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Antigen presentation by dendritic cells for B cell activation

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B cells are efficiently activated by antigens presented on cell membranes, which provide opportunity for receptor cross-linking and antigen capture. The two main cell types implicated in native antigen presentation to B cells are follicular dendritic cells (FDC), which reside in B cell follicles, and CD169$^+$ macrophages, which line the antigen-exposed surfaces of these follicles in both the lymph nodes and the spleen. There is mounting evidence, however, that conventional dendritic cells (cDC) can also participate in native antigen presentation to B cells. This underappreciated role, largely hidden by the simultaneous need for cDC to participate in T cells priming, appears to be primarily mediated by the type 2 subset of cDC (cDC2), but may also be a function of cDC1. Better understanding of how cDC participate in B cell priming is likely to improve our capacity to develop effective humoral vaccines.

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Introduction

Antibodies are the hallmarks of humoral immunity, acting via various heavy chain isotypes to provide a plethora of immune effector functions, from simple neutralization of pathogen-infectivity, to opsonization of microbes for destruction by cell-mediated or complement-mediated processes. To tailor effective antibody responses, germinal center (GC) reactions are evoked upon initial pathogen encounter, promoting B cell somatic hypermutation and isotype switching, eventually yielding long-term, high affinity, antibody-producing plasma cells of the appropriate isotype. Because of their potential to cause pathology to the host, antibody production must be carefully regulated, especially during GC reactions, where somatic hypermutation has the capacity to generate new specificities, potentially against self. One important control mechanism here is the B cells requirement for T cell help. Because T cells are rendered tolerant to self during thymic selection, specificities for self are rare and, as a consequence, so is help for B cells that mutate to autoreactive specificities.

To receive CD4$^+$ T cell help, B cells must process and present antigen on MHC II. B cell-mediated antigen presentation is also important for the subsequent differentiation of helper T cells into the T follicular helper lineage (Tfh cells), which are the crucial subset that provide help within GCs. Upon antigen recognition on B cells, Tfh cells deliver various signals including the soluble factors IL-21 and IL-4, and membrane-bound signals such as CD40 ligand, which aid B cell survival, proliferation, and differentiation [1]. Tfh cells must also express the appropriate chemokine receptors, such as CXCR5, for localization within B cell follicles where they provide this help. Eliciting Tfh cell responses is complex but essentially proceeds in two steps: first, naïve T cells are activated by cDC, which present their cognate antigen in the context of MHC II and provide costimulatory signals through receptors on T cells such as CD28 and ICOS. Then, these pre-Tfh cells migrate to the T–B border, where they recognize antigen presented by antigen-specific B cells, leading to full Tfh maturation. Thus, for T-dependent B cell responses, T cells are required to recognize antigen first on DC, then on B cells. As a consequence, preventing DC-driven activation of CD4$^+$ T cells, effectively blocks T-dependent humoral immunity. This critical requirement for DC in the priming of T cells has largely hidden another potentially important role for DC in promoting humoral immunity, namely, the direct presentation of intact antigen to B cells. This underappreciated function is receiving growing recognition and will be discussed in this short review.

Membrane antigens activate B cells more effectively than soluble antigens

To appreciate the potential importance of DC in presentation of antigen to B cells, it is first worth revisiting

some old studies that show membrane-bound antigens are more effective than soluble antigens at eliciting B cell responses [2–4]. In studies examining transgenic B cell tolerance to H-2K antigens encountered in the periphery by mature B cells, membrane-bound antigen efficiently deleted antigen-specific B cells whereas soluble monovalent antigen was ineffective [2,5]. Similarly, while membrane-bound hen egg lysozyme (HEL) effectively deleted developing B cells, soluble HEL was only able to induce anergy, presumably because of its weaker cross-linking capacity [3]. Examination of the uptake of various forms of HEL by B cells revealed that B cell recognition of membrane-bound HEL led to formation of an immunological synapse that resulted in efficient acquisition of antigen [4]. Importantly, in this system, membrane-bound HEL was shown to be several orders of magnitude more effective than its soluble counterpart at inducing B cell activation. Thus, membrane-bound antigens provide a strong cross-linking signal that efficiently facilitates both B cell activation and antigen acquisition.

It is interesting to speculate as to why membrane-bound antigens have evolved as the preferred antigen source for B cells. This may be because B cells are normally bathed in a sea of soluble self-antigens that are best ignored to minimize autoimmunity. Alternatively, it may relate to a need to concentrate antigens for efficient B cell recognition, with membrane association providing the platform for concentration. Whatever the reason, membrane-tethered pathogen-derived antigens appear to be superior at evoking humoral immunity. Membrane-bound antigens may naturally exist within the pathogen membranes, providing the desired multi-arrayed membrane-associated context important for B cell activation. Alternatively, pathogen-derived antigen can be captured and concentrated on the surface of host cells via receptors, for presentation to B cells. This latter context appears to be a common mechanism associated with induction of humoral immunity.

The many ways to provide Ag in a membrane-associated form

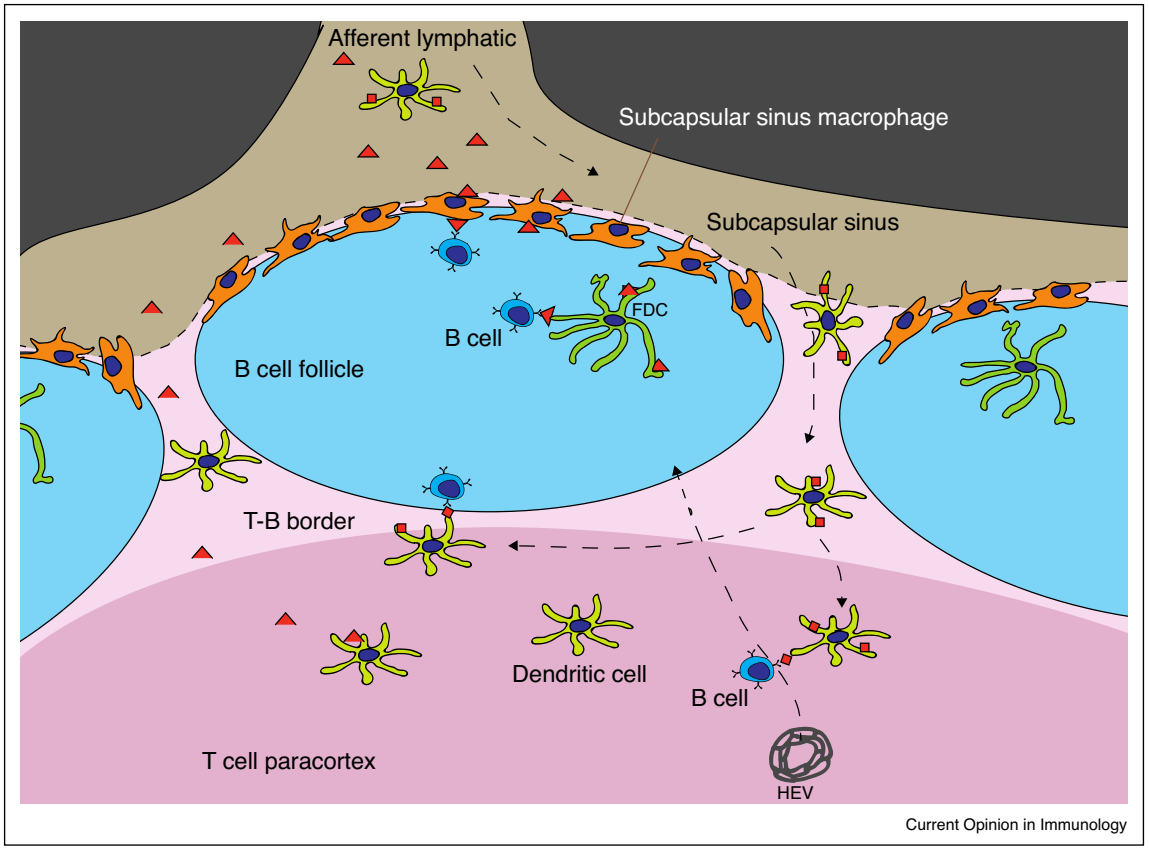
Given the superior capacity of membrane-associated antigens to activate B cells, there are numerous mechanisms that enable tethering of pathogen-associated antigens to membranes for presentation to B cells. A variety of complement receptor (CR1, CR2, CR3 (CD11b-CD18), and CR4 (CD11c-CD18)), expressed by the cell types implicated in presentation of native antigen to B cells, serve to capture and display complement-opsonized antigens or microbes. Pathogens can fix complement directly via the alternative pathway or indirectly by engaging the classical or lectin pathways [6,7]. Carbohydrates on the pathogen surface can bind mannose-binding lectin, ficolin or pentraxins for activation of the lectin pathway, whereas binding of pre-existing antibodies or polyreactive (natural) antibodies initiates the classical pathway. Antibody-coated antigens can also be bound by Fc receptors (FcR), which may lead to phagocytoses, but those captured by FcγRIIb are trafficked through non-degradative intracellular compartments and recycled as native antigens to the cell surface where they can be displayed to B cells [8•]. While the capture and display of opsonized antigen is well established, other mechanisms are also operative. For example, the capture of vesicular stomatitis virus (VSV) in the lymph node is independent of C3 complement or secreted immunoglobulin [9•]. Although the precise mechanism of VSV capture is unknown, an array of cell surface receptors bind microbial components and may contribute to their capture and presentation [10,11]. In this vein, for UV-inactivated influenza virus, the C-type lectin SIGNR1 receptor was shown to be involved in the capture of this virus and its presentation to B cells [12•]. In humans, the closely related C-type lectin, DC-SIGN has been shown to interact with multiple pathogens [13], including HIV, where it is involved in internalization of intact virus and its recycling to the cell surface [14].

In summary, an array of receptors expressed on various cell types may be involved in the capture of opsonized and non-opsonized microbes and their antigens for the presentation to B cells. In this review, we will restrict discussion to primary B cell responses, with a focus on the requirements for initial antigen recognition before GC formation, rather than to downstream affinity selection or secondary responses.

Antigen presentation by FDC and macrophages for initiating B cell activation

There are two main cell types implicated in presentation of antigen to B cells during primary responses: macrophages and FDC (Figure 1). Antigens entering the lymph nodes from the afferent lymphatics first access the subcapsular sinus (SCS) and if sufficiently small (<70 KD) can drain through conduits that supply interfollicular, medullary [15] or follicular [16] regions, or they can diffuse directly into the follicles through gaps in the floor of the SCS [17,18]. Larger antigens are captured by macrophages [19,20•,21•] and cDC [22] within the subcapsular sinus (SCS). $CD169^+$ SCS macrophages, which line the follicle-proximal side of the SCS, have been shown to capture immune complexes, particulate antigens and viruses [19,20•,21•]. $CD169^+$ SCS macrophages, unlike macrophages found in the medullary sinuses, are poorly endocytic, express only low levels of lysosomal enzymes, and, therefore, possess only limited degradative capacity [23]. Thus, instead of degrading antigens, SCS macrophages display these antigens to both cognate and non-cognate B cells via cellular protrusions that extend into follicles; under inflammatory conditions SCS macrophages may even move into the follicles [19,24]. Antigen-recognition on SCS macrophages by cognate B cells leads to B cell activation and migration to the T–B border in the pursuit of T cell help. Alternatively, non-cognate B cells can

Figure 1



Potential pathways for membrane-associated presentation of native antigen to B cells.
Antigen enters the lymph node either as free antigen (red triangles) or associated with migratory cDC (red squares). Free particulate antigens can be captured by subcapsular sinus macrophages and either presented to specific B cells within the follicles or shuttled via non-cognate B cells to FDC for presentation. Particulate antigens may also be captured by lymph node-resident cDC in the subcapsular sinus and transported into the paracortex. Though not formally demonstrated, these cDC have the potential to present to B cells either at the T–B border or as B cells enter the lymph node via HEV. Small, soluble antigens can directly drain into the lymph node for access by resident cDC, macrophages or FDC, which may present them to specific B cells. Migratory cDC can also carry antigens into the lymph node and present these antigens to specific B cells at the T–B border or as they egress from HEV to migrate into the follicles.

acquire immune complexes from CD169$^+$ SCS macrophages using CR1/2, then ferry these complexes into the follicle for handover to FDC [20•]. The capacity of FDC to retain native antigen for prolonged presentation to B cells is well documented and known to play a critical role in the GC reaction, driving GC-mediated somatic hypermutation and affinity maturation for effector and memory formation. However, antigen displayed by FDC within the follicle can also become the initial source of antigen recognized by cognate B cells, particularly when the B cells arrive in the follicle after antigen has already been acquired by FDC [25]. FDC efficiently capture complement opsonized antigens via CR1/2 [20•], and antibody-opsonized antigen via receptors such as FcγRIIb [26], but their role in the display of non-opsonized antigen is unclear. While non-cognate B cells that capture immune complexes from SCS macrophages can deliver antigen to FDC, these non-cognate B cells can also potentially present antigen to cognate B cells, during their migration through the follicle. Importantly, SCS macrophages and FDC are cell types shown to present membrane-bound antigen for initial recognition by cognate naïve B cells.

Like the lymph nodes, the spleen contains B cell follicles capable of generating humoral immunity. These follicles make up a part of the white pulp, which is bordered by the marginal zone. In the mouse, arterial blood enters the spleen via terminal arterioles that either release into the marginal sinus before blood flows slowly into the red pulp, or they release directly into the red pulp, blood later exiting the spleen via the venous system. As such, the marginal zone acts as one initial entry point into the spleen, filtering antigenic material

from the blood. The marginal zone is occupied by several cell types, including two subsets of macrophages, as well as stromal cells, cDC and marginal zone B cells (MZ B cells). Marginal zone metallophilic (MM) macrophages are a population of $CD169^+$ splenic macrophages similar in phenotype to SCS macrophages of the lymph node [27]. They line the marginal sinus adjacent to the white pulp, abutting B cell follicles. MM macrophages can capture various forms of antigen during immune responses and contribute to T-dependent humoral responses to some particulate antigens [28–30]. However, their direct provision of antigen to specific B cells, as seen for SCS macrophages in lymph nodes, while likely, has yet to be demonstrated. The outer marginal zone is occupied by marginal zone macrophages (MZ macrophages) and by MZ B cells, the latter a unique spleen-resident subset of B cells that migrates back and forth between the marginal zone and the B cell follicles, depositing opsonized antigens from the blood onto FDC within B cell follicles [31–33,34•]. The close association between MZ B cells and MZ macrophage may help facilitate this process.

Interestingly, despite the apparent capacity of various macrophage populations to provide antigen for B cell responses, their depletion has only a limited effect on T-dependent humoral immunity [12•,28,29,35–37], sometimes even resulting in enhancement [38]. One study that did suggest SCS macrophages play a crucial role in antigen presentation, showed that inflammatory signals associated with infections disrupted the SCS macrophage niche, coinciding with impaired humoral responses to newly encountered antigens [24]. However, inflammatory signals can also downregulate phagocytosis by DC and thus impair their ability to capture new antigens and stimulate T cell help [39], raising an alternative (macrophage-independent) explanation for impaired T-dependent humoral immunity post-inflammation. While an important role for $CD169^+$ macrophages in humoral immunity was also supported by reduced B cell responses when these macrophages were depleted by chlodronate or diphtheria toxin (DT) (for mice expressing the DT receptor in $CD169^+$ cells) [24], these treatments may also affect DC [40] and other cell types [41], leaving open alternative explanations. Overall, most studies suggest that macrophage-dependent pathways of B cell activation are not essential, and that significant redundancy must exist. While FDC play an important role in displaying opsonized antigen, their capacity to handle non-opsonized antigen for initial B cell activation is questionable. Thus, both the redundancy of macrophages in the presentation of antigen and the unmet need of presenting non-opsonized antigens by FDC, suggests that other cell types play a significant role in antigen presentation to B cells. One major candidate for this antigen-presenting role are DC.

The role of DC in presentation of antigen to B cells

DC can be divided into conventional DC (cDC), monocyte-derived DC and plasmacytoid DC, but it is the cDC that are largely responsible for initiating cellular and humoral responses [42–44]. Conventional DC can be further subdivided into cDC1 and cDC2, with the lymph nodes containing both migratory and resident counterparts of each population. There are finer intricacies to these divisions, but for the purpose of this review, these divisions will suffice.

As mentioned earlier, the role of cDC in the presentation of native antigen to B cells in T-dependent antibody responses is difficult to elucidate due to their essential role in priming T cells. However, a variety of studies support a role for DC in this process. DC were first implicated in the presentation of native antigen to B cells in studies that showed they could retain native antigen and, upon adoptive transfer, induce antibody responses [8•,45,46•,47••,48–50]. DC exposed to immune complexes, for example, were shown to capture antigen via the inhibitory Fc receptor, FcγRIIB, and retain this antigen in non-degradative compartments for recycling to the cell surface [8•]. Such DC pulsed with immune complexes were able to prime T-independent B cell responses in a FcγRIIB-dependent manner, implicating direct antigen-presentation to B cells, though antigen handover to other cell types cannot be formally excluded. In one of the earliest studies [47••], naïve B cells were cultured *in vitro* with antigen-pulsed DC to show a requirement for B cell-DC contact in the induction of isotype-switched antibody responses, implicating B cell recognition of antigen on DC. Similarly, immature DC from blood were shown to capture intravenously injected *Streptococcus pneumoniae* and present this *in vitro* to specific B cells [49]. *In vivo*, these immature DC trafficked to the marginal zone, where they associated with B cells and initiated T-independent plasmablast development.

Clear evidence for direct recognition of antigen on DC by specific B cells was formally obtained when transgenic B cells were visualized interacting with antigen-pulsed DC *in vitro* [51•] and *in vivo* [52••]. These studies used HEL as a model antigen and showed DC captured this soluble form of antigen and were able to display it on their surface, possibly via electrostatic interactions [52••]. While *in vitro* imaging of B cell-DC interactions in collagen matrices showed prolonged associations between antigen-specific B cells and antigen-bearing DC, implicating antigen presentation [51•], conclusions could be questioned for *in vivo* relevance. *In vivo* imaging [52••] was key to demonstrating that B cells entering the lymph node were able to recognize antigen directly on adoptively transferred antigen-pulsed DC, forming B cell-DC contacts within regions surrounding high endothelial venules (Figure 1). Antigen-specific recognition led to prolonged DC–B cell interactions, calcium fluxes in specific B cells, and upregulation of

CD86 and CCR7 on B cells. These contacts enabled B cells to acquire antigen and become activated, initiating their migration and antigen-presentation programs. This led to localization of activated, antigen-bearing B cells at the T–B border where they could present processed antigen to helper T cells. While this landmark study provided novel insight into B cell–DC interactions *in vivo*, the use of adoptively transferred DC left open some question of relevance to antigen presentation *in situ* when DC obtain antigen naturally. In support of these findings, however, DC are found naturally in regions adjacent to HEV [53], amenable to B cell surveillance.

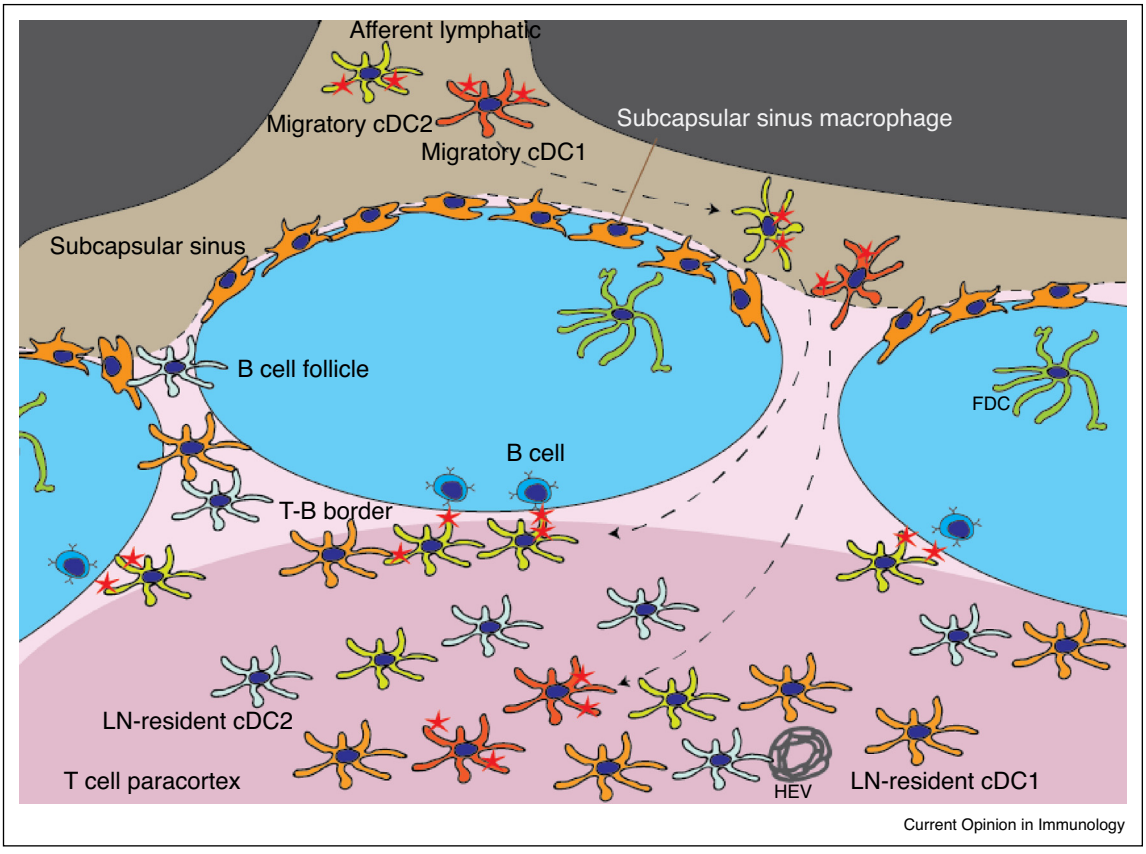
One approach to examine DC presentation of antigen to B cells *in situ* was to target the B cell antigens NP or HEL to DC via linkage to an antibody specific for DCIR2 expressed on cDC2 [54••]. Transgenic B cells specific for NP or HEL were activated within a few hours of targeting-antibody injection, in a manner that did not require T cells, MHC II or CD40, but did require DC. Specific B cells initially accumulated in the bridging channels of the spleen, an area where DCIR2$^+$ cDC2 normally reside [55,56,57••]. B cell activation depended on antigen targeting, as isotype control antibodies bearing the same antigens were not effective. These findings implied direct presentation of DC-targeted antigen to B cells. Antigen targeting did not lead to GC formation, however, unless appropriate adjuvants were co-administered, suggesting DC and/or B cells required additional stimuli (together with targeted antigen) for full elicitation of humoral immunity. Interestingly, B cells moved from the bridging channels to the T–B border soon after activation, presumably searching for T cell help. One possible role for adjuvant, not considered at the time, is to induce DC upregulation of CCR7 and EBI2, enabling these DC to migrate with B cells and localize at the T–B border [57••]. We speculate that this co-localization of antigen-bearing DC with activated B cells may be important to maintain DC-supplied antigen during T–B collaboration. Importantly, however, cDC2 can present antigen to B cells *in situ*, though how general this finding is with respect to natural antigens, is not clear.

Numerous studies have described antigen capture by lymph node-resident DC, particularly after antigen administration by the intradermal route [22,58,59]. For example, influenza virus injected intradermally drains to the subcapsular sinus, and is eventually captured by subset of cDC2 expressing SIGNR1 that migrate into the medullary interfollicular region of the lymph node [59]. A requirement for SIGNR1 for humoral immunity [12•], together with evidence that some of the SIGNR1$^+$ DC could also migrate toward follicles for potential delivery of antigen to FDC [12•], implicated these DC in presentation of viral antigen to B cells. A different scenario, though still involving cDC2, was seen when influenza virus was delivered intranasally [60•]. Here, free virus did not drain but instead was carried into the lymph node by migratory cDC1 and cDC2 trafficking from the lung. This latter report speculated that the drainage to lymph nodes seen after intradermal injections was largely an artefact of hydrostatic pressure generated by the injection forcing viral particles into the lymph. Despite the capture and transport of antigen by both cDC subsets after intranasal virus administration, cDC2 were essential for T_{FH} cell generation and T-dependent antibody production. This was demonstrated using DOCK8-deficient mice, where migration by cDC2 but not cDC1 is defective. In these mice, virus-bearing cDC1 still migrated to the lung-draining lymph nodes, but humoral immunity was poor. In wild-type mice, where both cDC subsets trafficked from the lung, migratory cDC2 bearing influenza virus accumulated at the T–B border region of the lymph node, while cDC1 remained primarily in the mid T cell zone, away from this border (Figure 2). This localization at the T–B border is reminiscent of cDC2 within the spleen after activation and may be a general requirement for their contribution to humoral immunity [57••].

Given the preferential ability of cDC2 over cDC1 to present MHC II-restricted antigens [61,62] and to localize to the T–B border after activation [57••,60•,63,64], together with the poor capacity of cDC1 to contribute to humoral immunity after intranasal influenza virus challenge [60•], it is tempting to discount cDC1 as capable of generating B cell responses. However, various strategies that target antigens either predominantly [65,66•,67] or uniquely [68] to cDC1 were shown to induce humoral immunity. Antibody responses occur at doses when the untargeted antigen is poorly immunogenic, implicating the targeted cDC1 in driving these responses. As an example, targeting antigen to the surface receptor Clec9A, expressed at high levels by cDC1 and to a minor extent by plasmacytoid DC, leads to efficient T_{FH} cell generation [69] and humoral immunity [66•], even in the absence of adjuvant. Such targeting leads to antigen-specific GC formation, isotype-switching and affinity maturation [70], and does not require plasmacytoid DC [69], implicating targeting of cDC1. In these and other cDC1-targeting studies, direct presentation of antigen to B cells by cDC1 is yet to be demonstrated, leaving open the possibility that antigen is transferred to other cell types. Future studies will need to clarify this issue. In all, however, these finding suggest that antigen captured by cDC1 can lead to B cell responses under some circumstances.

Given the demonstrated role of cDC1 in antibody responses to targeted antigens, it is difficult to explain why this subset does not induce humoral immunity to captured influenza virus [60•]. This defect may relate to cDC1 location, with influenza-bearing cDC1 localized centrally in the T cell zone, away from the T–B border [60•]. This explanation, however, does not explain why cDC1-bearing influenza virus do not present this antigen to B cells as they traffic from the blood, via HEV to the

Figure 2



Migratory cDC2 preferentially migrate to the T–B zone.
Administration of influenza virus (red stars) intranasally does not result in free virus drainage to the lymph node but leads to capture of virus by cDC1 and cDC2, which migrate from the lung to the draining lymph node. Here cDC2 migrate to the T–B border and induce Tfh cells and B cell responses, whereas cDC1 bearing virus migrate deep into the T cell paracortex and do not participate in humoral immunity. Note that lymph node-resident cDC1 can be located adjacent to B cell follicles in the steady-state and may potentially present targeted antigens to B cells in follicles or to B cells migrating from HEV to follicles.

follicles [52 a]. This failure may relate to whether the receptors used to capture antigen (whether virus, targeted or other) have the capacity for cell-surface display. Clearly complement receptors and FcγRIIb are able to recycle antigens to maintain surface display, while other receptors such as DEC205 largely traffic antigens for degradation [71]. Perhaps SIGNR1 expression by cDC2 enables surface display of influenza virus by this subset, while the receptors on cDC1 used to capture this virus favor degradation, precluding antigen-presentation to B cells. Thus, while differences in location within lymphoid tissues might be a consideration for B cell activation by cDC subsets, specific receptors involved in antigen capture and display might also contribute.

DC presentation to B cells is worthy of further consideration

Highly cross-linked antigens, particularly in membrane-associated forms, very efficiently activate B cells. As a consequence, antigen-presenting cells are generally required to provide B cells with membrane-displayed native antigen to initiate humoral responses. In the past, two cell types have been primarily implicated in this process, that is, macrophages and FDC, but mounting evidence suggests that cDC are also potential participants. Many antigens and pathogens are able to be opsonized by complement or natural antibodies, which likely brings into play both macrophages and FDC as major participants, but not all antigens can be opsonized in this manner and many pathogens or their products are likely captured from peripheral tissues where cDC are the only option for trafficking antigen into draining nodes. Thus, once we begin to broaden the assortment of antigens and microbes assessed for B cell-stimulation pathways, it is likely that a growing role for cDC will emerge. Furthermore, while cDC2 appear to be major players when it comes to humoral immunity, showing preferential location within lymphoid tissues for

participation in such responses, cDC1-targeted antigen can induce antibody responses, raising the possibility that this subset also has potential. Finally, consideration should be given to how antigens or microbes are captured by cDC and whether the receptors involved allow surface display or lead to degradative pathways. Many studies have implicated macrophages and FDC in presenting native antigen to B cells, but limited exploration of cDC in this role, and the somewhat limited range of antigens tested so far, suggest that this area is worthy of greater exploration.

Conflict of interest statement

Nothing declared.

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