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Antigen presentation to B cells

Naomi E Harwood and Facundo D Batista*

Address: Lymphocyte Interaction Laboratory, London Research Institute, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3LY, UK

* Corresponding author: Facundo D Batista (facundo.batista@cancer.org.uk)

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Abstract

B cells are capable of mounting responses to a bewildering range of potentially pathogenic antigens through the production of high-affinity antibodies and the establishment of immunological memory. Thus, regulated B-cell activation is critical for protection against a variety of bacterial and viral infections, as well as cancers. Here, we discuss a number of recent imaging studies that have provided new insights into the variety of mechanisms by which B-cell activation is initiated in the lymph node *in vivo*.

Introduction and context

The initiation of B-cell responses involves two distinct events that are separated both spatially and temporally. Initially, specific recognition of antigens by the B-cell receptor (BCR) triggers intracellular signalling and antigen internalization [1,2]. Secondly, processing and presentation of internalized antigens to helper T cells facilitates maximal B-cell activation and ultimately the generation of plasma cells capable of antibody secretion [3,4].

Typically, the events of B-cell activation *in vivo* take place in specialized secondary lymphoid tissues such as the lymph nodes to increase the likelihood of a B cell 'finding' its cognate antigen [5]. Lymph nodes are supplied with lymphatic fluid through the afferent vessel. The lymphatic fluid contains a representative sample of the soluble and cell-bound antigens found in the interstitial fluid. Upon entry to the node, the lymphatic fluid is not allowed to diffuse freely around the cortex but rather is moved around the subcapsular sinus (SCS) towards the central medullary region through trabecular sinuses. This restricted movement raises questions as to how follicular B cells in the lymph node interior gain access to lymph-borne antigens. In fact, until relatively recently, the mechanisms by which B cells initially encounter antigen and become activated *in vivo* remained enigmatic.

Major recent advances

The mechanism by which lymph-borne antigens gain access to the lymph node interior to activate follicular B cells *in vivo* is dependent on characteristics of the antigen itself and, in particular, its molecular mass. As such, Marc Jenkins and colleagues observed that fluorescently labelled antigen below 70 kDa was able to diffuse into the interior of the draining lymph node within minutes of administration [6]. They demonstrated that according to their vicinity to the SCS, follicular B cells were able to acquire antigens and subsequently migrate to the border of the T-cell zone to receive help from $CD4^+$ T cells. It was suggested that antigens gained access to follicular B cells through small pores in the SCS, which had been previously observed by electron microscopy [7-9]. However, as the existence of such pores has remained controversial, the authors have suggested in hindsight that it is more likely that the diffusion of small soluble antigens occurs through a novel follicular conduit network, which has been elegantly identified by the lab group led by Michael Carroll [10]. The source of smaller antigens *in vivo* remains questionable, though some recent evidence suggests that serum proteases may be involved in liberating antigens from the surface of pathogenic invaders [11]. Interestingly, the conduit network may offer the opportunity to transport chemokines such as CXCL13 to regulate the migration of follicular B cells towards the sites where

they are likely to encounter antigens [10]. In addition, it seems that this conduit network occupies a strategic position to allow follicular dendritic cells (FDCs) deep in the follicle to gain access to small antigens in the lymphatic fluid [12].

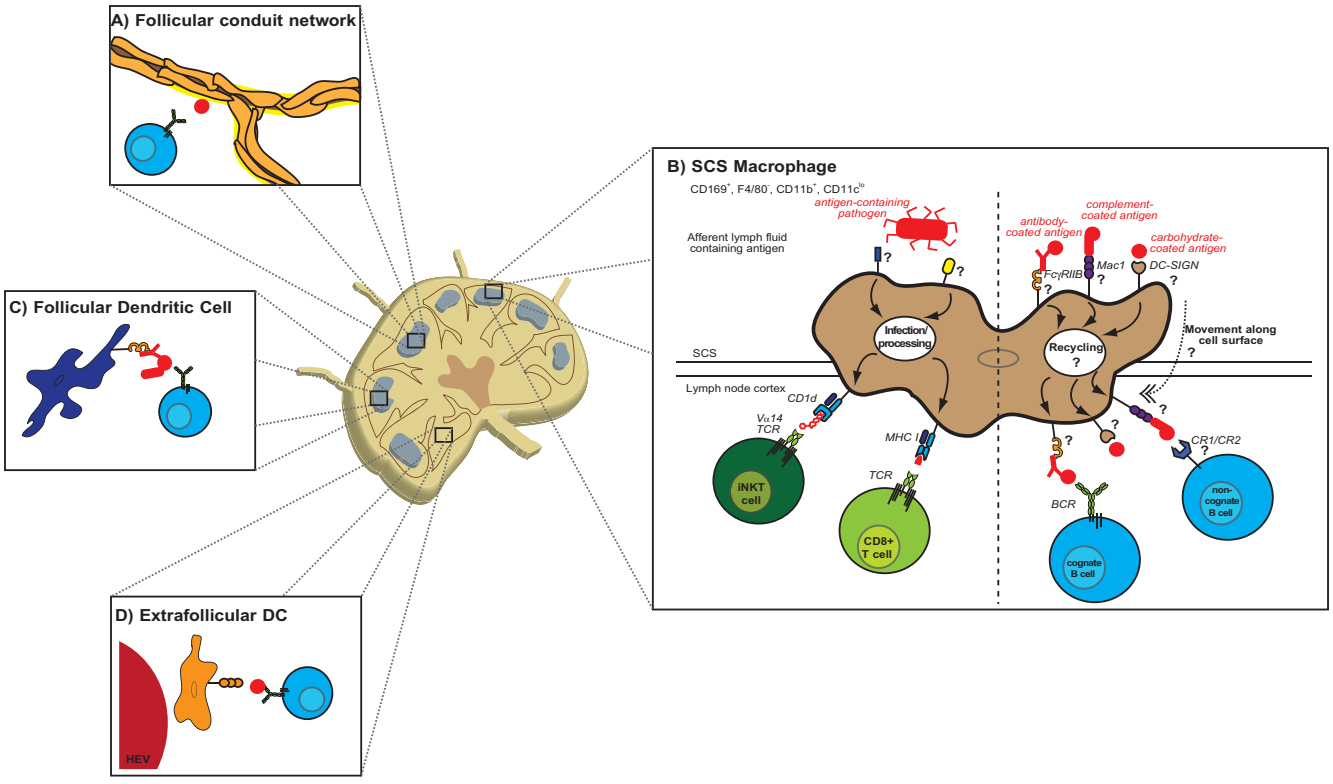
While the follicular conduit network provides a mechanism for small antigens to access B cells in the follicle, like its counterpart in the paracortex [13,14], the network precludes the free diffusion of larger antigens into the lymph node interior [10]. Three independent studies have demonstrated a role for a layer of $CD169^+$ macrophages positioned at the SCS in the acquisition of larger antigens in the form of viruses, particulates, or immune complexes [15-17]. In each of these cases, follicular B cells were observed arresting in proximity to SCS macrophages within an hour of antigen administration and accumulating antigens prior to migration to the follicular T-cell zone border. It seems that these macrophages also play a general role in retaining antigens at the SCS, as their removal through clodronate liposomes not only impairs local B-cell activation but also leads to systemic dissemination of viruses [16,18]. Furthermore, it has been demonstrated very recently that SCS macrophages also participate in the prevention of central nervous system infection by neurotropic viruses through the production of type 1 interferon [19]. Interestingly, these macrophages also display intact antigens to non-cognate B cells so that they can transport larger antigens from the SCS to FDCs in a complement-dependent manner [17]. While it is known that this transport is important for affinity maturation [20], there is some evidence that antigens arrayed on the surface of FDCs might also mediate activation of naive B cells [21]. Indeed, this type of presentation would provide an elegant means to improve the chances of extremely rare B cells encountering cognate antigens.

The discovery that macrophages present intact antigens to B cells was somewhat surprising given their usual role as specialised phagocytes. However, as SCS macrophages express low levels of lysosomal enzymes, it has been suggested that they exhibit reduced phagocytic capacity compared with medullary macrophages [20]. Furthermore, it has been noted that SCS macrophages lack expression of the mannose receptor – though it is not clear how this impacts on the propensity for phagocytosis [22]. Intriguingly, however, it has been observed that $CD8^+$ T cells relocalize around infected cells in the SCS [23] and that memory $CD8^+$ T cells engage in prolonged interactions with infected SCS macrophages [24]. This implies that SCS macrophages may also be capable of presenting processed antigens. Indeed this was formally established through the demonstration that $CD169^+$ SCS macrophages present processed lipid antigens to initiate the activation of lymph node invariant natural killer T cells [25]. In view of these apparently contradictory lines of evidence, it seems that SCS macrophages are capable of presenting both intact and processed antigens to different immune cells. However, it is unclear at this stage which factors govern the decision to recycle intact antigens to the cell surface or to target these antigens for processing and presentation in complex with major histocompatibilty complex molecules. It seems likely that the cell surface receptor that binds the antigen might play a role in this decision; for example, in the case of SCS macrophages this might involve FcγRIIB, Mac1 or SIGN-R1 as receptors for binding of immune-complexed antigen, complement-coated antigen, or carbohydrate-coated antigen, respectively [26].

Macrophages are not the only cell capable of presenting native antigens to follicular B cells in lymph nodes. Early observations suggested that dendritic cells (DCs) were capable of interacting directly with B cells *in vitro* to initiate activation [27]. In line with this, Ron Germain and colleagues have demonstrated that DCs endocytose and present antigen both *in vitro* and *in vivo* [28]. Furthermore, they showed that antigen-specific interactions with DCs can occur outside of the follicle when B cells enter the lymph node through high endothelial venules. This atypical location for activation of follicular B cells leaves them perfectly positioned to receive help from cognate $CD4^+$ T cells and, in line with this, 'trios' of B cells, T cells, and DCs have been observed in this site [29]. Another very recent study has highlighted a role for medullary DCs in capturing influenza virus and the induction of specific humoral immune responses [18]. Unlike uptake by the SCS macrophages that requires opsonization with mannose-binding lectin, capture by DCs was dependent on binding to SIGN-R1.

Regardless of the mechanism that enables B cells to initially encounter antigens, the second phase of B-cell activation involves the migration of B cells to the B-cell–T-cell border to receive help from cognate $CD4^+$ T cells [30,31]. Activated B cells then either form extrafollicular plasma cells capable of low-affinity antibody production [32] or enter specialized germinal centres (GCs) to undergo affinity maturation to generate extremely high affinity antibodies [33]. In GCs, after the initial 'wave' of antigens in lymphatic fluid has passed, antigen presentation to follicular B cells continues and B cells are selected for on the basis of their affinity for antigen accumulated on the surface of FDCs. Three dynamic, recent investigations have visualized B-cell dynamics in the GC during affinity maturation [34-36]. These independent studies demonstrated that B cells in the GC exhibit an unusual morphology and are highly motile, rarely making prolonged contact with FDCs. Thus, it appears that FDC-mediated presentation of antigen

Figure 1. B-cell activation in the lymph node



Lymphatic fluid containing antigens (red) enters the lymph node (centre) through afferent vessels and moves around the subcapsular sinus (SCS; brown), gaining access to B cells through a variety of recently elucidated mechanisms. **(A)** Smaller antigens are moved through the follicular conduit network and can gain access to cognate follicular B cells. **(B-D)** Larger antigens, such as immune complexes, are excluded from the conduit network and can be presented on the surface of **(B)** SCS macrophages (light brown) or **(C)** follicular dendritic cells (dark blue) to cognate B cells in the follicles (grey). Alternatively, **(D)** extrafollicular dendritic cells (orange) may present antigens to cognate B cells as they arrive in the node through the high endothelial vessels (HEV; red). The SCS macrophages, shown in **(B)**, have been the particular focus of a number of recent studies. These macrophages accumulate larger antigens potentially through a variety of cell surface receptors according to the nature of the antigen itself. Accumulated antigen might either move along the cell surface or enter intracellular vesicles and be recycled intact to the cell surface for presentation to cognate B cells through the B-cell receptor (BCR), or to non-cognate B cells through complement receptors. In addition, antigen may enter into processing compartments such as lysosomes for processing and presentation to $CD8^+$ T cells or, in the case of lipid antigens, to invariant natural killer (iNKT) cells. MHC I, multi histocompatibility complex class I; TCR, T-cell receptor.

to B cells potentially occurs through a different mechanism than through SCS macrophages or medullary DCs. Furthermore, examination of the rates of B-cell movement between the two GC zones suggests that non-absolute functional segregation between zones occurs, calling into question the classical model of selection in the GC [33].

Future directions

Taken together these recent studies reveal a plethora of alternative mechanisms by which follicular B cells can encounter cognate antigen, such that we are beginning to understand the initial events of B-cell activation *in vivo*. Future investigations involving characterization of the precise molecular requirements for antigen presentation to B cells by SCS macrophages, DCs, and FDCs, and the direct visualization of antigen throughout the process of affinity maturation, will provide new insights into the processes underlying B-cell activation *in vivo*. Alongside these investigations, *in vitro* characterizations at the single cell level using high-resolution imaging methodologies will be exquisitely useful in dissecting the different intrinsic molecular and cellular requirements for recognition of soluble and membrane-bound antigens. Indeed, through these types of studies, a critical role for the B-cell cytoskeleton in the recognition of antigens on a constrained surface is beginning to emerge, despite being previously overlooked due to characterizations carried out with soluble antigen stimulation [37].

Abbreviations

BCR, B-cell receptor; DC, dendritic cell; FDC, follicular dendritic cell; GC, germinal centre; SCS, subcapsular sinus.

Competing interests

The authors declare that they have no competing interests.

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