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HIGHLIGHTS

Basic

REVIEW

Reprogramming dendritic cells through the immunological synapse: A two-way street

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Dendritic cells (DCs) bridge innate and adaptive immunity. Their main function is to present antigens to prime T cells and initiate and shape adaptive responses. Antigen presentation takes place through intimate contacts between the two cells, termed immune synapses (IS). During the formation of IS, information travels towards the T-cell side to induce and tune its activation; but it also travels in reverse via engagement of membrane receptors and within extracellular vesicles transferred to the DC. Such reverse information transfer and its consequences on DC fate have been largely neglected. Here, we review the events and effects of IS-mediated antigen presentation on DCs. In addition, we discuss novel technological advancements that enable monitoring DCs interactions with T lymphocytes, the main effects of DCs undergoing productive IS (postsynaptic DCs, or psDCs), and how reverse information transfer could be harnessed to modulate immune responses for therapeutic intervention.

Keywords: Antigen presentation · Dendritic cells · Immunological synapse · Intercellular communication · Postsynaptic dendritic cells

Introduction

Immune synapses (ISs) established between DCs and T cells during antigen presentation by DCs are essentially communication events. During these events, both cell types come in the close proximity and start to physically interact. Because this process instructs $CD4^+$ T cells to become activated in response to antigens, the cross-talk between T cells and mature DCs is considered the cornerstone of adaptive immunity. These contacts rely on the formation of a stable antigen-dependent supramolecular structure termed "immune synapse" [1]. The molecular events that occur on the T cell and its subsequent activation and fate following IS formation have been studied in much detail [2, 3]. Conversely, the molecular events that occur upon antigen presentation and the fate of DCs following IS formation have been much less documented [4]. Some recent studies have described the events taking place on the DC when it establishes productive IS with T cells and the functional consequences of the formation of the IS, prompting us to provide a critical update on the state of the art of the field. Since this is a very active field, it is also necessary to discuss some of the recent technological advancements and models that allow studying the effects and functional consequences of IS formation on the DC; and how this may be used to modulate immune responses in therapeutic schemes.

Molecular dynamics on the DC side of the immune synapse

The IS of naïve $CD4^+$ T cells with mature DCs occurs at the T-cell region of lymphoid organs. Antigen-loaded, active DCs

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migrate from the periphery into this region guided by chemotactic signals, for example, CCL19/21. In vitro, the formation of IS requires about 30 min [5]. In vivo, it appears that T cells establish brief interactions that do not exceed 30 min within the first 8 h. Although this may suggest that productive IS are not taking place, these interactions lead to upregulation of activation molecules on T cells, indicating that antigen presentation has ensued. However, T cells start secreting IL-2 and IFN-γ sometime after this first wave of contacts [6, 7]. On this second phase, more stable and longer-lasting interactions between T cells and DCs occur [8]. However, after 24 h, T-DC contacts are again shorter lived, coinciding with the initiation of T-cell proliferation [6, 7]. The dynamics of these interactions are shaped by antigen dose [9, 10].

The IS prompts DCs to mirror Tcell molecular dynamics

Stable interactions that lead to IS formation involve the dynamic rearrangement of surface molecules into three Supra Molecular Activation Clusters (SMAC, (c)entral, (p)eripheral, and (d)istal) on the T-cell side. This is internally regulated by the reorganization of cytoskeletal structures and organelles [11, 12]. This has been extensively characterized on the T-cell side. On the DC, a mirrored organization of ligands for the polarized T-cell receptors enables productive binding, also involving polarization for the targeted delivery of signals, EVs, cytokines, and chemokines to the T cell. MHC molecules on DCs form clusters opposite of the CD3/TCR complex that polarize towards the contact on T cells. This is an actin-dependent process [13] boosted by polarization of endosomal compartments containing MHC-II molecules that move along microtubules [14]. Other molecules that relocate towards the IS are ICAM-1 to form bonds with LFA-1 found at the pSMAC of T cells; CD40 [15], CD70, and Plexin A1 (a Rho GTPase) opposite the cSMAC [16, 17]. Actin and phosphoinositide dynamics are essential on both sides of the IS. Disruption of F-actin dynamics on the DC prevents IS formation [18, 19]. Phosphatidylinositol 4,5-bis-phosphate accumulates at DC:T-cell contact site on the DC side [20]. WASP-deficient DCs fail to form stable IS and activate $CD4^+$ T cells [21, 22]. IS formation leads to recruitment of signaling kinases that accumulate at the DC side, indicating that the IS leads to the activation of specific signaling pathways within DCs [16]. Furthermore, the microtubule-organizing centre (MTOC) is polarized in DCs towards the contact site in a similar manner as in T cells. MTOC polarization is needed for the delivery of cytokines such as IL-12 for T-cell differentiation [19, 23]. In general, cytoskeleton remodeling on the DC side contributes to the stabilization of DC:T-cell contacts [18] and fine tunes antigen specificity and the dynamics of co-stimulatory molecules [16, 17, 19] (Fig. 1).

The IS acts as a platform for bidirectional communication between DCs and T cells

The strength of the engagement of membrane receptors during IS together with co-stimulatory signals stemming from cytokines and chemokines secreted by the DC fine tune T-cell activation [1, 2]. On the DC side, membrane receptor engagement also activates downstream signaling cascades. For example, CD40 engagement on the DC induces TRAF6 recruitment and activation of MAPK, JNK, and NF-κB (through the noncanonical pathway [24, 25]), as well as RANK-RANKL signaling [26]. The activation of these signaling cascades results in the production of proinflammatory cytokines and enhanced cell survival [27]. Information can also be exchanged through the IS via extracellular vesicles (EVs). These EVs contain specific molecules [28–30] that act as "signal four" of T-cell activation. A specific example is the transfer of DC telomere fragments within EVs, which promotes T-cell long-term memory [31]. However, EVs are also secreted by the T cell, traveling toward the APC [32]. For example, during the germinal centre reaction, T-cell EVs promote antibody production by contacting B cells [33]. Also, the transfer of T-cell oxidized mitochondrial DNA to DCs induces a cGAS/STING/IRF3-dependent IFN-like response on the receiving DC. This event renders receiving DCs more resistant to viral infection after IS formation [34]. This also occurs through noncontact dependent, paracrine transfer of EVs from other infected DCs residing nearby [35]. EVs from other sources, such as tumor cells, can also be captured by DC, influencing the outcome of cancer [36]. The IS also induces mitophagy and mitochondrial polarization towards the IS on the DC side [37]. A recent study describes a novel type of EVs with a distinct size and cargo, enriched in RNA-binding and ubiquitination-related proteins, and microRNAs. These EVs, named trans-synaptic vesicles (tSVs), are released from T cells in a model of immune synapse formation using supported lipid bilayers [38]. In summary, IS acts as a platform for the bidirectional communication of T cells and DCs, in which DCs receive specific information from the T-cell side that influences their postsynaptic fate and effector function (Fig. 1).

Antigen presentation promotes long-term DC survival

The lack of studies focusing on the DC side and the consequences of the synapse on DCs could be due to the common misconception that the ultimate fate of DCs after antigen presentation at the draining LN was to die and be scavenged *in situ* [16, 39]. This is the true fate of most DCs that arrive to the LN, mainly due to apoptosis or cytotoxic T cell-dependent killing [16, 40]. However, lineage tracing studies have indicated that DCs that migrate to LNs can reside there up to 15 days, after which they remain functional as evidenced by their ability to induce IFN-γ production and proliferation of $CD4^+$ T cells [41, 42]. The IS delivers antiapoptotic signals to DC, promoting their survival independent of antigen specificity [43]. Mechanistically, IS formation leads to the activation of Akt1 and the translocation of transcription factors NF-κB to the nucleus and FOXO1 to the cytoplasm, inhibiting mitochondrial-mediated apoptosis. However, the triggers of these antiapoptotic signals are unclear. It appears that CD40 engagement at the IS induces Akt activation [43, 44]; however, it could also depend on CD40-dependent activation of

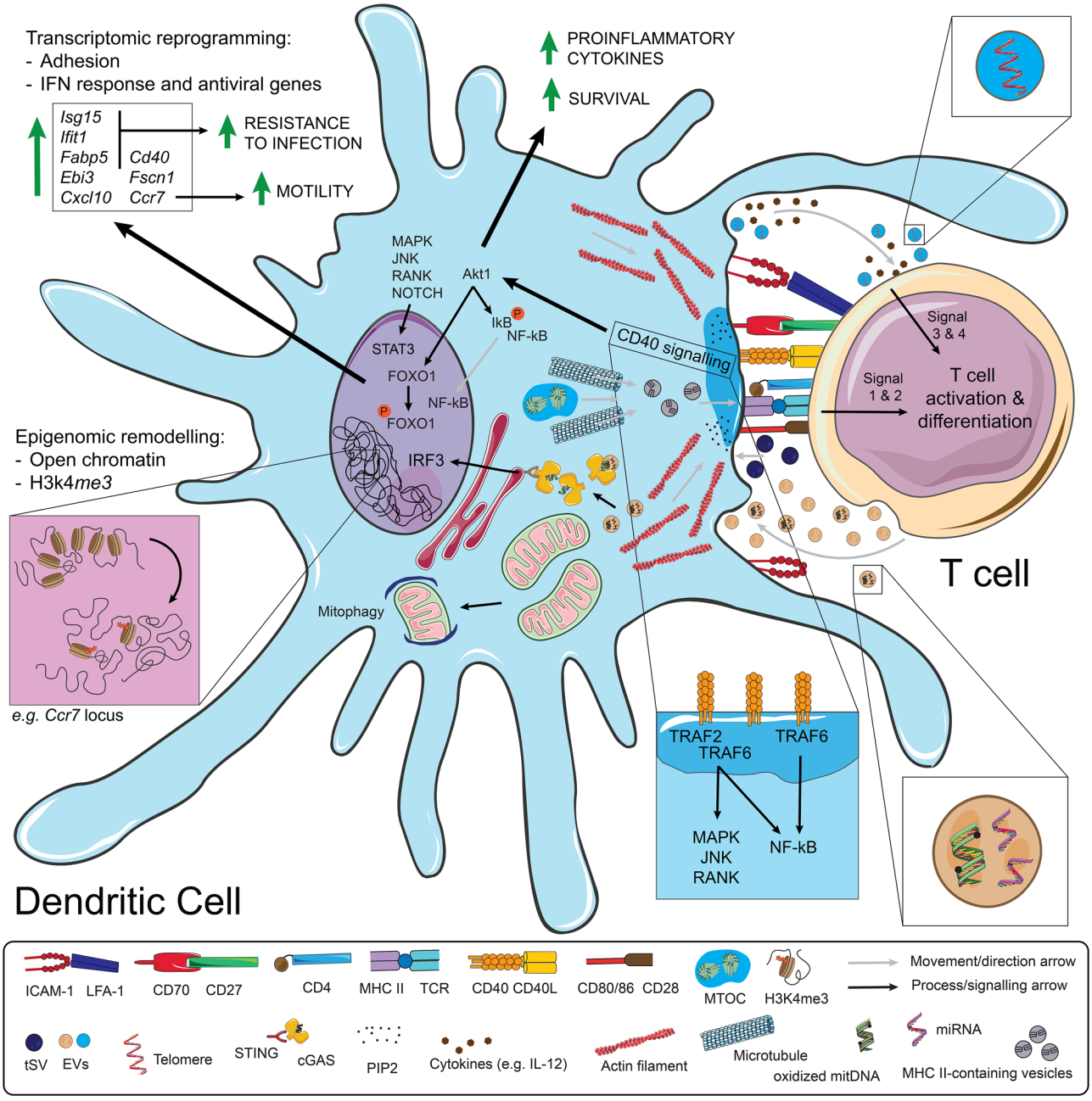

Figure 1. Molecular dynamics on the DC side of the immune synapse. IS formation involving the engagement of diverse membrane receptors at the interface between the DC and T cell. These signals, together with cytokines secreted by the DC side provide the three canonical signals that trigger T-cell activation and differentiation, together with other players such as EVs containing telomeres. At the T:DC interface, EVs containing miRNAs, oxidized mitDNA, and/or tSVs are transferred from T cells to the DC. These induce cGAS-STING signaling. On the DC side, the MTOC, actin and microtubule cytoskeletons polarize towards the IS, and mitophagy is induced. Within the nucleus, epigenetic remodeling includes enhanced chromatin accessibility and H3k4 trimethylation. Engagement of surface molecules on the DC side of the IS triggers CD40 signaling. These signals induce the transcription of specific genes, leading to enhanced survival, secretion of proinflammatory cytokines, increased motility, and resistance to infection.

RANK-RANKL [27, 45], or Notch-dependent induction of STAT3 [16, 46]. Finally, the fact that DCs can develop a memory-like phenotype following immunization [47] also indicates that post-IS DCs are not immediately cleared from lymphoid tissues, remaining there to perhaps enhance other immune processes (Fig. 1).

DC reprogramming through synaptic interactions: the "postsynaptic" DC paradigm

The data discussed in the previous section serve as the conceptual framework for branding DCs that have undergone antigen

presentation as "postsynaptic" DCs (psDCs). In this section, we discuss the methodological advances that underlie those studies and support future studies addressing the possible applications of psDCs in therapy.

In vitro model to study psDCs

psDCs can be generated in vitro by co-culturing BM-derived DCs (BMDCs) with $CD4^+$ T cells bearing a transgenic TCR (OT-II) specific for $OVA_{323-339}$ in the context of the MHC class II molecule $I\text{-}A^d$ [48]. This system is used to prove that IS formation induces transcriptomic reprogramming of psDCs. Genes upregulated included *Ccr7, Ifit1, Isg15, Tlr3, Fscn1, Cd40, Ebi3, Il12b, Ccl22*, or *Cxcl10* (Table 1). These changes are exclusively dependent on the interaction of DC with T cells in an antigen-dependent manner. Importantly, these changes are robust as they ensued when DC contacted with $CD4^+$ or $CD8^+$ T-cell bearing OT-II or OT-I TCRs, respectively. However, the extent of the upregulation is dependent on whether the interactions takes place with $CD4^+$ or $CD8^+$ T cells. The upregulated genes are involved in triggering an "alert" state that induce an antipathogen state against viral infection similar to that induced by T-cell-derived EVs [34]. Furthermore, the transcriptomic signature of psDCs reflects the acquisition of a memory-like phenotype that correlated with enhanced chromatin accessibility around the localization of the genes upregulated and increased trimethylation of H3K4 [48]. However, whether epigenomic remodeling is sustained in time remains unknown. Functionally, upregulation of *Ccr7*, increased chromatin accessibility at the promoter region of *Ccr7*, and increased H3K4*me3* of the *Ccr7* gene correlate with increased CCL19-guided migratory ability of psDCs (Fig. 1). psDCs migrate faster and with enhanced directionality toward CCL19 in vitro and reach draining LNs more efficiently when injected in vivo [48]. This model indicates a functional reprogramming of psDCs and suggested that DCs may acquire novel functional capabilities following IS formation. For instance, other genes upregulated include *Fscn1*, which enhances antigen presentation [49, 50]; *Apol7c*, which may be related to the increased survival of psDCs [51]; or *Dll4*, involved in Th1, Th17, and $CD8^+$ T-cell priming [52]. No other study to date has addressed the effect of psDC functional reprogramming beyond their motility.

In vivo models to study psDCs

One of the major challenges of the in vitro system is that, although it compares two experimental conditions in which no DCs have interacted in the absence of antigen versus DCs that have performed antigen presentation in the presence of antigen, psDCs cannot be identified specifically. This has been resolved using two novel approaches. On the one hand, stably interacting DC:T-cell pairs could be isolated prior to single-cell RNA sequencing of T:DC pairs [53]. As two or more cells of two different lineages are being sequenced as one, novel mathematical methods were developed to deconvolute the gene signatures and assign the observed gene changes to the DC or the T cell [54]. This technique, termed "sequencing of physically interacting cells" (PIC-seq) has not only allowed identifying which T cells preferentially interact with DCs in different contexts [55], but also recording the transcriptomic changes of interacting DCs in vitro or in vivo in draining LNs from immunized mice (Table 1).

On the other hand, a novel technique based in the intercellular labeling of antigen-dependent interactions termed "Labeling Immune Partnerships by SorTagging Intercellular Contacts" (LIPSTIC) employs an engineered bacterial transpeptidase Sortase A (SrtA) with decreased affinity that enables proximity-based labeling restricted to the distance range of the immune synapse [56, 57]. Although this technique can be used to study a variety of ligand-receptor interactions, it was specifically engineered and characterized in the context of DC:T-cell interactions. In this system, the CD40L molecule is fused to SrtA ($Cd40lg^{SrtA}$) and the CD40 surface molecule on DCs is fused to a polyglycine chain ($Cd40^{G5}$) that increases its efficacy as a peptide receptor. In the presence of a biotinylated substrate peptide for SrtA during DC:T-cell interactions, the SrtA covalently transfers the peptide to the CD40 molecule on the DC, which becomes biotinylated. It is important to underline that CD40L-SrtA molecules need to be upregulated and/or mobilized towards the synapse, something that only occurs late after IS formation, rendering this approach limited to antigen-initiated interactions. Also, nonspecific antigen-dependent CD40-CD40L interactions and labeling may take place at longer timepoints [56]. This system allows tracking DC:T-cell interactions *ex vivo* and in vivo [58], and was used to demonstrate that antigen-dependent interactions induce a specific activation state on interacting DCs that is essential for immune control of tumor progression [59]. It was also used to show that specific subsets of DCs underlie food tolerance and these populations become deregulated during gastrointestinal infection [60]. Of note, many of the genes identified using the LIPSTIC and PIC-seq models coincide with those identified in the in vitro BMDC model. These include *Cd40, Il12b, Ebi3, Ccl22, Dll4, Fscn1, or Cxcl10* (Table 1). This strongly supports the robustness of these events, as they remain consistent across models (Fig. 2). An interpretative caveat is that despite the disparity of the models, they all rely on the presence of OT-II $CD4^+$ T cells as the interacting partner of the DC [43, 48, 56, 59–61]. To increase the degree of generality and robustness, it would be important to determine whether postsynaptic reprogramming occurs using "natural" $CD4^+$ T-cell partners, that is, T cells bearing TCRs of various affinities for a wider array of peptide-loaded MHC-II molecules. Novel techniques, for example, universal LIPSTIC technology [62] and PIC-seq [54] may allow ascertaining the effect of polyclonal synaptic interactions on psDCs. Giladi and co-workers used PIC-seq to show that the genes upregulated in psDCs upon immunization with the helminth *Nippostrongylus brasiliensis*, which include *Fscn1* or *Ccl22* [54], may be conserved regardless of the clonality of the interaction. In this regard, the interacting DC population in the tumor microenvironment ("mature DCs enriched in immunoregulatory molecules" or mregDCs), display

Table 1. Summary of postsynaptic DC models.

References	DC population	Main upregulated genes	Immune synapse formation	Sequencing technique	Interacting T cell population	Immunization	DC source	Antigen presented
Alcaraz-Serna et al. [48]	Bone marrow-derived DCs (antigen-loaded DCs vs not loaded DCs)	*Ccr7*, *Tlr3*, *Fscn1*, *Cd40*, *Isg15*, *Ifit1*, *Cxcl10*, *Ebi3*, *Dll4*, *Il12b*, *Ccl22*, *Irf1*, *Gbp5*, *Irf4*, *Ccl17*, *Apol7c*	Yes, unknown how many of the fraction	RNA-seq	OT-II $CD4^+$ T cells	Antigen presentation in vitro	Bone marrow-derived DCs	$OVA_{323-339}$
Giladi et al. [54]	Phisically interacting cells in vitro	*Tmem123*, *Fscn1*, *Ccl22*, *Il12b*, *Cd40*, *Dll4*, *Il1rn*, *Bcl2l14*, *Cst3*, *Csf2rb2*, *Csf2rb*	Yes, all cells	PIC-seq	OT-II $CD4^+$ T cells	Antigen presentation in vitro	Splenic DCs	$OVA_{323-339}$
Giladi et al. [54]	Migratory phisically-interacting DCs in vivo	*Tmem123*, *Fscn1*, *Ccl22*, *Il12b*, *Cd40*, *Ccl17*, *Ebi3*, *Dll4*			Endogenous T cells	Intradermal ear immunization with inactivated *Nippostrongylus brasiliensis*	Draining auricular lymph nodes	Antigens from *Nippostrongylus brasiliensis*
Pasqual et al. [56]	Biotin$^+$ DCs (biotin$^+$ vs biotin$^-$ DCs)	*Apol7c*, *Serpin6b*, *Ctsb*, *Gm8221*, *Tmem39a*, *Ccl22*, *Fscn1*, *Dll4*, *Mab21l3*, *Cryaa*, *Uaca*, *Cd40*, *Rnf19a*, *H2-M2*, *Acpp*, *Fblim*	Yes, all cells	RNA-seq	Adoptively transferred $Cd40lg^{SrtA/Y}$ CD4-Cre OT-II $CD4^+$ T cells	Footpad immunization with DCs loaded with $OVA_{323-339}$	Draining popliteal lymph nodes	$OVA_{323-339}$
Curato et al. [61]	Nanoparticle-carrying DCs (OVA/CpG-NP vs CpG-NP)	*Ccr7*, *Cd274*, *Cd40*, *Cxcl10*, *Il12b*, *Irf1*, *Irf7*, *Irf8*, *Stat1*, *Stat3*, *Isg15*	Unknown	RNA-seq	Adoptively transferred OT-II $CD4^+$ and OT-I $CD8^+$ T cells	Hock immunization with OVA/CpG nanoparticles	Draining inguinal and popliteal lymph nodes	$OVA_{323-339}$ and $OVA_{257-264}$
Blecher-Gonen et al. [64]	Migratory DCs	*Ccr7*, *Fscn1*, *Apol7c*, *Ccl22*, *Nudt17*, *Ccl17*, *Cacnb3*	Unknown	Massively parallel scRNA-seq	Endogenous T cells	Intradermal ear immunization with inactivated *Mycobacterium segmentatis*, *Nippostrongylus brasiliensis*, or *Candida albicans*	Draining auricular lymph nodes	Antigens from *Mycobacterium segmentatis*, *Nippostrongylus brasiliensis*, or *Candida albicans*
Blecher-Gonen et al. [64]	Antigen-positive DCs (cluster 1 vs cluster 2)	*Ebi3*, *Dll4*, *Cd40*, *Sem7a*, *Cd200*, *Ccl9*, *Ccl17*, *Ccl22*						

Table summarizing the in vitro and in vivo models of postsynaptic DCs, the DC populations studied, genes up and downregulated, and experimental conditions underlying data generation.

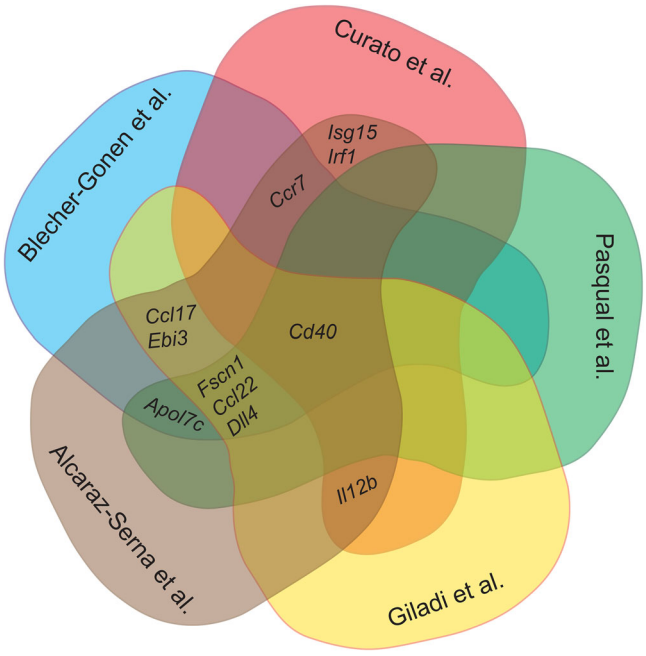

Figure 2. Gene signatures shared across postsynaptic DC models. Venn diagram of upregulated genes obtained from the indicated references ([48, 54, 56, 61, 64]) and depicted in Table 1. Genes shared between two or more datasets are indicated, with *Cd40* appearing in all datasets.

an upregulated gene signature that includes postsynaptic genes such as *Fscn1, Ccr7, Ccl22*, and *Ebi3* [55]. Together, these observations indicate that the postsynaptic signature may be conserved irrespective of the triggering antigen, although the strength of the TCR:MHCII interaction may modulate it. This is of particular importance when designing vaccines and antigen-specific immunotherapies, bringing up a novel and crucial factor that needs to be considered during antigen selection.

Finally, other systems that may be used for studying interacting DC:T cells in vivo include intravital imaging, histocytometry, and *in situ* photoactivation [63].

Indirect evidence of postsynaptic reprogramming

Despite the development of models that enable the study of interacting DCs to generate psDC populations, other approaches provided indirect evidence of psDCs reprogramming. For instance, comparison of DCs from LNs from animals immunized with OVA versus an irrelevant antigen in the presence of OT-II T cells revealed that most DCs increased expression of genes such as CD40 (Table 1). This indicates that the observed changes may be due to interactions produced during the IS [61]. Many of the upregulated genes are the same as those observed in psDCs [48, 54, 56] such as *Isg15, Ccr7, Cxcl10*, or *Il12b* (Fig. 2). However, they could not be directly attributed to psDCs, as interacting DC populations were not isolated. Hence, these changes could be produced in noninteracting DCs localized in the same anatomical region. Other studies have performed single-cell analysis of antigen-bearing DCs upon immunization. Interestingly, they also reported upregulation of genes such as *Ccr7, Fcsn1, Ccl22*, or *Ccl17* in migratory DCs [64] (Table 1 and Fig. 2). If these observations are put in context together with the studies that used LIPSTIC or PIC-seq, it seems clear that a growing body of evidence shows that IS formation induces psDC reprogramming.

Perspectives on the applications of postsynaptic reprogramming

The description of psDCs as a novel population that acquires specific transcriptomic and epigenomic traits raises the question of their physiological role in immune responses. It also prompts researchers to interrogate their usefulness in therapeutic or prophylactic settings. Future studies will surely explore the functional role of psDCs as well as the molecular mechanisms governing their generation and function. The current state of the art indicates that psDCs are somewhat protected from apoptosis [43], suggesting that psDCs may play additional immune functions after antigen presentation to $CD4^+$ T cells that can be harnessed therapeutically. Increased psDC survival may enable them to interact with other T cells, promoting their activation and reinforcing the ongoing adaptive response. The enhanced ability of psDC to migrate in a CCR7-dependent manner agrees with this possibility [48]. Indirect evidence suggests that influenza infection increases the conventional DC population in mediastinal LNs, promoting effector T-cell generation. However, whether these DCs are mostly psDCs is currently unknown [65].

Epigenomic remodeling and the activation of transcription networks related to antiviral responses and IFN networks [34, 48] indicate that psDCs may display enhanced innate functions. This is supported by the description of a DC subpopulation that develops a memory-like phenotype in a model of protective vaccination against *Cryptococcus neoformans*, an opportunistic pathogen that triggers pneumonia in immunocompromised individuals. Memory-like DC generation seems to be based on cytokine recall responses upon subsequent pathogen challenge and is abrogated by inhibition of histone modifications [47], in line with the role of epigenetic and metabolic remodeling as part of immune training beyond T cells [66, 67]. Such a DC population could be trained using antigen-cognate interactions, meriting further investigation. Also, whether psDCs reprogramming requires a metabolic switch similar to that seen in other innate trained cell population merits further research.

Postsynaptic reprogramming could be useful during immunization, as innate training confers heterologous protection [68–70] from other diseases [71, 72] due to the nonspecific trained immunity-based effect of vaccines [73, 74]. Indeed, this effect could also increase the efficacy of existing vaccines [75], or bring forth a novel ground-breaking generation of trained immunity-based vaccines to fight infection [76–80] or allergy [81]. The underlying rationale would be that psDC could enhance innate and adaptive responses. Trained DC subsets have been described in protective vaccination prime-boost schedules in macaques

using MVA [82]. Of note, it would be also interesting to assess whether psDCs could increase the efficacy of existing DC-based vaccines [83, 84]. A major hurdle is to generate psDCs using human cells, as prior research has been restricted to genetically engineered murine systems. Trained DCs could be obtained from mixed lymphocyte reactions with T cells from another donor, or with T cells enriched and restimulated with common peptides, for example, those from EBV, CMV, rotavirus, etc. [85–88]. Alternatively, human T cells can be modified to carry specific TCR, similar to CAR-T [89], or antigen presentation can be reproduced with cell-size lipid vesicles [90] or lipid bilayers [38, 91–93]. These contexts likely provide a better grip on the experimental conditions, enhancing reproducibility that is essential in clinical protocols including state-of-the-art isolation and purification of the resulting populations.

Conclusions

The DC side of the IS has been largely overlooked. However, it is now clear that major events with physiological consequences also take place on that side. DCs undergo major cytoskeleton remodeling that mirrors the classically described events on the T-cell side, which include the activation of signaling cascades via engagement of membrane receptors. The IS is no longer considered a one-way street from the DC to the T cell. Reverse communication via receptor engagement and EV transfer decisively shape DC fate. The development of novel approaches, such as in vitro BMDC systems, the LIPSTIC mice, and PIC-seq, has allowed tracking and studying the effects of IS formation on DCs both in vitro and in vivo during immunization. psDCs display a remarkably consistent signature of gene upregulation following antigen presentation associated to epigenomic remodeling. However, the functional consequences on psDCs still remain to be elucidated. Importantly, the contribution of these functionally reprogrammed DC population towards immune responses and the molecular mechanisms that govern psDC reprogramming could be a crucial steppingstone for the design of novel approaches that promote innate and adaptive immune responses, thus, improving DC-based therapies and providing a novel rationale for the design of more efficient vaccination technologies.

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Abbreviations: **BMDCs**: bone marrow-derived DCs · **EVs**: extracellular vesicles · **IS**: immunological synapse · **LIPSTIC**: Labeling Immune Partnerships by SorTagging Intercellular Contacts · **MTOC**: microtubule-organizing centre · **PIC-seq**: sequencing of physically interacting cells · **psDCs**: postsynaptic DCs · **SrtA**: sortase A · **SMAC**: SupraMolecular Activation Cluster · **tSVs**: trans-synaptic vesicles

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