Review

B cell localization: regulation by EBI2 and its oxysterol ligand

Dominique Gatto[1,2] and Robert Brink[1,2]

[1] Immunological Diseases Division, Garvan Institute of Medical Research, Darlinghurst, NSW 2010, Australia
[2] St. Vincent's Clinical School, University of New South Wales, Sydney, NSW, Australia

During antibody responses, B cells undergo a series of migratory events that guide them to the appropriate microenvironments for activation and differentiation. Epstein–Barr virus-induced molecule 2 [EBI2; also known as G-protein-coupled receptor (GPR)183] is a key chemotactic receptor guiding B cell localization. EBI2 and its ligand, 7α,25-dihydroxycholesterol, direct the migration of activated B cells to interfollicular and outer follicular regions of secondary lymphoid tissues. Moreover, modulation of EBI2 expression is crucial for the generation of extrafollicular plasma cell responses and germinal center formation. Here, we review the current findings that have delineated the function of EBI2 and its ligand and discuss how they collaborate with conventional lymphoid chemokine systems to position B cells optimally during immune responses.

Microenvironmental niches for naïve and activated B cells

Secondary lymphoid organs, such as the spleen and lymph nodes, are structurally and functionally compartmentalized to provide a set of microenvironmental niches that support the survival of resting B cells, as well as the activation and differentiation of B cells participating in antibody responses. In secondary lymphoid organs, B and T cells segregate to form B cell follicles that surround a central T cell zone (Figure 1). In the spleen, B cell follicles are surrounded by the marginal zone, an area rich in macrophages that also harbors a population of resident marginal zone B cells. Dendritic cell (DC) populations are localized in T cell areas as well as in interfollicular areas and the splenic marginal zone bridging channels, which are breaks in the marginal sinus where the T cell zone abuts the red pulp. Central to the compartmentalization of lymphoid organs is the localized production of chemokines and other guiding cues by networks of stromal cells (Table 1). The chemokine CXC ligand (CXCL)13 is made by follicular stromal cells, which comprise the marginal reticular cells in the outer follicle and a central network of follicular dendritic cells (FDCs) [1]. Naïve recirculating B cells respond primarily to this chemokine as a result of their high expression of chemokine CXC receptor (CXCR)5 and therefore migrate to B cell follicles [2]. By contrast, the fibroblastic reticular cell network in T cell zones is characterized by expression of the chemokines CC ligand (CCL)19 and CCL21, which mediate the migration of chemokine CC receptor (CCR)7-expressing T cells to these areas [3].

During antibody responses, B cell migration is orchestrated to facilitate: (i) access to antigen; (ii) interactions with helper T cells; and (iii) homing to the specialized microenvironments that promote rapid or long-term antibody production. Naïve B cells patrol secondary lymphoid organs for the presence of antigen by rapid, random movement within follicles. After antigen encounter, B cells move to the boundary between B cell follicles and T cell areas where they interact with T helper cells and undergo initial proliferation (Figure 2) [4]. This movement occurs about 6 h after activation and is directed by the antigen-mediated upregulation of CCR7; the receptor for the T cell zone chemokines CCL19 and CCL21 [5]. Early in the response, proliferating B cells begin to follow one of two alternate fates by differentiating into short-lived plasma cells or germinal center (GC) B cells. B cells recruited into the former pathway migrate to extrafollicular sites (Figure 2) where their proliferation and differentiation into plasmablasts and plasma cells is sustained [6]. Alternatively, B cells can localize to the FDC-dense areas in the center of follicles where they seed GCs (Figure 2) [7,8]. As the response progresses, B cells leave GC reactions either as long-lived plasma cells, which take up residency in the bone marrow, or as memory B cells [8].

It has only recently been appreciated that antigen-activated B cells undertake two additional transient migrations during the early stages of T-dependent antibody responses. In the first of these, antigen-activated B cells migrate rapidly (within 2–3 h) to the outer regions of the follicle before they relocate to the T–B boundary to receive T cell help (Figure 2) [9]. After interacting with T cells at the T–B boundary, activated B cells have also been shown to relocalize to interfollicular and outer follicular regions prior to their differentiation into early plasmablasts or entry into GCs (Figure 2) [10–13]. It transpires that both of these early B cell migratory steps are mediated by the chemoattractant receptor EBI2 (also known as GPR183) and its oxysterol ligand [9,14–17]. In addition, modulation of chemotactic signal delivered to responding B cells through EBI2 plays a critical role in delivering them to the correct microenvironments to undergo plasmablast versus GC B cell differentiation. Thus, EBI2 is part of a network of chemoattractant receptors that regulates B cell localization, providing an extra dimension to the migratory cues provided by conventional chemokines

Corresponding author: Brink, R. (r.brink@garvan.org.au).
Keywords: EBI2; oxysterol; B cell migration; antibody response.

1471-4906/$ – see front matter
 http://dx.doi.org/10.1016/j.it.2013.01.007

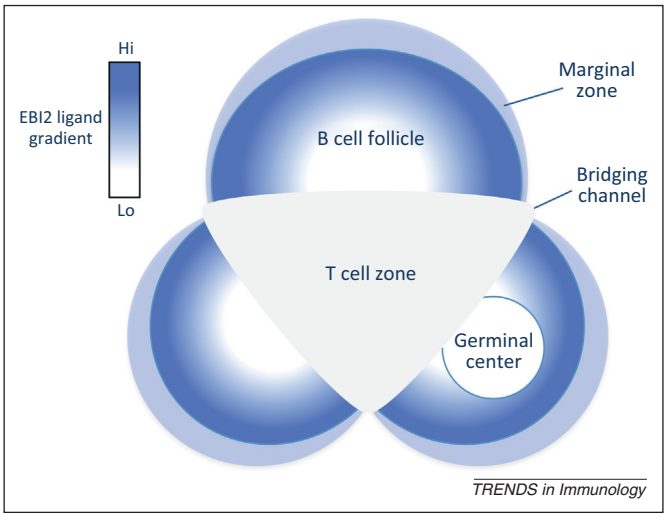

Figure 1. Epstein–Barr virus-induced molecule 2 (EBI2)-ligand gradients within the spleen. A stylized view of a cross-section of the splenic white pulp is depicted indicating the B cell rich follicle, the surrounding marginal zone, central T cell zone, and the intervening bridging channel regions. The putative distribution of EBI2 ligand 7α,25-dihydroxycholesterol (7α,25-OHC) is shown, indicating a gradient from the outer follicular regions to the central follicle. Following initiation of T-dependent immune responses, germinal centers form in the central follicular regions where EBI2-ligand concentrations are low. Not shown is the distribution of the ligands for chemokine CXC receptor (CXCR)5 and CCR7, which localize principally within the follicle and T cell zones, respectively (Table 1).

and their receptors. Here, we review the role of EBI2 and oxysterols in controlling B cell migration and discuss how EBI2 collaborates with the chemokine receptors CXCR5, CCR7, and CXCR4 to position B cells appropriately during T-dependent antibody responses.

EBI2 structure and expression

The gene encoding EBI2 was originally identified together with *EBI1 (CCR7)* as the most highly upregulated gene in Epstein–Barr virus-infected Burkitt's lymphoma cells [18]. EBI2 belongs to the rhodopsin-like subfamily of seven transmembrane GPCRs [19]. Although it is not highly homologous to any other known GPCRs, sequence alignments have clustered EBI2 with lipid receptors and indicated highest sequence similarity with GPR18 [19,20]. Studies of the activity of EBI2 have shown that it signals through the pertussis-sensitive $G\alpha_i$ protein [19].

Expression of EBI2 is most abundant in lymphoid tissues, with B cells, T cells, and various myeloid cells displaying high levels of *EBI2* mRNA [15,18,19]. EBI2 is expressed constitutively by mature B cells [17] and displays a characteristic expression pattern linked to B cell differentiation in both humans and mice. The already high constitutive EBI2 expression of naïve B cells is transiently increased after activation [9,14,17,21,22]. By contrast, GC B cell differentiation is associated with the shutdown of EBI2 expression [14,17,21,22]. Plasma cells maintain expression of EBI2, albeit at lower levels than do naïve B cells [14,17].

Little is known about the transcriptional and molecular regulation of *EBI2* expression. *In vitro*, cytokines such as interleukin (IL)-4, IL-6, and IL-10 have been shown to modulate *EBI2* expression, whereas lipopolysaccharide (LPS) stimulation upregulates *EBI2* transcription in most leukocyte populations [16,22,23]. Similar to chemokine receptors, cell surface EBI2 expression is downregulated by ligand binding through β-arrestin-mediated receptor internalization [15,16,24].

Oxysterols: the endogenous ligands for EBI2

Although EBI2 was initially suggested to have constitutive activity [19], it was subsequently deorphanized through the screening of tissue extracts with classic analytical methods and was shown to be the first GPCR activated by oxysterols [15,16]. The oxysterol 7α,25-dihydroxycholesterol (7α,25-OHC) was found to be a potent and selective agonist of EBI2 and its most likely endogenous ligand [15,16]. 7α,25-OHC is an oxygenated derivative of cholesterol and was previously identified as an intermediate product in the synthesis of bile acids [25]. Conversion of cholesterol into 7α,25-OHC is carried out by the biosynthetic enzymes cholesterol 25-hydroxylase (CH25H) and oxysterol 7α-hydroxylase (CYP7B1) [25], which are highly expressed by lymphoid stromal cells [15,24]. Although 7α,25-OHC appears as the major endogenous ligand for EBI2, inactivation of 7α,25-OHC synthesis via deletion of the *Ch25h* gene does not completely remove EBI2-ligand activity [24]. Thus natural oxysterols other than 7α,25-OHC may trigger EBI2 *in vivo*, consistent with the capacity of the receptor to bind and respond to several cholesterol derivates with high structural homology to 7α,25-OHC [15,16].

Expression of both CH25H and CYP7B1 by lymphoid stromal cells is required for the synthesis of 7α,25-OHC in lymphoid tissues and the correct positioning of B cells. Production of EBI2 ligand by hematopoietically derived cells appears limited [24] but may occur under some circumstances, given that both *Ch25h* and *Cyp7b1* can be expressed by hematopoietic cells [24]. Degradation of 7α,25-OHC is mediated by the enzyme 3β-hydroxy-Δ^5-C_{27} steroid oxidoreductase (HSD3B7) [25], which is also expressed in lymphoid stromal cells and in T cell zone DCs [24]. Differential expression of CH25H, CYP7B1, and HSD3B7 in stromal cells present in distinct compartments of secondary lymphoid organs appears to establish a gradient of 7α,25-OHC. The biosynthetic enzymes are more highly expressed in stromal cells at the perimeter of the follicle compared to stromal cells and FDCs in the center of the follicles, therefore, EBI2 ligand is higher at the follicle perimeter than in the inner follicle (Figure 1) [24]. By contrast, the degradation of 7α,25-OHC is increased in T cell zones, due to high levels of HSD3B7 expression in stromal cells within these areas, keeping levels of EBI2 ligand low [24]. HSD3B7 expression by splenic DCs, in particular of the $CD8^+$ subset, helps maintain low EBI2 ligand concentrations in the T cell zone. Levels of 7α,25-OHC have been shown to be increased in mouse lymphoid tissues upon LPS injection in a CH25H-dependent manner [15,16], suggesting that EBI2 ligand production may be regulated during infection, as has been observed for lymphoid chemokines [26].

Guidance of B cell localization by EBI2

EBI2-mediated distribution of B cells in follicles

Within follicles, B cells are exposed to multiple competing chemotactic stimuli as a result of their coexpression of

Table 1. Chemokine receptors and ligands controlling B cell migration in secondary lymphoid organs.

Receptor	Ligand	Ligand production
CXCR5	CXCL13	B cell follicles
CCR7	CCL19, CCL21	T cell zone
CXCR4	CXCL12	GC dark zone, splenic red pulp, lymph node medullary cords
EBI2	7α,25-OHC	Interfollicular and outer follicular areas, marginal zone bridging channels

CXCR5, CCR7, and EBI2, each of which mediates chemotaxis towards the source of its respective ligand (Table 1). The position of B cells in follicles is defined by a hierarchical integration of the various chemotactic signals. EBI2 is active in naïve B cells, as indicated by chemotaxis of naïve B cells to 7α,25-OHC [15,16]. Nevertheless, the organization of B cells into follicles does not appear to require EBI2, because the structure of spleen and lymph nodes of EBI2-deficient mice is normal [14,17,27]. The activity of CXCR5 is dominant in naïve B cells and overshadows the function of EBI2. This was demonstrated by studies showing that the removal of CXCR5–CXCL13 signaling, such as in *Cxcr5*$^{-/-}$ or *Cxcl13*$^{-/-}$ mice, results in the normal organization of splenic B cells becoming dependent on EBI2 [17,27]. Although B cells do not form normal follicular structures in the absence of CXCR5, they do still localize around T cell areas but in an abnormal ring-like structure. By contrast, when B cells also lack EBI2, they are unable to organize into these structures and instead disperse into the marginal zone area [17,27]. Similarly, the B cell aggregates that form in areas rich in splenic reticular fibroblasts and fibers in mice deficient for both CXCR5 and CCR7 are also lost when mice also lack EBI2 [27].

Although EBI2 only plays a secondary role in the formation of follicular structures, its expression levels greatly affect the position of B cells within follicles. EBI2-deficient B cells accumulate in the center of follicles in FDC-dense areas when transferred into wild type hosts or in mixed bone marrow chimeras containing a mix of EBI2-deficient and wild type B cells [14,17]. Conversely, overexpression of EBI2 in B cells by retroviral transduction promotes their localization to the outer areas of B cell follicles [17]. Wild type naïve B cells transferred into EBI2-deficient recipients distribute strikingly to the periphery of follicles; possibly as an effect of competition with the more numerous EBI2-deficient B cells unable to migrate to these areas [9]. Thus, the relative levels of EBI2 expression between B cells of the same compartment play a key role in determining their position within the follicular microenvironment. Existing evidence does not indicate a role for EBI2 in lymphocyte egress from the spleen and lymph node and this process seems primarily dependent on the sphingosine-1-phosphate receptor S1PR1 [28].

EBI2-dependent B cell positioning in the early stages of antibody responses

The best-characterized modulation of EBI2 on B cells is during the course of a T-dependent antibody response (Table 2), when EBI2 acts in concert with the migratory signals delivered by CXCR5 and CCR7 to localize B cells at various positions within lymphoid follicles. EBI2 was first noted as one of the genes that are upregulated after B cell receptor (BCR) stimulation by antigen [21]. *Ebi2* mRNA levels are increased in B cells within 1 h after activation in a nuclear factor (NF)-κB-dependent manner [9,14,21]. The upregulation of *Ebi2* mRNA is only transient and returns to resting levels within 6 h. However, EBI2 surface expression stays elevated 2 days after B cell activation by T-dependent antigen *in vivo* and the *in vitro* chemotaxis of activated B cells to 7α,25-OHC also remains increased compared to endogenous naïve B cells [9,15,24].

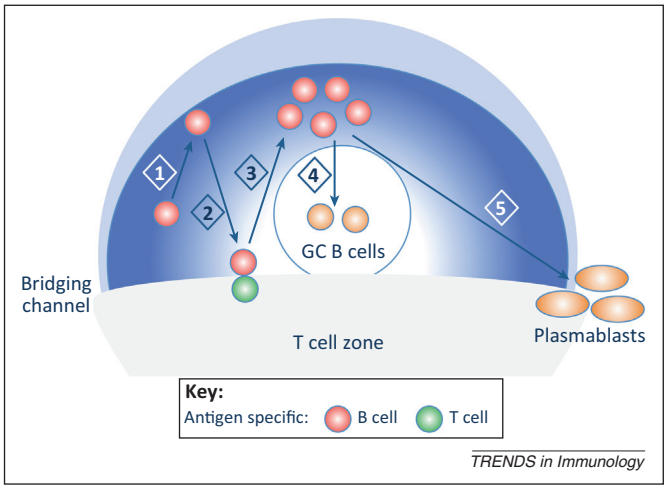

Figure 2. Epstein–Barr virus-induced molecule 2 (EBI2) and B cell migration during T cell-dependent antibody responses. Upon antigen encounter, activated B cells undergo a series of migratory steps that are mediated by the regulation of their responsiveness to chemoattractants expressed in the distinct lymphoid compartments. (1) Rapid upregulation of EBI2 expression results in the movement of antigen-activated B cells to the outer follicular regions within 2–3 h. (2) After 6 h, chemokine CC receptor (CCR)7 levels increase and B cells move back towards the T cell zone (red) to seek T cell help. EBI2 cooperates with CCR7 to distribute activated B cells uniformly along the T–B boundary. (3) 2–3 days after initial antigen exposure, B cells relocalize to interfollicular and outer follicular regions. Movement towards these areas high in 7α,25-dihydroxycholesterol (7α,25-OHC) concentration is mediated by EBI2, following downregulation of CCR7 on responding B cells. Here, activated B cells undergo proliferation and initial differentiation. (4) Some activated B cells commit to the germinal center (GC) differentiation pathway. These pre-GC B cells maintain chemokine CXC receptor (CXCR)5 but downregulate EBI2, which mediates their movement to the central follicular dendritic cell (FDC)-dense areas of follicles where they proliferate and form GCs. (5) Alternatively, other activated B cells maintain EBI2 expression but downregulate CXCR5, migrating to interfollicular regions and bridging channels where they differentiate into plasmablasts and subsequently plasma cells.

The rapid upregulation of EBI2 expression by antigen precedes that of CCR7, leading to a short interval following initial B cell activation (1–6 h) where EBI2 ligand provides the dominant migratory signals. Accordingly, the initial migration of activated B cells is towards the outer follicular regions where EBI2 ligand is known to accumulate (Figure 2) [9,24]. Although it is not clear why activated B cells undergo this initial movement to the outer follicle, it may be to acquire additional antigen presented in these regions by the macrophages that line the marginal sinus of the spleen and the subcapsular sinus of the lymph node [29,30]. Although CCR7-deficient B cells remain in these outer follicular regions following antigen activation [5,27], wild type B cells subsequently upregulate CCR7, resulting in their movement to the T–B boundary around 6 h after initial antigen encounter [5,9].

Once at the T–B boundary, signals delivered through EBI2 continue to influence the localization of the activated

Table 2. Responsiveness of B cell populations to EBI2-, CCR7-, CXCR5- and CXCR4-mediated chemotaxis.

Receptor	EBI2	CCR7	CXCR5	CXCR4
Naïve B cells	++	+	+++	+
Activated B cells (2–6 h)	+++	+++	++	+
Activated B cells (2–3 days)	+++	+	++	+
Dark zone GC B cells	–	–	++	+++
Light zone GC B cells	+	–	++	+
Plasmablasts and plasma cells	+	–	–	+++

B cells. Thus, in the absence of EBI2 expression, B cells cluster at the midline of the follicle/T zone interface rather than distributing along the length of the T–B boundary, with some cells entering T cell areas [9,14]. A similar observation has been made in CH25H-, CYP7B1-, and HSD3B7-deficient mice in which EBI2 ligand gradients are disrupted [24].

As the B cell response progresses, CCR7 expression subsides [9,13] and B cells once again become increasingly influenced by signals from EBI2 ligand due to the still elevated expression of EBI2 [24]. Thus, around 2–3 days after antigen encounter, EBI2 counteracts CCR7-mediated positioning of B cells at the T–B boundary and mediates B cell relocalization to interfollicular and outer follicular regions (Figure 2). This migratory event is promoted by interaction with T cells and CD40 engagement [9,17]. If B cells lack EBI2 or if EBI2 ligand production is disrupted, B cells fail to migrate to the periphery of follicles and localize to central areas of follicles [14,15,17,24]. Localization of B cells to interfollicular and outer follicular regions is important for the initial burst of B cell proliferation and GC commitment [10–12]. In interfollicular regions B cells engage in long-lived interactions with cognate T cells, which are thought to drive subsequent differentiation [11]. DCs localized in marginal zone bridging channels have also been shown to interact with activated B cells and promote their activation and extrafollicular plasma cell responses in situations in which antigen is targeted to this DC population [31]. However, the nature of the signals that B cells receive at the periphery of follicles and how these cues influence their commitment to extrafollicular plasma cells or GC B cells remains unclear.

EBI2 requirement for plasmablast generation

Although it has not yet been determined whether commitment of activated B cells to early plasmablasts takes place in interfollicular and outer follicular regions, it is now clear that B cell migration to these areas is crucial for subsequent antibody production. The early bifurcation of B cell differentiation to plasma cells or GC B cells is accompanied by changes in EBI2 and chemokine receptor expression (Table 2). B cells differentiating into plasmablasts retain EBI2 expression while downregulating CXCR5 and upregulating CXCR4 expression [13,17]. Expression of CXCR4 is required for localization of plasma cells in the splenic red pulp and lymph node medullary cords [32]. However, plasma cells generated from B cells deficient for CXCR4 retain the ability to access extrafollicular areas and accumulate at the perimeter of follicles [32]; possibly as a result of chemotaxis to EBI2 ligands concentrated in this area. By contrast, EBI2-deficient B cells fail to migrate to extrafollicular areas and to differentiate into plasmablasts, which leads to greatly reduced early antibody production [14]. Defects in early plasma cell generation have also been observed in mice deficient for enzymes required for establishing EBI2 ligand gradients, confirming the importance of EBI2-mediated chemotaxis to 7α,25-OHC for rapid antibody production to T cell-dependent antigens [15,24]. Interfollicular regions and marginal zone bridging channels are rich in DCs, which have been shown to associate closely with plasmablast and produce factors, such as IL-6 and a proliferation-inducing ligand (APRIL), which promote plasma cell growth and survival [33–35]. As discussed above, T cells also localize to interfollicular regions early in the response [11,12] and therefore may provide signals that are required for commitment of B cells to the plasmablast pathway. Furthermore, T cells undergo cognate interactions with plasmablasts in bridging channels and therefore might be involved in supporting plasma cell growth in these sites [13]. Plasma cell differentiation of EBI2-deficient B cells occurs normally *in vitro* and 7α,25-OHC has no effect on B cell proliferation and survival in culture [14,16], thus, it is unlikely that engagement of EBI2 by its ligand directly influences the generation of plasma cells. Rather, EBI2 is likely to mediate its effects by directing activated B cells to the distinct microenvironments that are conducive for induction, maturation, and/or survival of plasmablasts and plasma cells.

Downregulation of EBI2 and GC formation

GC B cell differentiation is characterized by a marked downregulation of EBI2 expression, which is mediated by the transcriptional repressor B cell lymphoma-6 (BCL-6) [14,17,21,22]. Expression of BCL-6 is first observed in interfollicular regions and it is in these sites that commitment of activated B cells to the GC pathway is thought to take place [10–12]. The loss of EBI2 expression that is brought about by the induction of BCL-6 leads to reduced responsiveness of activated B cells to local EBI2 ligands and consequent migration of pre-GC B cells from interfollicular regions to the FDC-rich center of follicles (Figure 2). This relocalization is most likely to be caused by increased chemotaxis to CXCL13 and by sphingosine-1-phosphate receptor $S1P_2$-mediated GC cell clustering in the centre of follicles [36]. The importance of BCL-6-mediated suppression of EBI2 for GC formation has been highlighted by the defects in GC formation of B cells with constitutive expression of EBI2. When B cells are made unable to downregulate EBI2 due to retroviral overexpression, their participation in GC reactions is reduced and activated B cells preferentially differentiate into plasmablasts [14,17]. Similarly, B cells hypomorphic for BCL-6 have been shown to be impaired in the entry to GCs, suggesting that BCL-6 upregulation in pre-GC B cells enables these cells to migrate into GC clusters [12]. These findings indicate that BCL6-induced EBI2 downregulation is a crucial component of the transcriptional changes that accompany GC formation.

GCs form normally in EBI2-deficient mice and the segregation of GC B cells into dark and light zones is

not affected. Although *EBI2* expression levels are low on GC B cells, EBI2 has been shown to be differentially expressed in GC dark and light zone populations, with light zone GC B cells expressing higher levels of *EBI2* than dark zone B cells [37]. Compartmentalization of GC B cells into dark and light zones is mediated by the differential abundance of the chemokines CXCL12 and CXCL13, and movement of GC B cells between zones is mediated by regulated expression of CXCR4 on dark zone and light zone GC B cells [7,8,38]. Changes in EBI2 expression in GC B cells may aid GC B cell movement during the process of affinity maturation and help position B cells in light zones towards a higher gradient of EBI2 ligand. Accordingly, EBI2 expression has been shown to influence the balance between dark zone and light zone GC B cell subsets and promote affinity maturation [27]. However, it is still unknown whether EBI2 plays a role downstream of GCs by regulating the generation, migration and/or function of the effectors of long-term humoral immunity, memory B cells, and long-lived bone marrow plasma cells.

EBI2 expression and B cell disease

Although chemoattractant receptors of the GPR family play essential roles in coordinating the migration of lymphocytes for efficient responses against pathogens, their dysregulation can result in the initiation or progression of inflammatory and autoimmune disorders. Involvement of EBI2 with inflammation has been suggested by the association of polymorphisms in the gene encoding EBI2 with susceptibility to type 1 diabetes and other inflammatory diseases [39]. In rats, EBI2 has been shown to regulate the inflammatory response of macrophages [39], but its role in regulating autoimmune B cells in diabetes has not yet been investigated. *EBI2* has also been found to be among the group of dysregulated genes in systemic lupus erythematosus patients, and is reported to be downregulated in peripheral blood cells from lupus patients compared to healthy controls [40]. Furthermore, *EBI2* maps to a chromosomal region that shows linkage in genome-wide scans of lupus patients [41,42].

Several studies have also linked regulation of EBI2 expression to human neoplastic diseases, such as acute myeloid leukemia, chronic lymphocytic leukemia, and diffuse large B cell lymphoma [43–46]. Gene expression profiling has indicated that *EBI2* expression is downregulated in follicular and GC B-like diffuse large B cell lymphoma [45,46]. It is yet to be determined whether this low expression of *EBI2* is somehow involved in cancer progression or simply reflects the expression of *EBI2* by the original cell type prior to transformation.

As mentioned above, infection of human B cells by Epstein–Barr virus induces high levels of EBI2 [18]. Upregulation of EBI2 on infected B cells is likely to mediate the observed propensity of Epstein–Barr virus-positive B cells to accumulate in interfollicular regions and avoid GCs [47,48]. During infectious mononucleosis this might be a strategy of Epstein–Barr virus to direct infected B cells to microenvironments conducive for their survival and to escape immune surveillance.

Concluding remarks

Although much has been learned about the migratory events and interactions that B cells undergo during antibody responses, only recently has the contribution of EBI2 and oxysterols in guiding B cells to niches that support their response become clear. EBI2-mediated chemotaxis acts in concert with the migratory signals delivered by lymphoid chemokines to localize B cells at various positions within lymphoid follicles during antibody responses. In the early stages of B cell responses EBI2 controls the migration of activated B cells to interfollicular and outer follicular regions; a relocalization step that is crucial for induction of antibody responses. Modulation of EBI2 on responding B cells recruits differentiating B cells to either the extrafollicular or the GC pathway of antibody production and therefore plays an important role in coordinating rapid versus long-term humoral responses.

Despite these new insights, future studies will be required to assess the significance of EBI2-mediated migration for B cell antigen encounter and to determine whether regulation of EBI2 expression plays a role in the movement of naïve B cells surveying the follicles. It will be important to dissect the nature of the signals and interactions that activated B cells seek in interfollicular and outer follicular niches and how these cues shape subsequent B cell differentiation. Furthermore, it will be interesting to investigate whether EBI2 plays a role in the localization of other leukocytes to interfollicular regions and to examine how EBI2 expression regulates their function and interactions with B cells. We also need a better understanding of how dysfunctional regulation of EBI2 may contribute to the induction and progression of inflammation and autoimmunity.

The GPR nature of EBI2 makes this receptor a highly suitable target for pharmaceutical intervention with small molecule drugs. The structural motifs critical for EBI2 function and the location and composition of its ligand-binding domain in EBI2 have started to be elucidated [49,50]. This information will facilitate future efforts to design novel therapeutic agents that may serve as agonists or antagonists for EBI2 to modulate inflammatory and autoimmune diseases or advance vaccine strategies.

Acknowledgments

The authors are supported by grants and fellowships from the National Health and Medical Research Council of Australia.

References

1 Cyster, J.G. *et al.* (2000) Follicular stromal cells and lymphocyte homing to follicles. *Immunol. Rev.* 176, 181–193
2 Förster, R. *et al.* (1996) A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* 87, 1037–1047
3 Cyster, J.G. (2005) Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. *Annu. Rev. Immunol.* 23, 127–159
4 Okada, T. and Cyster, J.G. (2006) B cell migration and interactions in the early phase of antibody responses. *Curr. Opin. Immunol.* 18, 278–285
5 Reif, K. *et al.* (2002) Balanced responsiveness to chemoattractants from adjacent zones determines B-cell position. *Nature* 416, 94–99
6 MacLennan, I.C. *et al.* (2003) Extrafollicular antibody responses. *Immunol. Rev.* 194, 8–18

7 Gatto, D. and Brink, R. (2010) The germinal center reaction. *J. Allergy Clin. Immunol.* 126, 898–907
8 Zotos, D. and Tarlinton, D.M. (2012) Determining germinal centre B cell fate. *Trends Immunol.* 33, 281–288
9 Kelly, L.M. *et al.* (2011) EBI2 guides serial movements of activated B cells and ligand activity is detectable in lymphoid and nonlymphoid tissues. *J. Immunol.* 187, 3026–3032
10 Coffey, F. *et al.* (2009) Initial clonal expansion of germinal center B cells takes place at the perimeter of follicles. *Immunity* 30, 599–609
11 Kerfoot, S.M. *et al.* (2011) Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. *Immunity* 34, 947–960
12 Kitano, M. *et al.* (2011) Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. *Immunity* 34, 961–972
13 Chan, T.D. *et al.* (2009) Antigen affinity controls rapid T-dependent antibody production by driving the expansion rather than the differentiation or extrafollicular migration of early plasmablasts. *J. Immunol.* 183, 3139–3149
14 Gatto, D. *et al.* (2009) Guidance of B cells by the orphan G protein-coupled receptor EBI2 shapes humoral immune responses. *Immunity* 31, 259–269
15 Hannedouche, S. *et al.* (2011) Oxysterols direct immune cell migration via EBI2. *Nature* 475, 524–527
16 Liu, C. *et al.* (2011) Oxysterols direct B-cell migration through EBI2. *Nature* 475, 519–523
17 Pereira, J.P. *et al.* (2009) EBI2 mediates B cell segregation between the outer and centre follicle. *Nature* 460, 1122–1126
18 Birkenbach, M. *et al.* (1993) Epstein–Barr virus-induced genes: first lymphocyte-specific G protein-coupled peptide receptors. *J. Virol.* 67, 2209–2220
19 Rosenkilde, M.M. *et al.* (2006) Molecular pharmacological phenotyping of EBI2. An orphan seven-transmembrane receptor with constitutive activity. *J. Biol. Chem.* 281, 13199–13208
20 Surgand, J.S. *et al.* (2006) A chemogenomic analysis of the transmembrane binding cavity of human G-protein-coupled receptors. *Proteins* 62, 509–538
21 Glynne, R. *et al.* (2000) B-lymphocyte quiescence, tolerance and activation as viewed by global gene expression profiling on microarrays. *Immunol. Rev.* 176, 216–246
22 Shaffer, A.L. *et al.* (2000) BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. *Immunity* 13, 199–212
23 Lam, L.T. *et al.* (2008) Cooperative signaling through the signal transducer and activator of transcription 3 and nuclear factor-κB pathways in subtypes of diffuse large B-cell lymphoma. *Blood* 111, 3701–3713
24 Yi, T. *et al.* (2012) Oxysterol gradient generation by lymphoid stromal cells guides activated B cell movement during humoral responses. *Immunity* 37, 535–548
25 Russell, D.W. (2003) The enzymes, regulation, and genetics of bile acid synthesis. *Annu. Rev. Biochem.* 72, 137–174
26 Mueller, S.N. *et al.* (2007) Regulation of homeostatic chemokine expression and cell trafficking during immune responses. *Science* 317, 670–674
27 Gatto, D. *et al.* (2011) EBI2 operates independently of but in cooperation with CXCR5 and CCR7 to direct B cell migration and organization in follicles and the germinal center. *J. Immunol.* 187, 4621–4628
28 Cyster, J.G. and Schwab, S.R. (2012) Sphingosine-1-phosphate and lymphocyte egress from lymphoid organs. *Annu. Rev. Immunol.* 30, 69–94
29 Cyster, J.G. (2010) B cell follicles and antigen encounters of the third kind. *Nat. Immunol.* 11, 989–996
30 Phan, T.G. *et al.* (2007) Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* 8, 992–1000
31 Chappell, C.P. *et al.* (2012) Extrafollicular B cell activation by marginal zone dendritic cells drives T cell-dependent antibody responses. *J. Exp. Med.* 209, 1825–1840
32 Hargreaves, D.C. *et al.* (2001) A coordinated change in chemokine responsiveness guides plasma cell movements. *J. Exp. Med.* 194, 45–56
33 Garcia De Vinuesa, C. *et al.* (1999) Dendritic cells associated with plasmablast survival. *Eur. J. Immunol.* 29, 3712–3721
34 Kabashima, K. *et al.* (2005) Intrinsic lymphotoxin-beta receptor requirement for homeostasis of lymphoid tissue dendritic cells. *Immunity* 22, 439–450
35 Mohr, E. *et al.* (2009) Dendritic cells and monocyte/macrophages that create the IL-6/APRIL-rich lymph node microenvironments where plasmablasts mature. *J. Immunol.* 182, 2113–2123
36 Green, J.A. *et al.* (2011) The sphingosine 1-phosphate receptor S1P(2) maintains the homeostasis of germinal center B cells and promotes niche confinement. *Nat. Immunol.* 12, 672–680
37 Victora, G.D. *et al.* (2010) Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell* 143, 592–605
38 Allen, C.D. *et al.* (2007) Germinal-center organization and cellular dynamics. *Immunity* 27, 190–202
39 Heinig, M. *et al.* (2010) A trans-acting locus regulates an anti-viral expression network and type 1 diabetes risk. *Nature* 467, 460–464
40 Ye, S. *et al.* (2003) Protein interaction for an interferon-inducible systemic lupus associated gene, IFIT1. *Rheumatology* 42, 1155–1163
41 Cantor, R.M. *et al.* (2004) Systemic lupus erythematosus genome scan: support for linkage at 1q23, 2q33, 16q12-13, and 17q21-23 and novel evidence at 3p24, 10q23-24, 13q32, and 18q22-23. *Arthritis Rheum.* 50, 3203–3210
42 Moser, K.L. *et al.* (1998) Genome scan of human systemic lupus erythematosus: evidence for linkage on chromosome 1q in African-American pedigrees. *Proc. Natl. Acad. Sci. U.S.A.* 95, 14869–14874
43 Aalto, Y. *et al.* (2001) Distinct gene expression profiling in chronic lymphocytic leukemia with 11q23 deletion. *Leukemia* 15, 1721–1728
44 Ley, T.J. *et al.* (2008) DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. *Nature* 456, 66–72
45 Alizadeh, A.A. *et al.* (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403, 503–511
46 Shipp, M.A. *et al.* (2002) Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat. Med.* 8, 68–74
47 Kurth, J. *et al.* (2000) EBV-infected B cells in infectious mononucleosis: viral strategies for spreading in the B cell compartment and establishing latency. *Immunity* 13, 485–495
48 Niedobitek, G. *et al.* (1992) Patterns of Epstein–Barr virus infection in non-neoplastic lymphoid tissue. *Blood* 79, 2520–2526
49 Benned-Jensen, T. *et al.* (2012) Molecular characterization of oxysterol binding to the Epstein–Barr virus-induced gene 2 (GPR183). *J. Biol. Chem.* 287, 35470–35483
50 Zhang, L. *et al.* (2012) Identification of structural motifs critical for EBI2 function and homology modeling of ligand docking site. *Mol. Pharmacol.* 82, 1094–1103