Molecular cues involved in the regulation of B cell dynamics: assistants of antigen hunting

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Abbreviations

7 α ,25-HC, 7 α ,25-dihydroxycholesterol; APC, antigen presenting cell; BCR, B cell receptor; Btk, Bruton's tyrosine kinase; cSMAC, central supramolecular activation cluster; DAMP, danger-associated molecular patterns; EBI2, Epstein-Barr virus-induced G-protein coupled receptor 2; ICAM-1, intercellular adhesion molecule-1; IS, immune synapse; LPS, lipopolysaccharides; LFA-1, lymphocyte function-associated antigen-1; MRC, marginal reticular cells; MTOC, microtubule organizing center; PAMP, pathogen-associated molecular patterns; PRR, pattern recognition receptors; pSMAC, peripheral supramolecular activation cluster; PIP₂, phosphatidylinositol-4,5-bisphosphate; PIP5KI, phosphatidylinositol-4-phosphate 5-kinase; PID, primary immunodeficiency; SLO, secondary lymphoid organs; S1P, sphingosine-1-phosphate; S1PR, sphingosine-1-phosphate receptor; TLR, Toll-like receptors; VCAM-1, vascular cell adhesion molecule-1; WASP, Wiskott-Aldrich syndrome protein; WIP, WASP-interacting protein; XLA, X-linked agammaglobulinemia.

Abstract

The ability of a cell to migrate, adhere and change its morphology is determinant to develop its functions; these capacities reach their maximum relevance in immune cells. For an efficient immune response, immune cells must localize in the right place at the right time; that implies crossing tissue barriers and migrating in the interstitial space of the tissues at high velocities. The dependency on trafficking abilities is even higher for B cells, one of the arms of the adaptive immune system, considering that they must encounter specific antigen for their clonal receptor in the enormous tissue volume of the secondary lymphoid organs (spleen, lymph nodes, Peyer patches). The regulated interplay between cell motility and cell adhesion allows B cells to reach distinct lymphoid tissues and, within them, to explore the stromal cell networks where antigen might be exposed. In this meeting-invited review, I summarize the current knowledge on the molecular cues and mechanisms that shapes B cell dynamics at the initial phase of the humoral immune response, including homeostatic chemoattractants and innate/inflammatory stimuli. I also revised the B cell behaviour alterations caused by BCR recognition of antigen and the molecular mechanisms involved.

Key words: B cells, chemoattractants, innate signals, motility, antigen, immune synapse.

Introduction

B lymphocytes are responsible for the neutralization and elimination of pathogens through the production of antibodies with high specificity for the antigens present on those microbes. The prompt onset of the humoral immune response is crucial to fight against the invaders, and depends on the B cell ability to search and detect antigen at the secondary lymphoid organs (SLO). The knowledge of “how, where and when” B cells find antigen *in vivo* experienced a great revolution in the last decade due to the development of new experimental models together with the application of multiphoton microscopy [1-3]. The visualization of the B cell immune response *in situ* and in real time revealed the intrinsic complexity of the process.

B cells are able to travel across tissue barriers and move through the interstitial space in response to external signals [4, 5]. Continuous migration allows them to seek for pathogen-derived antigens at SLO . The recognition of specific antigen halts motile B cells and leads to the formation of the immune synapse with the antigen-presenting cell (APC) [1, 2]; this long-lasting cell-to-cell interaction is critical for B cell activation [6-8]. B cells are also exposed to a variety of non-cognate signals such as innate/inflammatory stimuli that might modify cell motility/adhesion abilities and cell localization. B cell dynamics plasticity is thus intrinsic to B cell function and crucial for adaptive immune protection. This review focuses in the initial phase of the humoral immune response, particularly in the molecular cues that assist naïve B cells to search for antigen (chemoattractants, innate stimuli), as well as in the molecular mechanisms underlying the changes in B cell dynamics caused by BCR recognition of antigen. The processes referred as “B cell dynamics” in here include B cell motility and chemotaxis, cell adhesion, immune synapse formation, *in vivo* trafficking and tissue localization.

Steady-state B cell dynamics

B cells search continuously for antigen at the SLO, where they are organized in structures called follicles and migrate guided by the stromal cell network [5, 9]. They move with 6 $\mu\text{m}/\text{min}$ mean velocity and by random walk to efficiently explore the whole follicular volume [4, 10]. B cells use an amoeboid type of movement that allows fast migration, lacks strong adhesions with the substrate and keeps the integrity of the tissue where the cells move; amoeboid motility confers high ability for scanning and extracellular signal integration [11]. CXCL13 promotes B cell motility at the follicles signalling through its receptor CXCR5, expressed at the B cell surface. This chemokine is produced mainly at the network of follicular stromal cells, including marginal reticular cells (MRC) in the subcapsular region and follicular dendritic cells in the center, creating a chemokine gradient that drives B cell location at the follicles [5, 12]. CXCL13 accumulates in collagen fibers-rich zones and in association with glycosaminoglycan at the surface of stromal cells [13, 14], where integrin ligands (ICAM-1, VCAM-1) are present. The combination of surface-bound CXCL13 and integrin ligands might promote haptokinetic (surface-bound chemokine, adhesion assisted) B cell motility, which assists B cell scanning of the stromal cell surface.

Another pair of chemoattractant/chemokine receptor involved in B cell migration and location within the follicle is the lipid $7\alpha,25$ -dihydroxycholesterol ($7\alpha,25$ -HC) and its receptor EBI2 (also known as GPR183). EBI2 is expressed at the surface of all mature B cells, and its level increases within hours after antigen stimulation [15, 16]. EBI2 drives B cells towards the outer follicle and interfollicular regions of SLO. Its ligand $7\alpha,25$ -HC, identified in 2011, is a hydroxylated form of cholesterol [17, 18]. The enzymes responsible of $7\alpha,25$ -HC generation are highly expressed in stromal cells of interfollicular and outer follicular areas of lymphoid tissues, while their levels are lower in the central

follicular region [19]. The enzymes are also present in the T cell zone, but together with another enzyme that modifies $7\alpha,25\text{-HC}$ into a metabolite that does not bind EBI2 [19]. That defined enzyme expression compartmentalization might control $7\alpha,25\text{-HC}$ gradients in steady state. The importance of EBI2 as well as of CXCR5 in assisting B cell antigen encounter is consistent with the impaired antibody responses observed in mice lacking one of them [16, 20].

B cell exit from lymphoid organs is mainly regulated by another lipid, the lysophospholipid sphingosine-1-phosphate (S1P), and its receptor S1P-receptor-1 (S1PR1). S1P is synthesized by most cell types and it is abundant in plasma ($\sim 1\text{ }\mu\text{M}$) and lymph ($\sim 0.1\text{ }\mu\text{M}$) fluids, being carried by high density lipoprotein-associated apolipoprotein M and by albumen [21, 22]. Red blood cells, blood and endothelial cells are important S1P producers [23-25]. S1P half-life within interstitial fluids seems to be short, being its degrading enzymes associated to cell membranes. Then, a S1P gradient is established between lymphoid tissue (parenchyma, S1P concentration near nanomolar range) and lymphatic sinusses and vessels (S1P concentration in the micromolar range) that regulates the B cell egress [26-29]. S1PR1 expression can be detected at the surface of B cells isolated from lymphoid tissues but not in those obtained from blood, due to its notable sensitivity to ligand-induced downregulation [30, 31].

Local ligand production and/or accumulation within the follicle might promote homologous desensitization of the respective GPCR. This allows cyclical responsiveness of the receptors (CXCR5, EBI2, S1PR1) and thus B cell exploration of both the periphery and the center of the lymphoid follicle. In addition, heterologous desensitization mechanisms regulate GPCR function; EBI2 heterodimerization with CXCR5 at the B cell surface, in absence of $7\alpha,25\text{-HC}$, dampens CXCL13-mediated responses [32].

Modulation of B cell dynamics by antigen recognition

BCR encounter of antigen triggers B cell activation; while the BCR can bind soluble and membrane-bound antigens, the latter are more efficiently recognized *in vivo* and more effective in promoting B cell activation (reviewed in [33]). BCR recognition of membrane-bound antigen presented at the surface of the follicular stromal cell network leads B cells to adhere firmly to the APC and form the immune synapse (IS) [1, 2]. Antigen is gathered and accumulated with the BCR in a central cluster (central supramolecular activation cluster, cSMAC), surrounded by a peripheral domain enriched in the integrin LFA-1 and its ligand ICAM-1 (peripheral SMAC, pSMAC) [34]. The IS has important roles in several aspects of the B cell activation process [6, 7, 34]. *In vivo* data suggests that B cells experience several rounds of stop/IS in alternation with go/motile stages to reach full activation, exit the follicle and look for T cell help [1]. Regulation of B cell dynamics thus becomes critical for shaping antigen encounter and subsequent B cell activation. The interplay between BCR (stop/synapse) and CXCR5 (go/motile) signals has a key role in regulating B cell behaviour. *In vitro* studies showed that BCR signalling strength modifies CXCL13-mediated B cell migration, halting motile B cells above an antigen threshold [35]. In addition, CXCR5 signalling boosts BCR-triggered B cell activation by shaping B cell dynamics in at least two ways: it assists antigen gathering at the IS by promoting membrane ruffling and LFA-1-mediated adhesion and, in conditions of suboptimal antigen abundance, not enough to drive IS formation, CXCR5 signalling promotes the establishment of a LFA-1-supported migratory junction (named kinapse) that allows antigen encounter and BCR signalling integration while B cells are migrating [35].

Several reports gave insights into the molecular mechanisms used by CXCR5 and BCR to coordinate LFA-1 function and adhesion-site dynamics. Chemokine receptor and

BCR signalling activate LFA-1 by modulating integrin affinity (conformational change) and avidity (spatial distribution and clustering) for ICAM-1 [36, 37]. BCR-triggered IS formation needs of robust actin polymerization and cytoskeletal remodeling for the assembly of an F-actin-rich ring at the pSMAC. The signaling molecules Lyn, Syk, PI3K, Vav proteins, Rac and Rap GTPases and the Wiskott-Aldrich syndrome protein and its interacting protein (WASP/WIP) axis participate in the BCR-driven cytoskeletal rearrangements and LFA-1 activation [38-42]. Syk activation at the IS promotes the translocation of the scaffold protein vinculin from the cytosol to the pSMAC, where it stabilizes integrin clustering and strengthens B cell adhesion [43]. By binding to actin and talin, vinculin links the cortical actin cytoskeleton with adhesion sites at the plasma membrane; it regulates adhesion dynamics and transmission of mechanical forces [44, 45]. Lack of vinculin at the IS impairs appropriate pSMAC assembly, enabling B cells to move in response to CXCL13 whereas displacing the antigen cluster to the uropod [43]. Vinculin recruitment follows an early wave of phosphatidylinositol 4,5-bisphosphate (PIP₂) production by the phosphatidylinositol-4-phosphate 5-kinase (PIP5KI) [43]; PIP₂ is needed for vinculin activation and recruitment to the plasma membrane [46]. Bruton's tyrosine kinase (Btk) transports PIP5KI from the cytosol to the plasma membrane and thus facilitates PIP₂ generation [47]; Btk is recruited by PI3K-derived PIP₃ production [48]. Impaired Btk translocation to the membrane causes B cell inability to trigger IS formation and defects in F-actin polymerization and vinculin recruitment [49]. The Syk/Btk/PIP₂/vinculin molecular axis emerges as a major controller of B cell dynamics.

While the molecular mechanisms involved in halting B cell motility after antigen encounter to form the IS begin to be understood, the cues that drives IS disassembly to regain B cell migration are unknown. As mentioned above, *in vivo* data indicated several cycles of motility/IS stages interchange within the follicle before exiting towards the T

cell zone [1]. One possible mechanism would be the antigen consumption at the APC surface; that would reduce or even stop BCR signaling and allow CXCL13-driven motility, as described for suboptimal antigen abundance or affinity situation [35]. Diminished Syk activation would decrease local PIP₂ generation and vinculin recruitment, leading to pSMAC disassembly. Another question that remains opened is the following, if B cells are able to integrate BCR signals while migrating (kinapse stage), why do they stop and form the IS platform on the APC surface? Antigen acquisition and processing need of cell polarization events (MTOC/lysosomes translocation to the IS) and myosin-mediated mechanical force generation [50, 51]. Motile B cells might be inefficient to acquire antigen, what compromised its antigen presentation capacity to T cells and thus the B cell response. Kinapse/synapse stage regulation might be a way to determine B cell tolerance or lack of response *versus* immunogenic B cell responses.

Modulation of B cell dynamics by non-cognate signals

During the course of immune responses, B cells can be exposed to inflammatory/innate stimuli such as pathogen-associated molecular patterns (PAMP) and danger-associated molecular patterns (DAMP). Pathogen entrance through infection of or capture by subcapsular sinus macrophages might be an entry route to the follicle; pathogen-derived subproducts and dead-cell-derived ligands due to tissue infection/damage can be also source of innate signals within the follicle. B cells express a plethora of pattern recognition receptors (PRR), such as Toll-like receptor (TLR) family members (reviewed in [52]). TLR signals are important for the B cell response; they enhance antigen-presentation capacity, cytokine secretion, cell proliferation and differentiation into antibody-secreting cells [53, 54]. In addition, TLR signals regulate B cell trafficking and localization. TLR stimulation of B1 B cells facilitates their egress from the peritoneal

cavity [55]; marginal zone B cell location is also affected [56, 57]. TLR4-mediated B cell activation enhances homing to lymph nodes and localization to germinal centres [58].

The effects of innate signals in B cell dynamics and their consequences in antigen recognition and B cell activation are largely unexplored. B cell exposure to the pathogen-derived ligand LPS modifies cell dynamics over time [59]. TLR4-signals “primed” B cells for subsequent responses to CXCL13 and CCL21, promoting increased cell polarization, migration and directionality [59]. Increased directionality relates with Lévy walks type of motility, that improves target (antigen) seeking efficiency [60]. In contrast to the runs-and-pauses that featured random Brownian-like motility, generalized Lévy walks is characterized by straight tracks at fixed speed, promoting large displacements. MyD88-dependent signalling cascade and Rac GTPases were involved in those changes [59]. B cell exposure to lentiviral particles also enhanced migration in response to chemokine gradients or in haptokinesis models [61]. Then, pathogen sensing by innate receptors shapes B cell dynamics; more studies are needed to understand the consequences of that in the B cell antigen recognition process and in the kinapse/synapse balance. Two recent reports revealed TLR/BCR signalling crosstalk and, in particular, how TLR signalling sensitizes the BCR for antigen encounter by modulating the cortical actin cytoskeleton network [62, 63]. Another scenario is when the TLR ligand is linked to the antigen, and TLR and BCR signalling happen in the same time-frame. The consequences on synapse formation, assembly or stability that might shape the B cell activation process are still opened to exploration.

B cell priming by TLR ligands sets up a danger situation in which recognition of all kind of antigens (self- and non-self) might be affected. This mechanism could contribute to overcome tolerance to self-antigens in chronic inflammation. Antigen/TLR ligand physical association could help B cells to distinguish dangerous versus inert antigens.

Nonetheless, self-antigen/TLR7 ligand complexes also break tolerance and promote autoimmune diseases. The exposure time to the TLR ligand in both scenarios (priming and physical association) might be a key point to regulate the beneficial versus the dangerous side of TLR stimulation in B cells.

Relevance of B cell dynamics in pathology: primary immunodeficiency (PID)

B cell-related PID are a group of orphan diseases ($1:10^4$ - 10^5 prevalence) of variable severity, and represent the main group (~ 65%) of PID patients. B cell-PID patients have a compromised immune response against pathogens and develop autoimmune diseases and lymphomas; they are treated with unspecific gamma globulins administered subcutaneously or intravenously [64, 65]. The clinical studies performed on B cell-PID showed a high heterogeneity, making difficult the diagnosis [66, 67]. That stresses the necessity of a better understanding of the B cell-PID molecular basis. The progress in sequencing technology allows to identify the responsible gene in many cases; the data showed a major implication of actin cytoskeleton-related genes [68, 69]. Mutations in DOCK8 [7], DOCK2 [70], WASP [71], WIP [72], or RasGRP1 [73] have been found in PID patients. The X-linked agammaglobulinemia (XLA) patients present mutations in Btk [64], also involved in actin cytoskeleton rearrangements and adhesion-site dynamics [49]. Studies performed in mouse models underline the relevance of B cell motility, synapse formation, and tissue localization for the B cell response; actin cytoskeleton remodeling is the driving force of those cellular events. The finding that a high number of PID mutations affect genes related with actin cytoskeleton confirms the relevance of cell dynamics for B cell function also in humans. The design of diagnosis test to evaluate the B cell ability to polarize, move and/or form the IS would help in early PID diagnosis

and in personalized therapies, adjusting treatments depending on the clinical severity of the PID patient.

Concluding remarks

B cell dynamics regulation by distinct stimuli emerges as a critical aspect for B cell function at the initial stages of the humoral immune response. Chemoattractants (CXCL13, 7α ,25-HC, S1P) assist in B cell motility and tissue localization. Antigens halt B cell migration and promote stable B cell-APC contacts through the IS assembly to facilitate antigen acquisition and processing. Once antigen is consumed at the APC surface, B cells would recover motility and continue searching for more antigen over the follicular stromal cell network. TLR signaling modulates B cell trafficking and localization, and improves the B cell strategy to find antigen (Levy's walk motility). Understanding how changes in cell dynamics shape B cell capacity to encounter antigen and respond to it, will reveal essential aspects of the humoral immune response. This knowledge might have therapeutic use, being interstitial migration and cell localization potential targets for either avoiding pathologic immune responses (autoimmune diseases) or enhancing/boosting inefficient humoral responses (immunodeficiency, vaccines).

Authorship

Y.R.C. wrote this manuscript.

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Conflict of Interest Disclosure

The author declares no conflicts of interest.

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Figure Legends

Figure 1. Molecular cues involved in B cell dynamics regulation at the follicle. (Right panel) Scheme that summarizes the molecular signals affecting B cell behaviour within the follicle at the initial phase of the humoral immune response (antigen searching and recognition). Once B cells enter to the lymphoid tissue, CXCL13 mediates B cell entrance to the follicle and B cell motility within it, signaling through CXCR5 (light blue-B cell). The lipid chemoattractant $7\alpha,25$ -HC and its receptor EBI2 promote migration towards the interfollicular and outer follicular areas, facilitating B cell scanning of stromal cells at those locations; CXCL13 aids B cells to go back to the inner follicle. The presence of innate stimuli, such as LPS, can stimulate B cells and modify their pattern of motility (increased directionally, Lévy walks), enhancing their migratory response to CXCL13; by those mechanisms, TLR4 signaling improves B cell ability for seeking antigen. When no antigen is encountered, B cells will exit the follicle and the lymphoid tissue sensing a S1P lipid chemoattractant gradient through the S1PR1, and travel to another lymphoid tissue for continuing the antigen search. If B cells find antigen at the follicular stromal cell network and the BCR signaling is above a certain threshold, they will stop motility and establish the IS (dark blue-B cell), facilitating antigen acquisition; in case the antigen abundancy is not enough to trigger IS formation, B cells will keep moving while integrating BCR signals (kinapse stage; blue-B cell). It is unknown if B cells internalize antigen in the kinapse stage. (Left panel) Cropped image of the follicle showing the location of the subcapsular sinus macrophages and follicular dendritic cells (FDC), both APCs for B cells. Fibroblastic reticular cells (FRC), less abundant in follicles than in the paracortex (T-cell zone), embraced the lymphatic reticular conduits that go across the follicle.

Figure 2. Molecular cascade involved in halting motile B cells to establish the IS.

Model summarizing the molecular events that lead to stop motile B cells and establish the IS after BCR recognition of antigen. Motile B cells scan the follicular stromal cell network seeking for antigen; that is mainly mediated by CXCL13/CXCR5, aided by adhesion molecules (LFA-1/ICAM-1) to facilitate B cell close-contact with the stromal cell surface. The recognition of antigen through the BCR triggers Syk activation, which leads to Btk recruitment to the plasma membrane and activation (step 1). Btk brings PIP5KI from the cytosol to the plasma membrane together with it; PIP5KI produces a local increase in PIP₂ levels at the B cell:APC contact early during IS formation (first two minutes) (step 2). Local PIP₂ accumulation mediates the recruitment of vinculin and WASP, involved in adhesion platform assembly and actin polymerization, respectively (step 3). LFA-1/ICAM-1 segregate to the periphery of the B cell:APC contact site, while BCR/antigen clusters accumulate at the center, defining the pSMAC and cSMAC domains of the mature IS. Vinculin links the cortical F-actin ring with the adhesion molecules at the plasma membrane through its binding to talin and actin, giving stability to the adhesion platform (step 4). Non-muscle myosin-II (NM-II) activity is required to keep vinculin at the pSMAC; NM-II inhibition promotes vinculin dissociation, and pSMAC disassembly (step 4). While BCR signaling continues, the IS maintains its structure and B cells are stopped (step 5). Antigen consumption at the APC surface might attenuate or finish BCR signaling, and thus drive the synapse to take apart and regain cell motility in response to CXCL13 (step 6). The B cell internalized antigen while at the IS stage; acquired antigen is degraded and presented as antigenic peptides in the context of MHC class-II molecules at the B cell surface.

Figure 2. Carrasco YR.