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The unexpected contribution of conventional type 1 dendritic cells in driving antibody responses

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Summary

Antibodies are hallmarks of most effective vaccines. For successful T-dependent antibody responses, conventional dendritic cells (cDC) have been largely attributed the role of priming

T cells. By contrast, follicular dendritic cells and macrophages have been seen as responsible for B cell activation, due to their strategic location within secondary lymphoid tissues and capacity to present native antigen to B cells. This review summarizes the mounting evidence that cDC can also present native antigen to B cells. cDC2 have been the main subset linked to humoral responses, based largely on their favourable location, capacity to prime $CD4^+$ T cells, and ability to present native antigen to B cells. However, studies using strategies to deliver antigen to receptors on cDC1, reveal this subset can also contribute to naïve B cell activation, as well as T cell priming. cDC1 location within lymphoid tissues reveals their juxtaposition to B cell follicles, with ready access to B cells for presentation of native antigen. These findings support the view that both cDC1 and cDC2 are capable of initiating humoral responses provided antigen is captured by relevant surface receptors attuned to this process. Such understanding is fundamental for the development of innovative humoral vaccination approaches.

Introduction

Antibodies are essential for protection against numerous infectious diseases and are the effector arm of most vaccines. They have distinct heavy chains that determine their class and effector function, which varies significantly from direct neutralization of pathogens, to activating complement or opsonizing pathogens for destruction by immune cells. Antibodies are produced by B cells, and sometimes this can occur in the absence of T cell help (T-independent response), for instance when multivalent antigens efficiently cross-link the B cell receptor (BCR) or when antigen encounter occurs in the context of strong signalling via toll-like receptors (TLR) [1]. T cell help is required, however, for B cells to form germinal centres (GC) and undergo class switch recombination and affinity maturation *via* somatic hypermutation [2].

In the T-dependent humoral response, dendritic cells (DC) are primarily known for their role in priming $CD4^+$ T cells, which involves processing and presentation of antigen on MHC class II (MHC II) molecules in addition to providing appropriate costimulatory signals. Once activated, T helper cells then migrate to the T/B border in search of activated B cells that have captured the same antigen and have processed it for display in the context of MHC II. Presentation of antigen by B cells on MHC II further assists the development of follicular helper T cells (T_{FH}), which are the main T cell subset responsible for providing help during

GC reactions [2]. In contrast to T cells, which recognise linear peptide epitopes presented on MHC molecules, B cells generally recognise native antigens. While several cell types have been shown to provide native antigen to B cells[3], it is becoming increasingly clear that DC may also contribute to this process, inducing B cell activation. This less well-recognised role for DC will be the focus of this review.

1.1. Efficient activation of B cells by membrane-tethered antigens

It is well-known that membrane-bound antigen is more efficient than free antigen at activating cognate B cells [4]. This largely relates to the need for B cell receptor cross-linking for B cell activation, as shown by studies comparing responses to monomeric versus multivalent antigens [5][6]. Membranes are thought to act as platforms to concentrated antigen that enhance its capture by B cells [7][8]. Increases in local antigen density promote the formation of a synapse between the B cell and the cell presenting the native antigen, allowing for efficient BCR cross-linking and B cell activation [7]. This interaction culminates in the activated B cell migrating to the T/B border to acquire T cell help [9].

There are many receptors that have been utilised by cells to display pathogen-associated antigens on their membrane. Complement receptors, for example, can capture and display complement-opsonized antigens and facilitate B cell activation. Antigens or microbes can readily elicit complement fixation through several pathways [10], and thereby engage the complement receptors. Alternatively, Fc receptors (FcR) can capture antibody-coated pathogens, although this generally triggers phagocytosis. The inhibitory FcR known as FcγRIIb, however, binds antibody-antigen complexes, and these can be transported to non-degradative vesicles and then recycled back to the cell surface[11]. Such antigen recycling has been shown to participate in T-independent B cell responses[11].

1.2. Conventional ways B cells encounter antigen for their initial activation in lymphoid follicles

The key role of efficiently supplying B cells with the membrane-tethered native antigen is limited to only a few cell types and these cells are strategically placed to interact with naïve B cells [3]. **(Figure 1).** The spleen and lymph nodes filter the blood and lymph for pathogens and their products and, through highly organised structures, maximise the likelihood for a rare antigen-specific lymphocyte to encounter its cognate antigen. Circulating B cells enter these lymphoid compartments and congregate in follicles around the T cell zone. These B cells linger in the follicles for about a day, randomly moving around, presumably scanning this antigen-rich environment for their cognate antigen [2],[3]. Small antigens (<70 KD) entering the lymph node *via* the afferent lymphatics, can freely circulate through the conduit system and gain access to the B cell follicles [12][13]**(Figure 1).** By contrast, larger antigen complexes cannot freely circulate and require transport by accessory cells[14]. **(Figure 1).** Two cell types, the subcapsular sinus (SCS) macrophages and follicular dendritic cells (FDC), are well-known to play key roles in presenting native antigen to B cells. Specifically, the $CD169^+$ SCS macrophages reside in the follicular-proximal side of the SCS and can capture immune complexes and particulate antigens[15][16][17]. **(Figure 1).** $CD169^+$ SCS macrophages have a low propensity for degrading antigen and possess projections that reach into the follicles to display the captured antigen to B cells[15][17]. **(Figure 1).** There is strong evidence that antigen presenting $CD169^+$ SCS macrophages can efficiently activate B cells. It has been shown that cognate B cells accumulate in regions containing $CD169^+$ SCS macrophages displaying particulate antigen before their activation and localization to the T/B border[15]. Moreover, macrophages have the capacity to localize to B cell follicles following immunisation[16] and are capable of inducing B cell production of antigen-specific IgM and IgG[16]. $CD169^+$ SCS macrophages have also been shown to contribute to B cell activation *via* antigen hand-over to non-cognate B cells, which migrate into B cell follicles[17]. **(Figure 1).**

FDC are localized in the central regions of lymphoid follicles and capture antigens mainly *via* the complement receptors CR1 and CR2[17] and Fcγ receptor II[18][19]. **(Figure 1).** FDC are a common source of native antigen and play an essential role in supporting affinity maturation in the GC, after initial B cell activation[20]. However, FDC can also be the initial source of antigen for naive B cells arriving in the follicle [20]. A number of cell types can provide FDC with native antigen. Firstly, marginal zone B cells (MZ B cells) have been shown to capture IgM-containing immune complexes in a complement receptor dependent manner, then migrate to the follicles and deposit these complexes onto FDC[21]. Secondly, non-cognate B cells that express high levels of complement receptors can also transport immune complexes to FDC[17][22]. Finally, antigen displayed on projections of $CD169^+$ SCS macrophages can become a source of antigen, transported by non-cognate B cells to

FDC[17]. In summary, both SCS macrophages and FDC have the capacity to display native antigen on their surface as a source of membrane-associated antigen for B cells, contributing to their initial activation **(Figure 1)**. While antigen is only retained transiently on SCS macrophages, FDC act as a long-term source of native antigen for the germinal centre reaction and affinity maturation.

1.3. The role of DC in the initial activation of B cells in a humoral response

For induction of humoral immunity, DC are first required to activate naive T cells, enabling their subsequent participation in T-B collaboration. Because of this prominent role in initiation of antibody responses, DC have only rarely been considered as playing an important role in initial B cell activation. Studies published by MacPherson and colleagues were some of the first to provide compelling indirect evidence that DC can present native antigens to B cells to facilitate humoral responses [23]–[25].

Direct interactions between antigen-bearing DC and transgenic B cells were then revealed *in vitro* when DC pulsed with Hen Egg Lysozyme (HEL) were cocultured with HEL specific-B cells [26]. Later, in a landmark study, using two-photon microscopy and adoptive transfer, the interaction between DC and B cells was visualised *in vivo* [27]. Qi and colleagues, showed that HEL-transgenic B cells interacted with adoptively transferred HEL-pulsed DC in regions adjacent to high endothelium venules (HEV) of the lymph nodes [27]. This prolonged *in vivo* interaction resulted in B cell calcium fluxes and upregulation of the co-stimulatory receptor CD86 and the chemokine receptor CCR7 [27]. These phenotypic changes enabled activated B cells to localize to the T/B border and present processed antigen to $CD4^+$ T cells, ultimately supporting T-dependent antibody responses.

A series of studies have also illustrated that DC-pulsed with antigen retain native antigen on their cell surface and elicit antibody responses upon adoptive transfer [11],[23][28][29][30]. DC exposed to immune complexes, for instance, are capable of endocytosis *via* FcγRIIB and shuttling antigen into non-degradative intracellular vesicles, allowing their recycle to the cell surface[11]. This form of native antigen presentation resulted in the induction of T-independent B cell responses. The role of DC in presenting native antigen to B cells was also addressed by Gonzalez and colleagues, showing that antigen captured *via* SIGN-R1, a lectin receptor expressed by DC residing in the subcapsular and medullary sinuses, can become a potential source of antigen to B cells [31]. In their study, these DC utilised SIGN-

R1 to capture lymph-borne influenza virus, then migrated toward FDC, potentially participating in antigen handover [31]. This study suggests that upon activation, DC can be a source of antigen not only to T cells but also to B cells.

When considering the role of DC in the direct activation of B cells, it is important to highlight that the DC network is comprised of multiple subsets with specialised functions. Based on ontology, DC are divided into monocyte-derived, plasmacytoid DC and classical DC (cDC) [32],[33], and it is the latter cDC that have been reported to induce humoral responses[32],[33][34][35],[36].These cDC can be further divided into type 1 (cDC1) and type 2 (cDC2), each with migratory and lymphoid-tissue resident sub-populations[32],[33].

1.4. The role of cDC in priming Th cells in humoral responses

cDC2 have traditionally been linked to humoral immunity primarily due to their efficient capacity to process and present antigen in the MHC II pathway for priming of $CD4^+$ T_H cells[37]. In addition, their localization in the spleen and LN has been described as favouring the sampling of antigens for activation of $CD4^+$ T cells and generation of humoral immunity [38]–[40]. In the spleen, cDC2 are localized within the bridging channels[41],[42] [43], and in the LN, they are closely associated with the lymphatic sinus–proximal regions [38],[40],[44]. **(Figure 1).** These locations, respectively, facilitate sampling of circulating and lymph-borne antigens [40]. In addition, $CD4^+$ T cells were shown to rapidly cluster and become activated in regions rich in cDC2 following immunisation, suggesting that this is the area that MHC II presentation occurs [40],[44]. Furthermore, cDC2 express higher levels of Cxcl13 (the CXCR5 ligand) than cDC1, a chemokine that potentially attracts newly formed pre-T_{FH}[39]. By contrast, cDC1 were reported to be localized deeper in the T cell zones of the spleen and LN away from the B cell zones, where they have been shown to predominantly cluster with early activated $CD8^+$ T cells following immunisation [39][40],[44]. **(Figure 1).**

The pivotal role of migratory and lymphoid resident cDC2 in driving T_{FH}-dependent humoral immunity has been demonstrated in multiple vaccination studies [39] [44] [45] [46]. Most of these studies clearly show that the importance of cDC2 in humoral immunity hinges on their ability to prime of $CD4^+$ T cells and generate T_{FH}.

As previously indicated, the antigen presentation capacity and localization of cDC1 is thought to favour activation of $CD8^+$ T cells over $CD4^+$ T cells. However, while many studies have suggested cDC1 are poor at driving humoral immunity, these studies have generally involved the delivery of antigen to the cell surface receptor DEC205 [37],[47]. In contrast,

when other receptors on cDC1 were utilised, such as Clec9A, a clear capacity for inducing long-lasting humoral immunity is observed [48][49] [50] [51], both in mice and non-human primates, even in the absence of adjuvant [56]. Targeting antigen to Clec9A induced T_{FH} formation and potent antibody responses in the absence of adjuvant[52][49],[53]. Furthermore, Fossum and colleagues showed the benefits of targeting antigen to Clec9A over DEC205, both in terms of total production of antibodies, and in their superior capacity to neutralize influenza virus [54]. The basis for poor antibody responses following DEC205-targeting has not yet been fully elucidated, but might be attributed to several factors including: 1) the expression of DEC205 by multiple cell types, which leads to rapid antigen clearance following immunisation; and 2) rapid endocytosis and degradation of DEC205-target antigen, which limits presentation of native antigen to B cells [55]. Conversely, the mechanisms underpinning the efficient $CD4^+$ T cell priming following Clec9A-targeting are still to be elucidated, but cDC1 show no signs of activation following such immunisation[49], suggesting that the efficient generation of Tfh is not related to elevated expression of costimulatory molecules, as might occur with adjuvants such as TLR agonists. Persistent presentation of Clec9A-targeted antigen by cDC1 may be an important factor [53], but surface display of antigen (as shown below) and co-operation of B cells is also likely to contribute.

1.5. Unveiling the role of cDC1 in presentation of native antigen to B cells

Evidence relevant to which cDC subsets can present native antigen to B cells comes largely from studies that use monoclonal antibodies (mAb) to deliver antigen to specific molecules expressed selectively by subsets of cDC. Chappel et al. (2012), delivered antigen to cDC2 using mAb targeting DCIR2 and found it elicited robust antibody responses [56]. They found that DCIR2-targeting resulted in rapid B cell activation that was independent of T helper cells [56]. In addition, antigen delivered to FIRE (F4/80- like receptor) or CIRE (C- type lectin receptor), both expressed on cDC2, induced a strong humoral response[47]. In contrast, antigen delivered to DEC205, expressed largely by cDC1, induced poor humoral responses, unless co-administered with adjuvants such as TLR ligands, which are known to upregulate DEC205 on cDC2 [53]. Thus, while these comparisons suggested cDC1 may be poor at presenting native antigen to B cells, they generally employed DEC205-targeting to test cDC1, and failed to exclude the nature of this receptor as the basis for the outcome. Indeed, when antigens were delivered to XCR1, another receptor expressed on cDC1, robust antibody responses were elicited [54]. Arguably, the most extensively studied way to elicit antibody responses by targeting antigen to cDC1, is by utilising the surface receptor Clec9A

[49],[52],[57]–[60]. So far, we have ascribed this success to effective activation of $CD4^+$ T cells and the ability to elicit T_{FH} responses [52][49],[53]. However, robust antibody responses also require initial B cell activation, generally through recognition of membrane-bound antigen. For cDC1 themselves to present Clec9A-targeted antigen to B cells they would have to retain native antigen on their surface and then contact B cells, either as they migrate through HEV toward the follicles or potentially in regions bordering the follicles **(Figure 1).** We used several imaging techniques, including two-photon *in vivo* imaging, to decipher whether cDC1 ever randomly contact B cells [58]. Interestingly, we observed that cDC1 have ample opportunities for interactions in regions bordering B cell follicles in both lymph nodes and spleen [54] **(Figure 2).** Moreover, we have shown that a significant amount of antigen delivered to Clec9A with the use of mAb is retained in its native form on the surface of cDC1, allowing direct presentation to cognate B cells in regions bordering B cell follicles[58] **(Figure 1).** This enables rapid B cell activation and their subsequent localization to the T/B border, in a process that is independent of T cells[58]. Interestingly, antigen delivered to DEC205 is not retained on the surface of cDC1, which limits its capacity for B cell activation [58] **(Figure 1).** This observation, perhaps, explains the relatively poor capacity of DEC205-targeted antigens to induce antibody responses**.** Our study sheds light on the role played by cDC1 in humoral immunity and shows that previous efforts, employing DEC205, have underappreciated the capacity of this cDC1 subset to participate in humoral immunity. While the use of targeting strategies allows us to show the potential of cDC1 to present native antigens to B cells, further studies are required to decipher the role of this cDC subset in physiological responses initiated by infectious agents.

Conclusions

Taken together, studies suggest that efficient B cell activation requires native antigen display on the surface of accessory cells, coupled with the opportunity for these accessory cells to interact with B cells. It is now clear that both, cDC1 and cDC2, can interact with B cells and that their capacity to activate B cells is directly linked to their ability to display native antigen on a given receptor. [61]Thus, superior antibody production may require targeting antigen to DC receptors that not only facilitate efficient antigen processing and presentation for $CD4^+$ T cell priming, but also display native antigen on the cell surface for the direct activation of B cells.

Conflict of interest statement

Nothing declared.

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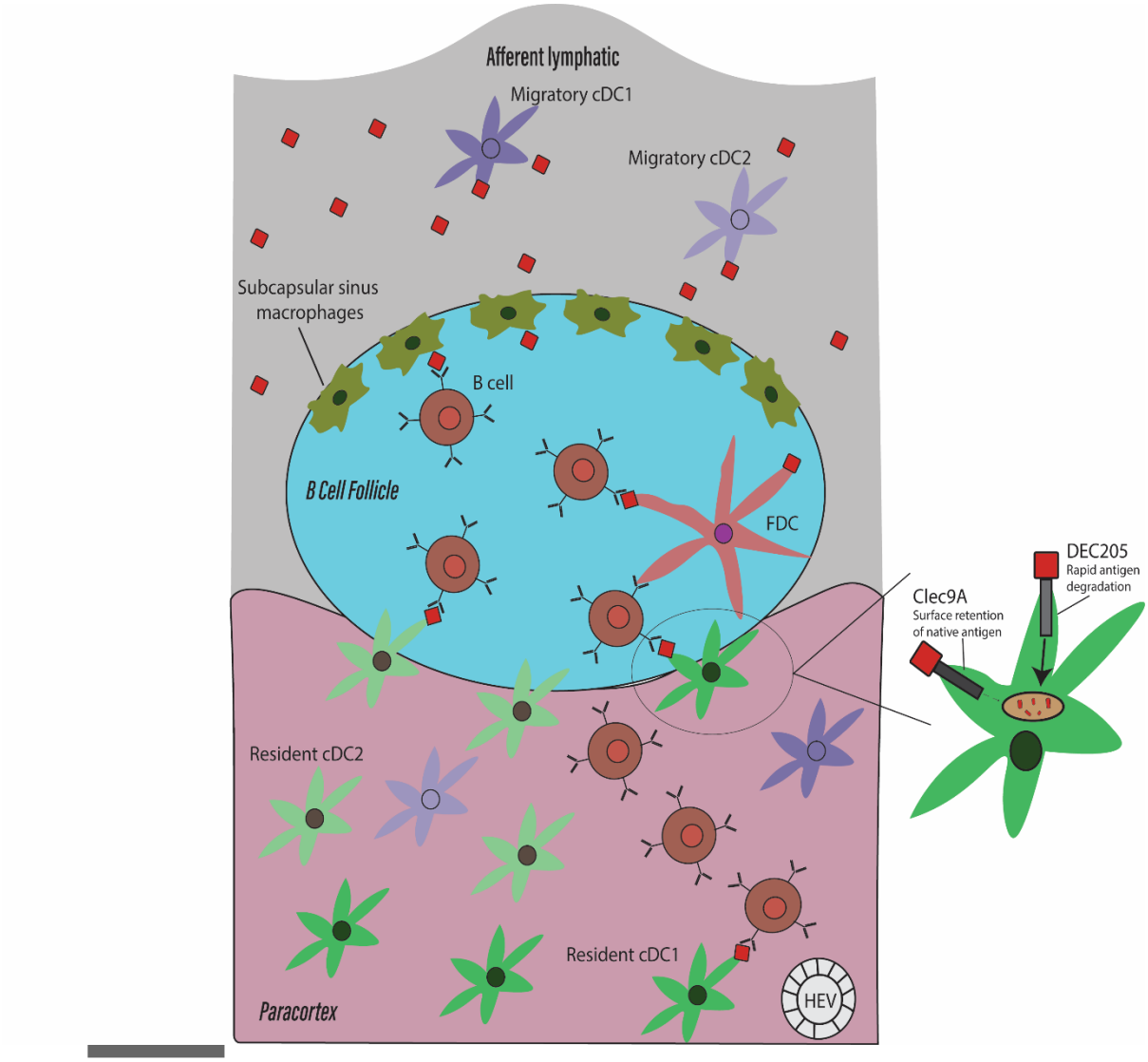

Figure 1. Pathways employed for native antigen presentation to B cells in lymph nodes. Antigen can access the lymph node as soluble free antigen or associated with migratory cDC (red squares). Sufficient small soluble antigen can directly diffuse into the lymph node and does not require migratory cDC for transport. Both pathways lead to antigen capture by subcapsular sinus macrophages and presentation to B cells or transportation to FDC via different methods for presentation. Alternatively, resident or migratory cDC can retain native antigen for presentation to B cells. cDC can present antigen to B cells in two moments, during migration from HEV to B cell follicles or in regions bordering the follicles (T/B border). In the figure is depicted two cDC1 receptors, Clec9A and DEC205. DEC205 is unable of retaining native antigen on the surface of cDC1 and thus fails to present antigen to B cells, whilst Clec9A retains antigen on the surface and efficiently activates B cells.

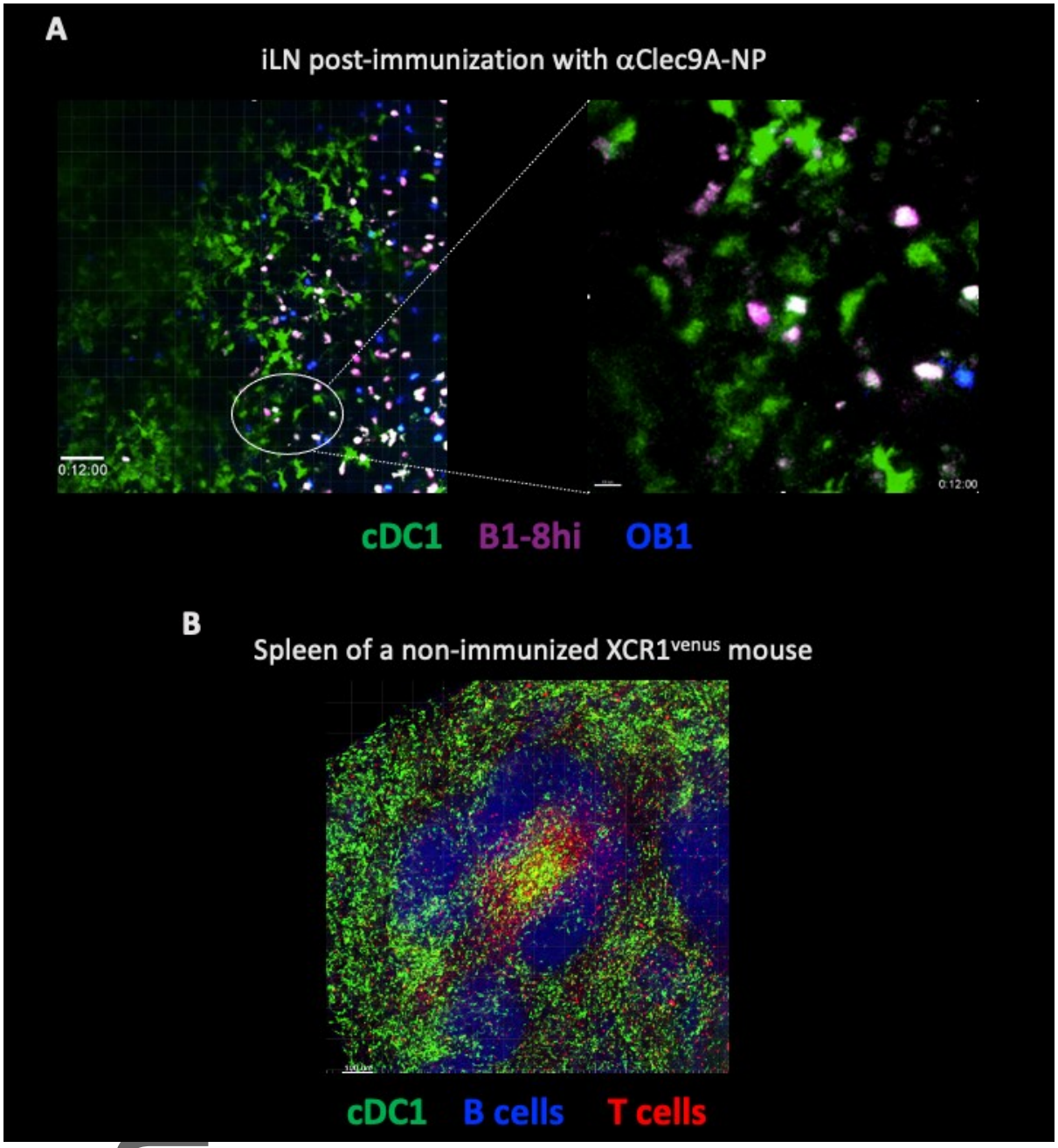

Figure 2. cDC1 have ample opportunities for interactions with B cells in regions bordering B cell follicles in the lymph nodes and spleen. A. Ovalbumin-specific OB1 transgenic B cells (labelled with CTV, displayed in blue) and NP-specific transgenic B1-8hi B cells (labelled with CTDR, displayed in magenta) cells were adoptively transferred into lethally irradiated B6 mice reconstituted with bone marrow cells from Xcr1$^{venus/+}$ mice. Intravital imaging of the inguinal Lymph node was performed after 1.5- 3h post-immunization with 0.5 μg of αClec9A-NP. **Left image:** depicts B1-8hi and OB1 in close proximity to cDC1 following immunisation. **Right image**: illustrates the close contact between B1-8hi B cells and cDC1 following immunisation. **B.** 24 x 10^6 CTV$^+$ polyclonal B cells (displayed in blue) and 5 x 10^6 CTDR$^+$ polyclonal T cells (displayed in red) were adoptively transferred into Xcr1$^{venus/+}$ mice (cDC1 displayed in green) 1 day before sacrifice to delineate B cell follicles and T cell zones for imaging of 200-250μm thick spleen vibratome sections. Image B illustrates a thick spleen section. Scale bar, 100μm except figure A left, 10μm.

Multiple cellular interactions are required for induction of T-dependent antibody responses. Here, we reveal that the type 1 conventional dendritic cells not only have the capacity to prime Tfh cells but can provide a scaffold for presentation of native antigen to B cells for their activation.