have been reported to interact directly with viral ssRNA [38].

The importance of inflammasomes in viral infections is further underlined by the presence of other inflammasome-inducing PRRs, such as AIM-2, which can directly detect viral dsDNA [39]. The recognition of viral dsDNA is also performed by other important PRRs, such as cGAS and IFI16 [40] (Figure 1A).

Despite having different cellular localizations and structures, the signaling pathways of these receptors all converge towards the same proinflammatory transcription factors such as NF-κB and various IRFs, or towards the inflammasomes, which results in the production of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-18, TNFα and IFN-I [41] (Figure 1A).

Together, these receptors constitute a defensive array that allows for not only the efficient recognition of multiple classes of viruses, but also the recognition of the same pathogen on multiple levels. During infection, viruses rapidly

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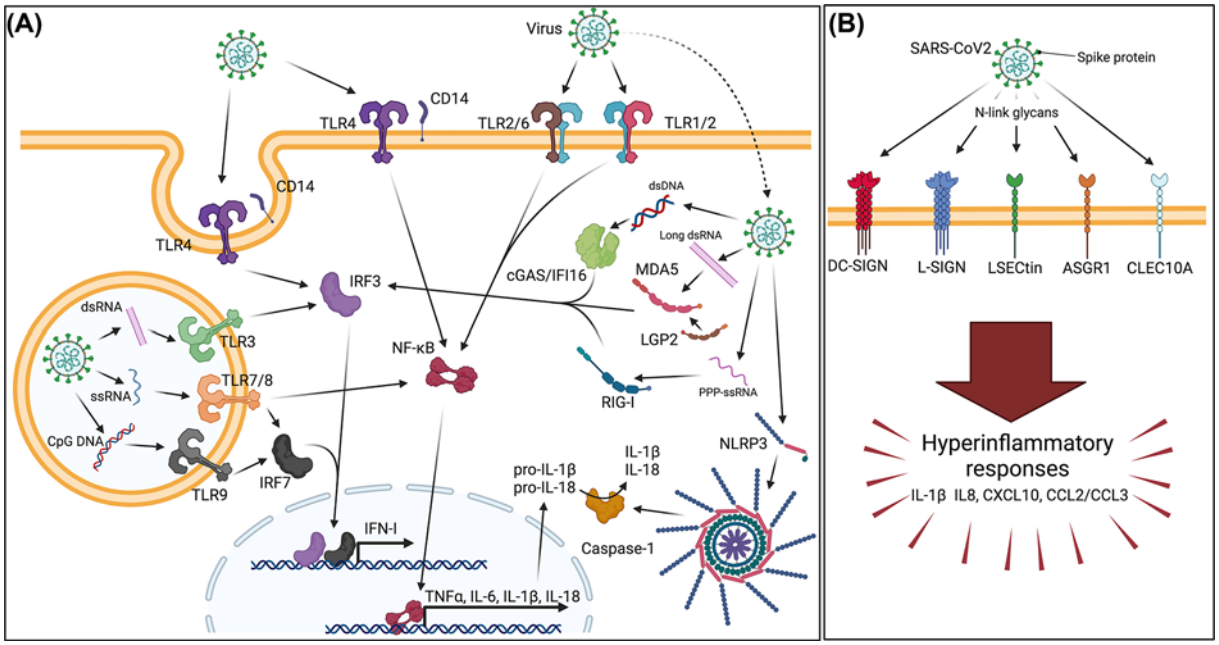

Figure 1. Cellular machinery involved in viral recognition and response

(**A**) Mechanisms of viral detection by immune cell through PRRs and the corresponding transcription factors activated downstream signaling. TLR4/CD14, TLR2/6 and TLR1/2 localize at the cell surface; TLR3, TLR7/8 and TLR9 in the endosomal or intracellular compartment, cGAS, MDA5/LGP2, RIG-I and NLRP3 are located in the cytosol. Black arrows indicate the transcription factors activated by each PRR. (**B**) Schematic representation of myeloid cell receptors capable to interact with SARS-CoV-2. Myeloid cells possess a large plethora of CLRs, including DC-SIGN, L-SIGN, LSECtin, ASGR1 and CLEC10A which have been described to bind SARS-CoV-2's spike protein via N-glycosylated regions outside of the canonical RBD. CLRs engagement by SARS-CoV-2 do not lead to active viral infection or replication, but rather to a hyperinflammatory response. Abbreviations: CLR, C-type lectin; DC-SIGN, C-type lectin DC-specific intercellular adhesion molecule-grabbing non-integrin; L-SIGN, liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin; LSECtin, liver sinusoidal endothelial cell lectin; RBD, receptor-binding domain; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2.

spread through blood vessels causing morbidity and, in some cases, mortality. Among the various immune cell types DCs are one of the earliest responders, as they have to activate adaptive immunity, control viral replication and reduce virus spread from the peripheral site avoiding multiorgans failure.

COVID-19 disease: it is not just a flu

Over the past 16 years severe outbreaks have been caused by three different coronaviruses (CoVs): severe acute respiratory Cov (SARS-CoV) in 2002, Middle East respiratory syndrome CoV (MERS-Cov) in 2012, and SARS-CoV-2 in 2019. SARS-CoV-2 is the causative agent of the COVID-19 which started in Wuhan, China in December 2019 and quickly spread all over the world becoming a still ongoing pandemic. The COVID-19 disease places a huge burden on the healthcare and economic system worldwide and it has been declared a public health emergency by WHO. SARS-CoV-2 has infected 224'511'226 individuals so far and it caused 4'627'540 deaths as reported by the WHO (updated 13 September 2021) [42]. The rapid spread of the disease is due to the efficient transmission of SARS-Cov-2 from subject to subject via droplets or direct contact and to the paucity of symptoms observed in most patients. Despite most individuals being mildly ill or even asymptomatic, a certain percentage of them develop severe pneumonia that can rapidly evolve into acute respiratory distress syndrome and multiorgan failure with fatal outcomes. The exacerbated immune response plays a major role in progression to severe illness [43].

SARS-Cov-2: mechanisms of the infection

SARS-CoV-2 has four structural proteins: nucleocapsidic, membrane, envelope and spike (S) proteins. The S protein is a highly glycosylated type I membrane protein anchored in the virus membrane. It is produced as a precursor that matures after a complex proteolytic process through the host secretory pathway. The S protein is composed of two

main functional domains, the N-terminal region named S1 and the C-terminal region named S2. S1 is essential for virus entry into the cells, as it contains the receptor-binding domain (RBD) which recognizes and binds to its specific surface receptor angiotensin-converting enzyme 2 (ACE2) [44,45]. S2 is responsible for the fusion process between the viral envelope and the target cell membrane which allows the genetic material of the virus to enter into the cell. S1 and S2 are separated by a multibasic protease cleavage sequence that can be recognized by multiple proteases, increasing the possibility of S protein activation. Among other proteins, transmembrane protease serine (TMPRSS)2 [45], TMPRSS4, furin and furin-like proteases operate the proteolytic cut in S1/S2 cleavage site. The initial step of SARS-CoV-2 entry is the binding of S protein mainly to ACE2, although other possible interactors have been proposed [46]. *In vivo*, the binding of RBD to ACE2 is strictly dependent on RBD accessibility which is regulated by host proteases that can induce an RBD open conformation, allowing its binding to the receptor. A first cleavage event between S1/S2 domains is necessary for RBD exposure; subsequently, a second cut in S2′ cleavage site, operated by TMPRSS2, proprotein convertase 1 (PC1), trypsin-like proteases or Cathepsins, generate the dissociation of S1 subunit to S2 and the irreversible and complex refolding of S2 into its post-fusion conformation. The latter structural rearrangements bring together host and viral membranes allowing their fusion [47]. Interestingly, a series of studies showed that both the uncleaved S precursor and the cleaved S1/S2 complex are present in the virus membrane, as well as the S2′ mature proteins [48,49]. Thus, the maturation process of S proteins and the virion assembly and budding take place in the lumen of the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC) [49] where furin proteases are widely expressed. Furthermore, it has been suggested that S precursor located in the virus membrane, can spontaneously mature independently of ACE2. Thus, both the pre-fusion (S1/S2 complex) and post-fusion (mature S2′) spikes can be present on the surface of mature virions with a ratio that can vary between different SARS-CoV-2 variants [48]. All these aspects contribute significantly to the infectivity and spread of SARS-CoV-2, since the maturation of S protein is essential for virus entry. The ACE2, TMPRSS2 and Furin proteins are highly expressed in the respiratory tract, particularly in the lung epithelium, explaining why COVID-19 is mainly a pulmonary disease, at least initially, and clarifying why SARS-CoV-2 transmission occurs predominantly through respiratory droplets. In spite of that, gastrointestinal symptoms are also often present in COVID-19 patients [50]. Indeed, ACE2, TMPRSS2 and TMPRSS4 are also widely expressed in the gastrointestinal tract [51] and SARS-CoV-2 efficiently and productively infects enterocytes [52], but the evidence of a possible oro-fecal transmission of the virus is not yet conclusive [50]. In the lung, in addition to pneumocytes, ACE2 is also expressed on the cell surface of endothelial cells and alveolar macrophages that represent extra targets of SARS-CoV-2 [53].

SARS-Cov-2: mechanisms of recognition and early immune response

The first consequence of SARS-CoV-2 entry into pneumocytes is the virus replication and new virions assembly. The host cell responds immediately to viral infection with the induction of pyroptosis, an inflammatory programmed cell death triggered by the assembling and activation of the inflammasome (Figure 2). This is a supramolecular machinery which acts as platform for the recruitment and activation of caspases (caspase-1, 4 and 5) leading to the processing of several pro-inflammatory mediators, including gasdermin D (GSDMD). The inflammasome-mediated cleavage of GSDMD results in the production of pore-forming N-terminus peptides which alter cell membrane permeability leading to lytic cell death. In this regard, a growing body of evidence is showing that SARS-CoV-2 is capable of triggering NLRP3 inflammasome activation in both epithelial/endothelial [54] and innate immune cells [55,56], and causing the rupture of cells via pyroptosis, leading to the release of all their components into the local environment (Figure 2). Moreover, in the immune cells the inflammasome also has other substrates including pro-inflammatory cytokines such as pro-IL-1β and pro-IL-18 which are released in the extracellular compartment in their mature form contributing to the exacerbation of inflammation [57]. SARS-CoV-2-induced pyroptosis has dramatic consequences for the host and is thought to be among the leading causes that contribute to severe COVID-19 pathogenesis. On one hand, pneumocyte pyroptosis compromises the alveolar epithelium, pouring into the neighborhood a massive amount of mature virions capable of infecting other cells and also viral PAMPs and DAMPs, such as host DNA, which can be recognized by immune and non-immune cells expressing PRRs [54,58] (Figure 2); on the other hand the activation of the NLRP3 inflammasome in innate immune cells is shown to be at the basis of the establishment of a hyperinflammatory phenotype and, at the same time, of an intense leukopenia, which are hallmarks of severe forms of COVID-19 [55,56]. However, what emerges from a recent study is that in spite of SARS-CoV-2 infection causing the lytic cell death of human monocytes, potentially leading to leukopenia, this event seems to be independent from the NLRP3 inflammasome [56]. This suggests that, conversely from other cells, SARS-CoV-2-induced pyroptosis possibly proceeds through an alternative inflammasome signaling pathway. Although there are several studies which report the capacity of SARS-CoV-2 to induce caspase-1 activation and IL-1β production in human monocytes/macrophages,

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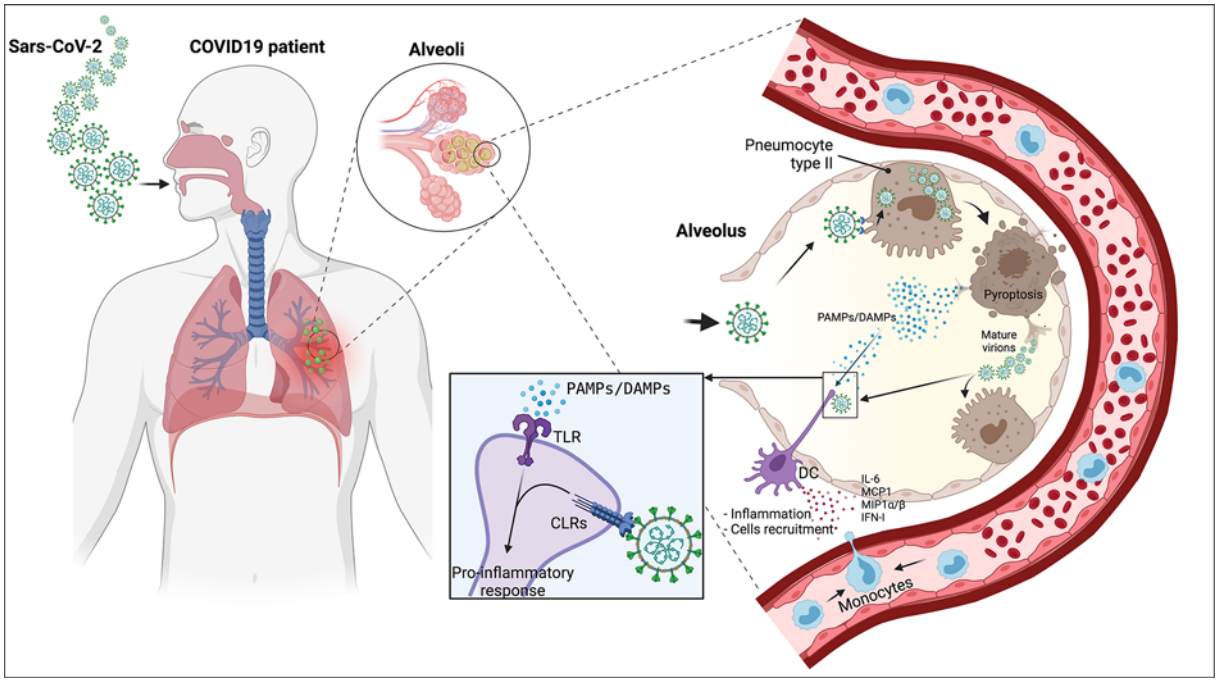

Figure 2. Inflammation triggered by SARS-CoV-2

Upon inhalation, SARS-CoV-2 reaches the lower respiratory tracts where it can infect ACE2-expressing cells, including type II pneumocytes. Active viral replication and the assembly of new virions can induce pyroptosis, resulting in a massive release of alarmins (DAMPs), viral PAMPs and mature virions into the local environment. PAMPs/DAMPs and virions can be recognized by DCs and other immune cells via PRRs, including TLRs and CLRs leading to the onset of a strong hyperinflammatory response that contributes to COVID-19 pathogenesis. Abbreviation: CLR, C-type lectin.

little to no evidence exists about its ability to activate the inflammasome in DCs. The role of DCs at this early infection phase is rather to be related to their capacity of sensing the virus or PAMPs/DAMPs released by dying infected cells, in order to recruit immune cells for the viral clearance and to promote T-cell activation. Indeed, DCs are equipped with a wide arsenal of PRRs responsible for the detection of several viral components. Among the PRRs involved in SARS-CoV-2 sensing are TLR7 [59], RIG-I [60], MDA5 [61] and possibly cGAS-STING [62]. Upon binding to their agonists, PRRs initiate a signaling pathway which culminates in the activation of IRF3, IRF7 and NF-κB. In the nucleus, these transcription factors regulate the expression of inflammatory mediators such as TNF, IL-6, monocyte chemoattractant (MCP1), macrophage inflammatory protein 1 α (MIP1α) and MIP1β and, importantly, induce the production of IFN-I, the main molecules involved in a strong antiviral response [58,63]. Indeed, the uncontrolled release of pro-inflammatory mediators due to the hyperactivation of the innate immune system observed in the first phase of SARS-CoV-2 infection results in the continuous migration of monocytes and neutrophils towards the lung tissue, further contributing to acute lung injury (ALI). For example, a high presence of neutrophils in the lung has been observed in severe COVID-19 patients. Activated neutrophils produce leukotrienes, reactive oxygen species (ROS) and neutrophil extracellular traps (NETs) that not only participate to ALI establishment or exacerbation but also cause endothelial lesions, which contributes to the systemic dissemination of the virus [64–66].

SARS-CoV-2: role of DC subsets in the antiviral response

DCs are widely distributed throughout the respiratory tract, where they act as tissue sentinels. In the lung, cDC1s are present in the mucosa and in the vascular wall, whereas cDC2s are found mainly in the lamina propria. The pDC population colonizes all of the lung including the parenchyma, the alveolar septa and the airways [67] (Figure 2). Another source of lung DCs is represented by circulating monocytes that extravasate into the lung tissue in inflammatory conditions and differentiate into moDCs [67]. Despite the crucial role of DCs in the activation of adaptive immune response, and the relevance of adaptive immunity in COVID-19 pathogenesis, our knowledge of their specific role in COVID-19 disease is still limited. Indeed, it is not yet clear whether DCs represent a target of SARS-CoV-2 or whether they are activated following virus infection of other cells that release PAMPs/DAMPs, or whether both

scenarios are true. We have recently described a peculiar and abnormal cDC2 activation after their direct stimulation with SARS-CoV-2. We have observed that DC2s and DC3s from mild and severe COVID-19 patients in spite of being able to produce inflammatory cytokines like IL-6, progressively lose their antigen presentation capacity as disease severity increases. Interestingly, cDC2 from healthy donors profoundly down-regulate key molecules involved in antigen presentation after SARS-CoV-2 infection *in vitro*, suggesting that the functional impairment observed in patients, reflect a direct interaction between DCs and SARS-CoV-2, and underlining the high impact of this anomalous DC maturation on T-cells activation [68]. ACE2 is poorly expressed in DCs and slightly increased only in some DC subtypes which reside in the interstitial space of the lung [69,70]. However, SARS-CoV-2 entry could be mediated by macropinocytosis or by innate immune receptors that may likely directly orchestrate virus entry or facilitate ACE2-dependent access. Particularly, the C-type lectin DC-specific intercellular adhesion molecule-grabbing non-integrin (DC-SIGN) has been shown to be directly involved in SARS-CoV infection in both an ACE2-dependent or independent manner [71,72] (Figure 1B). The current hypothesis for the role of DC-SIGN in SARS-CoV-2 recognition via glycosylated spike binding, is that this receptor could act as an attachment factor that might significantly enhance infection as already shown for HIV, hepatitis B virus (HBV) and HCV infections [72]. In this regard, a recent study showed that besides DC-SIGN myeloid cells possess a large array of C-type lectins (CLRs) capable of interacting with SARS-CoV-2's spike protein, which includes liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN), liver sinusoidal endothelial cell lectin (LSECtin), asialoglycoprotein receptor 1 (ASGR1) and CLEC10A (Figure 1B) [73]. This set of receptors can bind the spike protein through the recognition of N-glycosylated motifs which are located outside of the canonical RBD. CLRs engagement by SARS-CoV-2 do not lead to an active viral infection or replication, but rather to a hyperinflammatory response (Figure 1B) [73]. However, whether SARS-CoV-2 can directly infect DCs by binding the CLRs independently of ACE2 is still an open question. Furthermore, recent studies have expanded the family of receptors potentially involved in SARS-CoV-2 infection, corroborating the idea that the presence of other molecules are as important as ACE2 for virus entry. Among these, Neuropilin-1 has been recently identified as an unconventional receptor for SARS-CoV-2, as it binds the S1 subunit of the spike protein generated by furin [74,75]. Furthermore, an interesting study shows a new route of SARS-CoV-2 infection through the spike engagement of CD147 [76]. Notably, Neuropilin-1 and CD147 are well expressed by DCs [77]. Another important aspect to consider is the DC expression of proteases that act on spike maturation, significantly increasing the virus entry and infectivity [78]. For instance, furin enzyme is one of the proteins involved in spike maturation that acts by cleaving the S1/S2 complex at the specific multibasic site. mo-DCs, which have high amounts of furin and DC-SIGN, have been shown to be efficiently infected by SARS-CoV-2 despite having low levels of surface expression of ACE-2 and TMPRSS2. Although mo-DCs are permissive to SARS-CoV-2 entry, the infection is abortive as evidenced by the lack of increase in the virus genome after 72 h of infection. Interestingly, after virus infection, mo-DCs up-regulate both DC-SIGN and furin protease but not ACE-2 and TMPRSS2, highlighting the possibility that alternative factors are involved in virus access [79]. Notably, even though DC infection is abortive, it can contribute to SARS-CoV-2 spreading or can result in the loss of specific DC subsets. The few studies that directly describe DC functionality in COVID-19 disease showed a functional impairment of DCs as well as a reduced number of circulating conventional and pDCs. Firstly, the work of Sànchez-Cerillo demonstrated that cDC1, cDC2 and pDC frequency is massively decreased in the blood of both mild and severe COVID-19 patients. Of note, the analysis of DC frequency in leukocytes from bronchoscopy samples, revealed an increased infiltration of cDC2 subsets whereas cDC1s and pDCs were almost undetectable, suggesting that only the cDC2 subpopulation infiltrates lung tissue [80].

A second investigation of 17 acute and 24 convalescent COVID-19 patients enrolled in Hong Kong hospital conducted by Zhou and colleagues described a significant decrease in the frequency of circulating DCs. Moreover, the authors showed that cDCs from severe patients had a reduced expression of MHC-II and co-stimulatory molecules such as CD86 and were overall functionally impaired [81]. Interestingly, they also found an increase in the cDC/pDC ratio in SARS-CoV-2 infected individuals, speculating that the decrease in pDCs could explain, at least in part, the reduction in IFN production and the early decrease in innate immunity functionality [81]. Notably, the impairment in DC functions could directly contribute to alterations of the adaptive immune response. Recently, Kvedaraite and colleagues performed a high-dimensional flow cytometry analysis of the various blood DC subsets in mild and severe COVID-19 patients by which they confirmed a general decrease in circulating DCs and an affected maturation status, with lower levels of human leukocyte antigen DR (HLA-DR) and CD86 in all DC subsets [82]. The authors pointed out that the disappearance of DCs from circulation gives rise to their infiltration in inflamed tissues. Interestingly, this work revealed a phenotypic change in DC subsets that includes an IFNs response in cDC1 and a decrease in the coinhibitory molecule CD200R in pre-DCs, DC2, and DC3 subsets of severely sick patients [82]. In agreement, we reported a decreasing frequency of blood cDC1s and DC2 sin COVID-19 patients, but an increase in the DC3s, ascribed to the increment of the $CD14^{+}CD163^{+}$ inflammatory subset, that correlates with disease severity [68].

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In conclusion, despite the recent investigations definitively demonstrating an involvement of DCs in COVID-19 disease, our comprehension of their role in SARS-CoV-2 infection is still incomplete. There is still a lack of knowledge on various aspects of DC involvement in COVID-19 disease that need to be urgently addressed. Indeed, no study has investigated the potential mechanism of SARS-CoV-2 entry in DCs, an aspect that could improve our overall knowledge on SARS-CoV-2 infection and DC responses, and most importantly could have a potential impact on medical intervention and vaccine design.

IAV: it is not just a cold

SARS-CoV-2's transmission mechanism and clinical presentation are partially overlapped with those of other respiratory virus infections, including influenza virus. In this regard, the mechanisms by which DCs sense IAV, and the roles of these cells during IAV infections will be discussed in this paragraph.

IAV: mechanisms of recognition by DCs

The IAV is a negative sense ssRNA virus which can cause an acute infection in the respiratory tract. As described above, while SARS-CoV-2 infections are usually limited to the lower respiratory tract, potentially causing pneumonia, IAV infections can take place throughout the entire respiratory district, affecting both upper and lower airways [83]. The main IAV infection mechanism is based on the recognition of host sialic acid (SIA)-containing receptors via the viral hemagglutinin (HA) envelope protein. This physical surface interaction allows the virus to enter the cell through receptor-mediated endocytosis [84]. Although bronchiolar-alveolar epithelial cells are the prime IAV cellular target, DCs and other immune cells can also be susceptible to infection [85]. However, DCs possess a wide array of PRRs in order to promptly detect extracellular or internalized influenza viral particles. IAV being an RNA virus, the first PRRs involved in its recognition are endosomal TLR7 and TLR3, which recognize single- and double-stranded RNA respectively, as well as the cytosolic detectors RIG-I and NLRP3 [86,87]. TLR7 allows immune cells to sense IAV that enters cells via endocytosis. This receptor is highly expressed by pDCs which makes these cells optimal viral sentinels capable of initiating the antiviral response before IAV can proceed with the replication [88]. While TLR7 can detect the IAV genome as it is, TLR3 is responsible for the recognition of possible dsRNA intermediates generated during viral replication. Although previous studies have clearly shown that this PRR is activated during IAV infection and that it is critical for the dampening of IAV viral burden [89,90], the specific ligand and activation mechanism of TLR3 during IAV infections are currently unknown. RIG-I is responsible for detecting small cytosolic viral 5′-triphosphorylated RNA intermediates usually generated during IAV replication. Like RIG-I, NLRP3 also plays a key role in the detection of cytosolic IAV components and of virus-induced cellular stress. This intracellular receptor is strongly expressed by the majority of DC subsets and is responsible for the formation and activation of the canonical inflammasome complex [91]. Among the roles of NLRP3 inflammasome there is the secretion of pro-inflammatory mature IL-1β and the activation of pyroptosis, an inflammatory type of lytic programmed cell death crucial for antiviral and antimicrobial responses [92]. Several studies support the idea of a pivotal role for NLRP3 inflammasome in immune cell recruitment and the initiation of the adaptive immune response, placing this molecular complex among the main host anti-IAV defense mechanisms [93,94]. Contrary to what initially thought, DNA-sensing PRRs have also proven to be important for IAV detection, albeit with an indirect recognition mechanism. As mentioned above, the cGAS receptor is a sensor of cytosolic dsDNA molecules, which normally signals through the interaction with the downstream molecule STING. The dsDNA recognized by cGAS can derive from exogenous pathogens, including bacteria, DNA viruses [21] or retroviruses [95], but also from host mitochondria (mitochondrial DNA, mtDNA) [96]. A recent study reported that the envelope M2 protein of IAV can favor the translocation of host mtDNA into the cytosol, causing the triggering of the cGAS-STING pathway, thus initiating the host antiviral response [97]. Furthermore, IAV can also be detected by DCs via PRRs which recognize specific viral carbohydrate structures. Indeed, most DCs express several CLRs, such as DC-SIGN (CD209) and L-SIGN (CD209L) or the murine homolog SIGN-R1, which recognize glycans on IAV glycoproteins, making DCs even more effective sensors of the virus [98,99]. On the other hand, however, although IAV do not primarily infect DCs, the presence of CLRs on these cells makes them susceptible to viral infections. Indeed, there is evidence that CLRs represent a possible alternative entry route for IAV to sialylated receptors, serving as anchor point for infection [85].

IAV: role of DC subsets in the antiviral response

PRRs engagement by IAV-derived PAMPs and the activation of their respective signaling pathways result in the release of pro-inflammatory and chemoattractant mediators, ultimately leading to the establishment of an antiviral immune microenvironment. Among these factors, IFN-I can be released by both human and mouse respiratory epithelium in

response to infection and by resident alveolar macrophages upon viral recognition [100]. Resident $CD103^+CD11b^{lo}$ $langerin^+$ cDCs are also important sources of IFN-I, although their most prominent role is rather the initiation of the acquired immune response given by their migration to regional LNs in order to present viral antigens to naïve T lymphocytes [101]. IAV-induced IFN-I play a crucial role in hindering initial viral replication and in triggering the host antiviral response. In fact, the paracrine action of these cytokines primes the nearby cells against the virus by inducing them to produce a variety of antiviral proteins [102]. Moreover, IFN-I strongly contribute to the release of chemoattractant factors like chemokine (C−C motif) ligand (CCL)2, CCL5, chemokine (C−X−C motif) ligand (CXCL)9, CXCL10, CXCL11 in order to recruit immune cells, such as circulating monocytes and DC progenitors to the infection site [103]. Although the classification of DCs in cross-presenting cDC1s and $CD4^+$ T cell-polarizing cDC2s is well established at the steady state [10,11], a large body of recent studies performed in mice models of influenza infection supports the idea that this dichotomy becomes less and less clear during IAV-triggered inflammation [104–107]. This is largely due to the alteration of surface marker expression and to the appearance of inflammation-associated subsets with intermediate or hybrid phenotypes. The IAV-triggered inflammatory milieu is associated with the appearance of heterogeneous subsets of inflammatory DCs with monocyte or pre-cDC origin and with different functions depending on the origin [104,108]. On one hand, freshly recruited monocytes may be subjected to a differentiation process characterized by the up-regulation of several 'conventional' DC markers, like MHC-II and CD11c, which leads them to acquire an inflammatory phenotype to some extent closer to cDC2s [109]. The resulting mo-DCs, however, possess a reduced capacity to migrate to regional LNs, and therefore are poorly involved in the antigen presentation process [104,108]. The main task of mo-DCs is rather associated with the orchestration of the antiviral immune response in the trachea and lungs by producing pro-inflammatory cytokines and engaging effector cells or antibody-complexed viral antigens [104,108]. In support of this idea, recent studies performed in mice models showed that mo-DCs enter the antigen-exposing tracheal epithelium in response to IFN-I-induced release of CCL2, subsequently acting *in loco* by sensing IAV through SIGNR-1 and producing chemokines like CXCL9 and CXCL10 to recruit and activate NK cells [108]. On the other hand, *bona fide* pre-cDC-derived inflammatory DC2s (inf-DC2s) undergo a similar phenotypic change as mo-DCs, acquiring classical markers which define cDC1 and macrophage lineages. Although only few surface markers, like CD26, allow to distinguish inf-DC2s ($CD26^+$) from mo-DCs ($CD26^-$), contrary to the latter, inf-DC2s are capable of migrating toward draining LNs and are described as excellent activators of both naïve $CD4^+$ and $CD8^+$ T cells [104]. As mo-DCs, inf-DCs establishment is driven by IFN-I, which induces in DCs the up-regulation of both phagocytic receptors (CD64) and antigen-presenting molecules, including chemokine receptors, adhesion molecules (CD11b) and co-stimulatory molecules. This 'phenotypic switch' make inf-DCs excellent APCs capable of easily incorporating the IAV-antigens found in respiratory tracts, processing and presenting them via class II or class I MHC molecules to T lymphocytes in the LNs [104]. Although previous works have shown that cDC1s are the main cells responsible for CTL priming in IAV infections [100], a growing body of evidence strongly points towards the possibility that this role can be equally accomplished by inf-cDC2s [104,108]. Moreover, inf-cDC2s possess the ability to also prime $CD4^+$ T cells, inducing the polarization of naïve T lymphocytes towards IFN-γ-producing cells, thus favoring the establishment of a T_H1 immune response [104]. The emerging picture is that IAV-triggered inflammation drives a strong 'thinning' of the phenotypic differences between DCs and mo-DCs which are normally appreciable at the steady state. Therefore, particular attention should be paid to the inflammatory phagocytic cell classification, and indeed, these new insights might be exploited to re-evaluate the roles and functions previously attributed to particular cellular subsets.

HIV: the hitcher

As described above, a possible role for DC-SIGN in SARS-CoV-2 cell entry and viral dissemination has been proposed [71,72]. Besides, the involvement of DC-SIGN and other CLRs in viral infections has already been demonstrated in the case of HIV. In this regard, sensing of HIV by DCs and DC-dependent HIV dissemination and trans-infection will be discussed in the next paragraph.

HIV: mechanisms of recognition by DCs

The capacity of HIV to permanently insert a copy of its own RNA inside the host DNA genome, placed this virus among the *Retroviridae* family members. Although two subtypes of HIV are currently described, HIV-1 and HIV-2, HIV-1 is the most widespread form worldwide and it turned out to be more infectious [110]. Therefore, HIV will be discussed referring to the HIV-1 type. The main mechanism of HIV infection is based on the capacity of viral envelope glycoprotein gp120 to bind its primary receptor CD4 and co-receptor C−X−C chemokine receptor type (CXCR)4 or CCR5, triggering the fusion between the viral envelope and the target cell membrane, and ultimately

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leading to the HIV capsid release into the cell [111]. Therefore, the cellular target of choice for this virus is represented by cells that strongly express CD4, CXCR4 or CCR5 co-receptors, such as $CD4^+$ T lymphocytes, but also DCs [112] and macrophages [113]. As deeply described in the next paragraph, DCs are among the first immune cells to come into contact and to be infected by the virus. In order to establish an optimal priming of naïve T cells and trigger proper $CD4^+$ T_H1 and $CD8^+$ CTL antiviral responses, infected DCs have to efficiently detect HIV antigens [114]. In the case of HIV, NAs are the main viral PAMPs which alert the cell following infection. Upon uptake, the PRR machinery DCs can count on to detect these PAMPs is rich, and includes cell-intrinsic cytosolic receptors for both RNA molecules like RIG-I [115] and MDA-5, and cytosolic DNA like cGAS [116,117] and IFI16 [118]. HIV can also be recognized through cell-extrinsic mechanisms which involve endosomal TLRs like TLR7 [119,120] following internalization of virus-infected, apoptotic cells [114,121]. TLR7 detects ssRNA molecules and its engagement results in the activation of transcription factors IRF7 and NF-κB through the initiation of the MyD88-dependent pathway, ultimately leading to the release of massive amounts of IFN-I and other pro-inflammatory cytokines. Due to the high expression of TLR7/8, this signaling is particularly present in pDCs, making these cells crucial regulators of HIV spreading [119,122]. Despite the large array of PRRs, HIV detection in DCs can be hindered by the presence of a variety of intrinsic molecules strongly expressed in HIV target cells. This HIV-related peculiarity is among the causes of a suboptimal DC maturation, consequently leading to the expansion of antigen-specific T cells with poor antiviral activity. This, in addition to other HIV-intrinsic features, may explain the inability of the immune system to clear HIV infection.

HIV: role of DC subsets in the antiviral response

Due to the high expression of the CD4 receptor, $CD4^+$ T cells are to be considered the main cells in which HIV replication occurs. However, the frequency of these cells is low in anogenital tissues, which represent one of the main entry routes for sexually transmitted HIV. In this regard, a pivotal role for DCs in the initial HIV uptake, subsequent transmission and systemic dissemination is supported by several pieces of evidence. What emerges is that some DC subsets which reside in the epidermal layer of anogenital tracts can incorporate and support high levels of HIV load, becoming efficient vectors for virus spreading [123–125]. If this role was in the beginning assigned solely to LCs [126–128], a recent study clearly showed that another DC subset called epidermal $CD11c^+$ DCs, which normally reside in lower epidermal layers of anogenital tracts, is preferentially involved in sexually transmitted HIV dissemination [129]. Unlike LCs, epidermal $CD11c^+$ DCs do not express the virus-binding receptor langerin, but they possess a wide array of other CLRs, including the mannose receptor (MR, CD206), and higher amounts of CCR5, CD54 and CD80. These peculiarities make this subset particularly prone to interact with HIV and capable of efficiently transferring the virus to $CD4^+$ T lymphocytes (*trans*-infection) [129]. Moreover, during inflammation, an additional DC subset different from LCs has been described to populate the epidermis, thus potentially playing a crucial role in HIV dissemination: the inflammatory dendritic epidermal cells (IDECs) [130]. One of the proposed mechanisms at the base of DC-dependent HIV dissemination and trans-infection is the one mediated by CLRs. As in the case of IAV, CLRs may play a detrimental role for the host also during HIV infections. CLRs, such as langerin (CD207), MR, DC-SIGN, SIGLEC-1 and DC immunoreceptor (DCIR), are able to bind several viral envelope components, including glycoprotein gp120 [131–134] and gangliosides [135,136]. While this surface interaction allows DCs to sense and incorporate HIV, the failure of a proper viral internalization or the lack of new virions release may cause a progressive accumulation of HIV particles at the cell surface [123]. This event, combined with the expression of adhesion molecules (CD54) and the ability to migrate to LNs, make cDCs, and especially resident subsets, capable of transferring HIV to T lymphocytes, turning them into effective viral delivery vectors [124,137]. This transmission mechanism results strongly enhanced in patients not capable of spontaneously controlling HIV (progressors), in which the altered phenotype of several DC subsets is characterized by the overexpression of both CLRs and adhesion molecules [138]. Although the pDC phenotype has also been shown to be altered during HIV progression [139,140], HIV *trans*-infection seems to be limited to resident and cDC subsets [141,142]. The transfer mechanism described above, however, occurs irrespectively of the productive viral replication inside DCs, but is rather based on a CLRs-mediated viral delivery. In this regard, a recent study showed that a more efficient transfer to T lymphocytes can take place when DCs themselves are subject to a productive HIV infection (*cis*-infection) [129]. DC *cis*-infection may also occur as a consequence of the initial CLR-mediated virus–cell interaction, in those cases when the DC subset involved possesses high levels of CCR5 or CD4 co-receptors, as with epidermal $CD11c^+$ DCs. Although some DC subsets such as $CD141^+$ cDCs result intrinsically more resistant to HIV entry [143], the expression of the aforementioned receptors make also cDC and pDC subsets in principle susceptible to *cis*-infection. At this stage, what makes DCs particularly prone to effectively convey HIV to other cells, is their peculiar capacity to support high levels of viral

load. Indeed, despite the array of PRRs available, HIV detection in DCs can be hindered by the presence of a variety of intrinsic molecules strongly expressed in the myeloid cell lineage. Some DC subpopulations, for instance, possess high levels of the cytosolic SAM domain and HD domain-containing protein 1 (SAMHD1) protein which allows cells to keep the viral genome at low levels through the inhibition of the HIV retro-transcription process [144,145]. This reduces the accumulation of viral DNA intermediates, thus preventing their detection and the induction of IFN-I, allowing the cell to better withstand high viral loads [146]. While this mechanism appears in principle beneficial, it precludes the establishment of a robust antiviral immune response in the host and it may turn infected DCs into optimal HIV delivery vectors. Therefore, DC intrinsic mechanisms which target more downstream viral replication steps, such as the integration process or the release of new viral particles, represent a more efficient antiviral strategy. Indeed, these mechanisms cause the progressive build-up of retro-transcription intermediates allowing DCs to better detect viral PAMPs in order to potently perform antigen presentation [147]. As mentioned above, in progressors several DC subsets overexpress many surface markers, including CLRs, adhesion and co-stimulatory molecules [148]. In most cases, these HIV-induced alterations may affect DCs, changing their anatomical distribution or conferring them a constitutively active phenotype. In particular, these perturbations seem to be particularly relevant in the case of pDCs. For instance, it has been described that HIV pathogenesis correlates with a reallocation of circulating pDCs, which leave the blood in order to reach the LNs and other districts [149]. In spite of that, it appears that this reduced blood pDC frequency is a hallmark of disease progression or chronic infection rather than an event of initial antiviral response. In line with this idea, a recent study shows that during the early stages of HIV infection pDCs transiently increase in the blood before the onset of plasma viremia [122]. Although these cells show an activated phenotype with a strong IFN-I signature, compared with healthy patients or patients who control HIV progression (controllers) they turn out to be less sensitive to HIV or other viral PAMPs when stimulated *in vitro* [150]. Moreover, the refractory state of pDCs was seen to be maintained also during disease progression [151]. pDCs from progressors also showed a prolonged surface exposure of TNF-related apoptosis-inducing ligand (TRAIL) due to an altered cytosolic trafficking of the molecule. The lack of TRAIL surface removal allows this effector to trigger cell death in cells with which it comes into contact, including $CD4^+$ T cells, thus participating in the progressive depletion of these cells [139].

HHVs: enemies within

In the previous paragraph the double-edged role of DCs as tissue sentinels and vehicles for viral dissemination has been described. The same dynamics can also be found during the infection with other different classes of viruses, such as the dsDNA HHVs.

HHVs: mechanisms of recognition by DCs

The co-evolution of herpesviruses with their hosts has led to a complex interplay between viruses themselves and the host immune system [152]. DCs, as key components of the innate immunity, are one of the first cell types to interact with herpes viruses during an infection. On one hand, various evidence shows that DCs are necessary for the immune response to HHVs [153–156]. On the other, many HHVs are able to productively infect DCs and impair their correct function or use them as vectors for further viral dissemination [157–161]. In DCs, HHV sensing involves virus-derived dsDNA or glycoproteins, which are recognized by a plethora of plasma membrane, endosomal and cytosolic sensors such as TLR9 [162–164], TLR2 [164,165], TLR3 [166], DC-SIGN [159,167], cGAS [168,169], IFI16 [170,171], with contributions from many others. We have already extensively reviewed TLR9 signaling [86]. Briefly, TLR9 is an endosomal PRR highly expressed by pDCs, and, to a lesser extent, by myeloid DCs and is long known to recognize unmethylated CpG DNA motifs [172]. TLR9 exhibits a dual signaling pattern, with two distinct pathways leading to the activation of either NF-κB, or IRF7 [86]. While both pathways rely on the recruitment of toll-interleukin 1 receptor domain-containing adapter protein (TIRAP), MyD88, TNF receptor-associated factor 6 (TRAF6) and interleukin receptor-associated kinase (IRAK) 4/1/2 molecules, the differential and temporally distinct recruitment of various adapter proteins results in the bifurcated signaling of this receptor [86,173]. As such, NF-κB activation ultimately relies on the recruitment of transforming growth factor-β-activated kinase 1 (TAK1), TAK-binding protein (TAB)1/2, NF-κB essential modulator (NEMO), the deactivating phosphorylation of NF-κB inhibitor of NF-κB (IκB) and IκB kinase β (IKKβ). Instead, IFN-I production depends on the recruitment and phosphorylation of IRF7 [174]. TLR2 is a cell surface PRR reported to recognize several glycoproteins from various HHVs, such as Herpes simplex virus (HSV) [175,176], Varicella-Zoster Virus (VZV) [177], Human cytomegalovirus (HCMV) [178] and likely Epstein–Barr Virus (EBV) [179]. Other studies show that TLR2 is also able to recognize non-structural proteins, such as viral dUTPases [180,181]. The signaling of TLR2 closely recapitulates the NF-κB branch of TLR9 and involves the same adaptor proteins [182]. For a long time, TLR2 has been considered unable to induce an IFN-I response, but

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further inquiry revealed that some cell types are indeed able to produce IFN-I after TLR2 stimulation [183–186], although with different mechanisms in mice and humans [185,186]. TLR3 is one of the endosomal PRRs that recognize dsRNA. While herpesviruses have a dsDNA genome, the dsRNA intermediates that are generated during their replication are able to trigger TLR3 signaling [187]. Differently than other TLRs, TLR3 does not utilize TIRAP-MyD88, but interacts directly with TRIF and is able to induce the activation of NF-κB through a receptor-interacting protein 1 (RIP1) dependent pathway, while the IFN-I production depends on IRF3 [188]. DC-SIGN expression is restricted to DCs and IL-4 activated monocytes and macrophages. While Raf1 has been identified as a central mediator of downstream signaling, the outcome of DC-SIGN engagement depends heavily on the specific ligand. Often, this interaction leads to increased IL-10 production [189]. A very important PRR in HHV recognition is cGAS. cGAS is a dsDNA sensor that is able to generate cyclic cGMP-cAMP (cGAMP) dinucleotides upon activation. cGAMP molecules act as second messengers and lead to the activation of STING, which, in turn leads to the IRF3-dependent production of IFN-I and non-canonical NF-κB-dependent production of inflammatory cytokines [190,191]. The importance of this pathway is further evidenced by the plethora of cGAS-STING pathway-inhibiting HSV proteins [192–196]. Most of the HHV-sensing PRRs and their respective signaling pathways converge on NF-κB and IRF3/7 that allow for proinflammatory cytokines and IFN-I production, which limit viral replication and initiate an adaptive immune response [197,198]. In DCs, HHV recognition and response is dependent on the specific virus, as they share both similar, but also distinctive features. Furthermore, different DC subsets can sometimes interact very differently with the same virus, which adds an ulterior layer of complexity to the DC–HHV interaction.

HHVs: role of DC subsets in the antiviral response

Human herpesviruses can be classified into three groups: α, β and γ herpesviruses. We will hereafter describe the DC interactions with the prominent members of each group. HSV-1 and HSV-2 are α-herpesviruses that enter the body through mucocutaneous sites, such as the skin or, in the case of HSV-2, the genital mucosa [199]. In spite of the presence of tissue resident DCs (dermal DCs and LCs), their contribution to HSV control appears to be relatively minor. Indeed, the productive infection of these cells causes the impairment of their function through the down-regulation of CD83, CCR7, CXCR4 and the degradation of cytohesin-interacting protein (CYTIP), which results in the activation of β-integrin-mediated adhesion and a diminished migratory capacity [200–204]. Nonetheless, some studies suggest an active role for LCs, which, upon getting infected, migrate towards the dermis, where they transfer viral antigens to blood dendritic cell antigen 3 (BDCA3$^+$)/CD141$^+$ dermal DCs (the human equivalent of murine CD103$^+$ DCs) and DC-SIGN$^+$ DCs, possibly by undergoing apoptosis and getting phagocytosed [205,206]. Indeed, HSV can induce apoptosis in immature DCs [206,207], partly by causing the down-regulation of cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP), a key anti-apoptotic protein [208]. BDCA3$^+$ dermal DCs express higher levels of the damaged-cell uptake receptor CLEC9a when clustering together with LCs [205,206,208], which further reinforces the idea of antigen uptake from dying cells. This suggests that there is a relay of antigens from LCs to migratory DCs, which then migrate towards LNs and activate the adaptive response. In a recent study, Hor and colleagues show that the CD8$^+$ T cell response is indeed induced by a defined spatiotemporal sequence of events [209], albeit in a murine model, which might explain some of the differences. In this model, the migratory DCs that capture viral antigens in the skin relocate to the LNs, where they present the antigens to CD4$^+$ T cells but are not able to cross-present to CD8$^+$ T cells. Instead, antigens are passed to XCR1$^+$ DCs, which are known to have excellent cross-presenting capabilities. Coupled with CD4$^+$ T-cell licensing, it is XCR1$^+$ DCs that activate the subsequent CD8$^+$ T-cell adaptive response. Indeed, many studies highlight the importance of cross-presenting DCs in peripheral HSV infections in both humans and mice [205–214]. Another important subset of DCs with a remarkable antiviral function is the pDCs. Given their function it would be expected that they exert a primary defensive role in HSV infections. In fact, pDC-derived IFN-I are able to potently stimulate NK cells *in vitro* [215], yet their contribution in localized murine models of HSV infections also seems to be limited [216,217]. Nonetheless, murine and possibly human pDCs are of great importance in ocular, HSV-induced keratitis, their depletion resulting in severe disease [218]. One study shows that murine CD11c$^+$ DCs can even have a deleterious role in stromal keratitis and contribute to the exacerbation of the lesions by activating CD4$^+$ T cells in an autophagy-dependent manner [219]. In another report, an HSV-derived protein, infected-cell protein (ICP) 22, is shown to down-modulate the expression of CD80 and limit corneal scarring by dampening the immune response in mice [220]. In spite of these observations, the precise involvement of murine DCs in the aggravation of HSV-induced keratitis is still unclear, given that in other similar instances cornea-residing DCs are reported to limit scarring and pathological phenomena [221]. Interestingly, the autophagic degradation of the nuclear lamina in immature mo-DCs has been found to promote the nuclear

egress of HSV-1 capsids, thus facilitating the assembly of new virions [222]. This process is instead inhibited in mature DCs, suggesting that the maturation state of a DC can impact greatly on their susceptibility to HSV infections. In fact, another study shows that HSV-1 protein ICP34.5 interferes with murine DC autophagy and ultimately with antigen processing and presentation [223], representing one of the many immune evasion strategies employed by HHVs. For example, in murine DCs HSV-1 is able to up-regulate the expression of tripartite motif (TRIM)7/RNF90 and TRIM30α, E3-ligases of STING that cause its proteasomal degradation with subsequent diminished production of IFN-I and IL-6 [224,225]. In another study, an engineered HSV-2 lacking glycoprotein D (gD-2) is able to boost murine DCs' viability and function, which is instead impaired by WT strains due to an enhanced UPR response [226]. Furthermore, HSV-1 infected mature DCs not only have a reduced expression of CD83 and IL-6R but can also cause their down-regulation in bystander DCs. The mechanism of such peculiar DC impairment involves the transmission of viral proteins via L particles, that is viral particles loaded with viral proteins, but lacking a capsid and devoid of DNA [204,227]. In HSV as well as other HHV infections, the DCs are both the hunter and the prey. Given their role on the front lines, it is not unexpected that their interaction with multiple pathogens might result in a broad range of outcomes. For example, recent studies uncover a series of mechanisms through which HSV-2 increases DC sensitivity to HIV infection [228], which might in turn allow for further HIV dissemination [229]. It is therefore important to recognize the intrinsic limits of the various models employed in viral infection studies. In fact, one study highlights how HSV-2 infection of DCs in the genital mucosa seems to be promoted by the complement system-mediated opsonization of viral particles [230], which ulteriorly reinforces the idea that some results might need further validation in *in vivo* settings.

Another HHV that interacts extensively with DCs is the HCMV. As a β-herpesvirus with broad tropism, HCMV (HHV-5) also has the ability to infect various subsets of DCs [231], mainly through the interaction with DC-SIGN [159,232]. Protective immunity is associated with both cDCs and pDCs, via interferogenic and adaptive immunity-activating signaling pathways both in murine and human models [233–236]. Furthermore, some studies also highlight the importance of IFN-I-independent responses, such as in mo-DCs, which directly contribute to infection control in CMV-infected fibroblasts by releasing interferon-unrelated soluble factors [237]. While there is some evidence that HCMV impairs the migratory ability of infected DCs by inducing the degradation of CYTIP [238], other studies underline the role of myeloid DC migration to the LNs and their further recirculation in promoting viral dissemination. In fact, this phenomenon, present in both mice and humans, is promoted by CMV-derived proteins, M33 and US28, respectively [239,240]. Nonetheless, the negative modulation of DC activity seems to remain a cornerstone of the herpesviruses' escape mechanism, as one of the immediate-early proteins of CMV, IE2, directs the DC activation molecule CD83 towards proteasomal degradation [241]. Furthermore, in murine models of CMV a decrease in splenic DCs and cross-presenting DCs has been observed, which correlates with a decreased $CD8^+$ T cell response [242,243]. Although not completely clear, these studies suggest that this kind of DC depletion might be due to IFN-I. As with other viruses from the same family, the effects of CMV on the immune system and its ability to properly respond to stimuli go beyond the CMV infection itself. Some studies show that pulmonary CMV infections and concomitant stimulation with a low-potency allergen, such as OVA, sensitizes the animal to further exposure, an effect that is dependent on migratory DCs [244]. As such, CMV infections can represent a hidden threat to susceptible individuals.

The last group of HHVs are γ-herpesviruses with its two human-infecting members being EBV and Kaposi's Sarcoma-associated herpesvirus (KSHV). EBV (HHV-4) has a very broad prevalence in the human population. While asymptomatic in most cases, it can lead to infectious mononucleosis or even cancer, with EBV-attributed malignancies constantly increasing worldwide [245]. The role of DCs in EBV infections has been extensively reviewed by Münz [246,247], with the last years having seen only limited progress in the area. It is known that DC-mediated interferon responses are protective during EBV infections, either by directly targeting the EBV host cell type, the B cells, or by activating a protective NK immunity in an IFN-I-, IL-12- and IL-15-dependent manner. In the later stages, DCs also contribute to the activation of a CTL response against infected cells, with some subsets, such as the CD137L-expressing DCs, being more efficient than others [248]. pDCs are the most robustly activated DC subset in EBV infections, even though they are ultimately dispensable for primary infection control [156]. Nonetheless, pDC role might not be limited to IFN-I production, as they might also contribute to the activation of the adaptive immunity, possibly through a trogocytosis-like mechanism, that allows them to get cross-dressed with MHC class I molecules containing EBV antigens [249]. In line with this evidence on the role of pDCs in early EBV control, the presence of pDCs in the blood seems to be sensibly reduced during EBV infections [250,251]. Another tumor-inducing HHV is KSHV or HHV-8. As the name suggests, it is the etiologic agent of Kaposi's sarcoma, a vascular tumor. Differently from EBV, KSHV is able to productively infect DCs [252]. In the skin, both interstitial dermal DCs and LCs support

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productive infection with KSHV, which enter the cell by binding DC-SIGN and langerin, respectively [160]. In particular, DC-SIGN can bind the highly mannosylated viral glycoprotein B [253]. This type of infection leads to the signal transducer and activator of transcription 3 (STAT3)-mediated production of IL-6, IL-10 and IL-23 and induces an autophagic block, which can interfere with antigen processing and presentation [254,255]. Furthermore, KSHV can impair DC migration through the down-regulation of CCR6 and CCR7 [256].

Ultimately, HHVs are a group of viruses whose strategy relies on quantity instead of quality. While being relatively harmless for most immunocompetent individuals, their extremely high prevalence worldwide makes them a dangerous foe for many immunocompromised individuals, such as transplant recipients.

DCs: jacks of all trades, masters of some

As tissue sentinels endowed with migratory properties and tasked with cytokine production and adaptive immunity activation, DCs represent a crucial node of the immune system which can greatly influence the outcome of an infection. The DC-mediated antiviral response is characterized by a certain degree of overlapping pathways and the strategies of immune escape employed by the viral pathogens can vary, but often end up targeting the same cell functions. The common ssRNA genome of SARS-CoV-2, IAV and HIV makes them recognizable by such PRRs as TLR7, RIG-I and MDA5, while HHVs' DNA genome gets recognized by TLR9, cGAS and IFI16. Viral protein recognition by CLRs is similar across different types of viruses, with DC-SIGN being one of the main PRRs involved in this type of sensing. Furthermore, the viral life cycle often involves the temporary formation of novel molecules or their release from isolated compartments, as is the case for replication intermediates and mtDNA, which can lead to NLRP3 activation. Moreover, DNA-sensing cGAS-STING signaling appears to be also important in IAV infections and possibly SARS-CoV-2, while TLR3 signaling that recognizes dsRNA molecules plays a role in HHV infections. In spite of belonging to different phyla, kingdoms or even realms, SARS-CoV-2, IAV, HIV and HHVs have all evolved various mechanisms that inhibit DC activation in terms of down-regulation of the antigen presentation machinery, of the co-stimulatory molecules such as CD83 and CD86 and also of the migratory capacities. Moreover, the migratory ability of DCs can be cleverly exploited by HIV or HHVs, which attach to cell surface lectins such as DC-SIGN in order to promote their own dissemination throughout the organism. The fact that very different viruses, with different genomes of different sizes and organization, but also different tropisms, life cycles, pathological manifestations and prevalence in the population all have evolved similar forms to evade the immune system by downmodulating DC functions, underlines the central role of DCs, with their ability to activate adaptive immunity, in the antiviral response and makes them one of the key players in viral infections.

DC-targeted vaccines: the future is now

As carefully described above, DCs strongly emerge as crucial initiators and regulators of the acquired immune response against viral infections, thanks to their capacity of antigen presentation. This peculiarity can be exploited in order to design and develop DC-targeted vaccines usable in different clinical settings, including infectious diseases and cancer. There are two main vaccination strategies generally followed in clinic: preventative and therapeutic vaccination.

The first strategy aims to induce a humoral immune response in patients, through the generation of memory B cells capable of producing specific virus-targeting neutralizing antibodies to rapidly eradicate the pathogen in case of infection. The different vaccines currently distributed on the international market against SARS-CoV-2 virus, such as Pfizer (BNT162b2), Moderna (mRNA-127), AstraZeneca (Vaxzevria) and Johnson & Johnson's Janssen, are all designed and developed with this purpose. The therapeutic vaccination, instead, points to trigger a cell-mediated immune response through the activation and expansion of specific $CD8^+$ CTLs capable of detecting and removing infected cells. Thanks to their capacity of activating CTLs via antigen (Ag) cross-presentation, DCs are the focus of this second strategy. The general idea is to deliver a viral antigen to DCs and favor its presentation on to the MHC-I molecule in order to promote cross-presentation and specific $CD8^+$ CTLs generation.

Among the first attempts in this regard, is the *ex-vivo* generation of autologous DCs loaded with viral Ags and the re-injection of these cells into patients [257,258] (Figure 3A). Although the majority of studies and trials concerning this approach were carried out by using *ex-vivo* differentiated mo-DCs, which, as described above, are not a physiological human DC subpopulation; the safety, good tolerability and low side effects led the FDA to approve this therapeutic strategy for the treatment of specific types of cancer [259,260]. However, the high costs and the limited clinical feasibility often restricted this approach, driving translational medicine to design new strategies rather based on '*in situ*' generation of cross-presenting DCs. One of the first approaches developed consisted in the *in vivo* targeting of

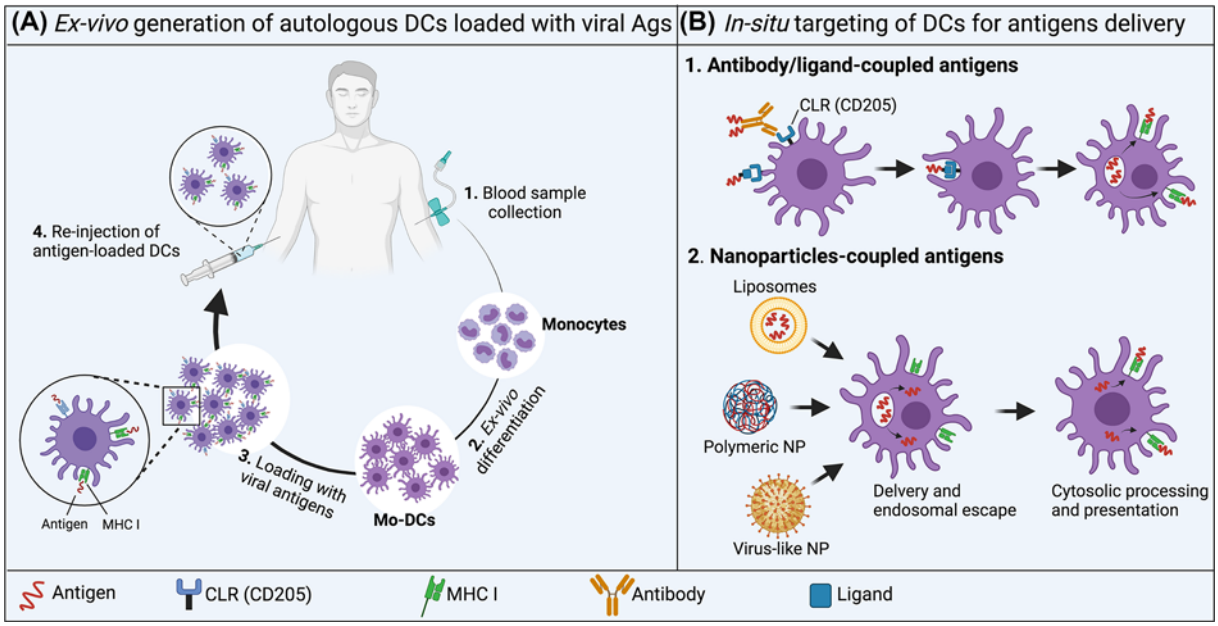

Figure 3. Graphic representation of the main DC-based vaccination strategies

(**A**) *Ex-vivo* generation of autologous DCs loaded with viral antigens. Monocytes are collected from patients' blood and differentiated *in vitro* to originate mo-DCs. Cells are then cultured and expanded in the presence of viral antigens for the presentation of viral peptides in association with MHCI molecules. Finally, mo-DCs loaded with the antigens are re-administered to the patient. (**B**) *In-situ* delivery of antigens to DCs. *In vivo* DC targeting may be achieved by administering antigens coupled or fused with antibodies/ligands capable to interact with DC-specific receptors such as CLRs (1) or by using NPs (2). The presence of NPs favors endosomal escape, allowing coupled antigens to reach the cytosol and undergo proteasomal degradation. This event strongly increases the loading of processed peptides on MHC-I molecules. Abbreviation: NP, nanoparticle.

DCs with easy to incorporate viral Ags coupled or fused with antibodies or ligands capable of favoring the internalization of the complex [261–263] (Figure 3B). The antibody/ligand-dependent uptake of viral components leads to an enhanced processing and loading of the resulting viral cargo on MHC molecules, ultimately boosting the presentation process [264]. Crucial for this approach are the identification and selective targeting of DC-specific receptors in order to allow a precise delivery of molecules into DCs. In this regard, CLRs, such as DEC205/CD205 receptor, have been identified as an effective entrance gate to selectively deliver Ags into DCs, which is also capable of boosting both the presentation [265,266] and cross-presentation processes [267–269]. Therefore, several chimeric monoclonal antibodies have been designed to target these C-type lectin receptors in order to establish a robust cell-mediated immune response. Although the efficacy of this strategy turned out to be particularly dependent on adjuvants co-administration to enhance the immunogenicity of the protein vaccine [270], this approach led to promising results at least in cancer, successfully passing both phase 1 [271] and phase 2 trials [272].

To further improve selective DC targeting and activation, novel approaches introduced the use of nanoparticles (NPs) as vehicles to deliver viral Ags [15,273] (Figure 3B). Indeed, these carriers constitute in all respects customizable platforms ready to be functionalized and/or encapsulated with everything needed for a focused targeting and activation of DCs. The most frequent nano-carriers used to deliver viral Ags are liposomes [274], biodegradable polymers like chitosan [275,276], protein-based nanocages [277], multilamellar vesicles [278] and virus-like particles [279] (Figure 3B). These NPs are designed to mimic viral entities in order to be easily internalized into APCs, thus favoring the delivery of viral Ags into the cell. Moreover, the presence of these carriers allows the encapsulated Ag to reach the cytosol, which makes them susceptible to proteasome degradation and thus available for loading on MHC-I molecules [274]. Although the underlying mechanism behind this process remains in part elusive, several studies show that the internalized NPs may alter endosome integrity favoring the endosomal escape of NP content into the cytosol [280–283]. Although nano-carriers are adjuvants *per se* capable of triggering DC maturation, the immunogenicity of the particle can be further enhanced by introducing specific adjuvants together with the appropriate

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antigen. Unlike the antibody–Ag approach, in which adjuvant and Ag are introduced separately inside the formulation, in the case of NPs the compounds are bound to the same macromolecules thus improving the immunostimulatory properties of the vaccine [284].

Although DC-targeted therapeutic vaccinations certainly are promising approaches, these methods required long fine-tuning, are expensive and therefore hardly compatible with the timing imposed by the COVID-19 pandemic. In this regard, while current vaccines are not designed to selectively target and interact with DCs, they are proving to be extremely effective. Indeed, both liposome-mediated vaccines like Pfizer (BNT162b2) and Moderna (mRNA-127) and adenoviral vector vaccines like AstraZeneca (Vaxzevria) have a broad tropism and are potentially able to enter into any cell present at the injection site to deliver their cargo through a non-specific mechanism. Although this strategy based on a non-specific delivery of viral antigens/mRNA could seem less effective compared with DCs-targeted vaccine approaches, it represents the fastest, cheapest, and readily scalable method suitable for a widespread vaccination campaign at the moment.

Moreover, these vaccines, in particular mRNA-based vaccines, are a relatively new pharmaceutical drug class that still needs to be tweaked and thus has ample room for improvement.

Therefore, the intriguing future challenge could be to invest on this new strategy, to both improve the capacity of these vaccines to target DCs and to extend their use also in other infections and disease. Encouraging results in this direction are to some extent already been achieved, including the development of both adenovirus-based vector vaccines [285] and RNA-loaded lipid carriers [286] capable of specifically targeting DCs.

Conclusions

The complex interplay between DCs and viruses is the result of a long-standing evolutionary arms race. The role of DCs as both master APCs and tissue sentinels makes them one of the first cell types to encounter viral particles as well as one of the first initiators of the immune response. As such, they are endowed with a vast array of PRRs that recognize many different classes of viral pathogens, with various DC subsets being ulteriorly specialized for various tasks, such as antigen capture, cross-presentation or production of inflammatory cytokines. Given their importance, it is unsurprising that viruses evolved various strategies of immune evasion or impairment of DC function. Indeed, the main viral recognition and downstream pathways are often targeted by viral proteins that aim to inhibit viral PAMP detection, DC activation, viral antigen presentation and cytokine production. Furthermore, some viruses, such as HHVs and HIV make clever use of DCs' PRRs and migratory capacity to promote their own dissemination. It is undeniable that DCs are one of the main actors in viral infections. Further inquiry into DC function and mechanisms can provide novel insights on antiviral immunity and offer new therapeutic approaches, especially for viruses with high social impact that still lack a definitive therapy, such as HIV, or even more so, SARS-CoV-2.

Data Availability

Data are not present in this review article.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ACE, angiotensin-converting enzyme 2; Ag, antigen; ALI, acute lung injury; APC, antigen-presenting cell; BDCA3, blood dendritic cell antigen 3; CADM1, cell adhesion molecule 1; CARD, caspase recruitment domain; CCL, chemokine (C−C motif) ligand; CCR, C−C chemokine receptor; CD, cluster of differentiation; cDC, conventional DC; cGAS, cyclic GMP-AMP synthase; CLEC, C-type lectin domain family; CLR, C-type lectin receptor; COVID-19, coronavirus disease 2019; CSF-1, colony stimulating factor 1; CTL, cytotoxic T lymphocyte; CXCL, chemokine (C−X−C motif) ligand; CXCR, C−X−C chemokine receptor; CYTIP, cytohesin-interacting protein; DAMP, damage-associated molecular pattern; DC-SIGN, DC-specific intercellular adhesion molecule 3-grabbing non-integrin; DC, dendritic cell; EBV, Epstein–Barr Virus; ER, endoplasmic reticulum; HA, hemagglutinin; HCMV, human cytomegalovirus; HHV, human herpes virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus;

IAV, influenza A virus; ICP, infected-cell protein; IDEC, inflammatory dendritic epidermal cell; IFI16, interferon-γ-inducible protein 16; IFN, interferon; IKKβ, IκB kinase β; IL, interleukin; infDC2, inflammatory DC2; IRAK, interleukin receptor-associated kinase; IRF, interferon regulatory factor; IκB, inhibitor of NF-κB; KSHV, Kaposi's sarcoma-associated herpesvirus; L-SIGN, liver/lymph node-specific intercellular adhesion molecule 3-grabbing integrin; LC, Langerhans cell; LGP2, laboratory of genetics and physiology 2; LN, lymph node; MDA5, melanoma differentiation-associated protein 5; MHC, major histocompatibility complex; mo-DC, monocyte-derived DC; MR/CD206, mannose receptor; mtDNA, mitochondrial DNA; MyD88, myeloid differentiation factor 88; NA, nucleic acid; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; NK cell, natural killer cell; NLRP3, NLR family pyrin domain containing 3; NLR, nucleotide-binding oligomerization domain (NOD)-like receptor; NOD, nucleotide oligomerization domain-containing protein; NP, nanoparticle; PAMP, pathogen-associated molecular pattern; pDC, plasmacytoid DC; PRR, pattern recognition receptor; RBD, receptor-binding domain; RIG-I, retinoic acid-inducible gene I; RLR, RIG-I like receptor; S protein, spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; STING, stimulator of interferon genes; Tip-DC, tumor necrosis factor/inducible nitric oxide synthase-producing DC; TIRAP, toll-interleukin 1 receptor domain-containing adapter protein; TLR, Toll-like receptor; TMPRSS, transmembrane protease, serine; TNFα, tumor necrosis factor α; TRAF6, TNF receptor-associated factor 6; TRAIL, TNF-related apoptosis-inducing ligand; XCR1, X–C motif chemokine receptor 1.

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