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Immunol Rev. Author manuscript; available in PMC 2013 May 01.

Published in final edited form as:
Immunol Rev. 2012 May ; 247(1): 36–51. doi:10.1111/j.1600-065X.2012.01114.x.

S1PR2 links germinal center confinement and growth regulation

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Summary

Germinal centers (GCs) are sites of rapid B-cell proliferation and somatic mutation. These ovoid structures develop within the center of follicles and grow to a stereotypic size. The cell migration and interaction dynamics underlying GC B-cell selection events are currently under intense scrutiny. In recent work, we identified a role for a migration inhibitory receptor, S1PR2, in promoting GC B-cell confinement to GCs. S1PR2 also dampens Akt activation and deficiency in S1PR2 or components of its signaling pathway result in a loss of growth control in chronically stimulated mucosal GCs. Here we detail present understanding of S1PR2 and S1P biology as it pertains to GC B cells and place this information in the context of a current model of GC function.

Keywords

S1P; chemokine; migration; affinity maturation; apoptosis; antibody

Introduction

Germinal centers (GCs) have long been noted for their distinctive histological appearance, high frequency of mitotic figures, and formation within the center of lymphoid follicles during immune responses (1), and more recently for their connection to the development of a high affinity antibody response (2). The hallmark structure of the T-dependent antibody response, GCs have attracted considerable interest as to how their complex microenvironmental organization and cellular dynamics could contribute to their functional output: antibody-secreting plasma cells and long-lived memory cells that express antigen receptors of high affinity for antigen present on the invading pathogen or immunogen (2, 3).

Antibody affinity maturation, which refers to the phenomenon in which the affinity of serum antibody for a foreign antigen increases over time after immunization (4), is strongly associated with the GC based on several observations. GC B cells accumulate increasing numbers of somatic mutations in their immunoglobulin (Ig) genes during the first two to three weeks after immunization (5, 6). These mutations are focused in antigen-binding regions of the Ig genes, and specific mutations known to increase the affinity for antigen appear with increasing frequency over time in GC B cells, suggesting that an ongoing selection mechanism is taking place within the GC.

In addition, a physiological role for GCs has been shown by studies using mouse models in which GC formation is disrupted. Mice deficient in CD28 or ICOS costimulatory molecule function (7, 8), and mice lacking the gene encoding the lymphotoxin receptor in stromal

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The authors have no conflicts of interest to declare.

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cells cannot form GCs (9). In each case, the absence of GCs correlates with low levels of class switching, reduced accumulation of somatic hypermutations, and low levels of antigen-specific antibodies. Some affinity maturation can take place in experimental systems in which GC formation is disrupted (10, 11) suggesting that GCs are not absolutely required for the competition and selection that leads to improved antigen affinity. However, repeated immunizations or strong adjuvants are used to induce antibody responses in these systems. Thus, the GC environment is likely optimized for the development of high affinity antibody responses.

B-cell antigen encounter and early movements

GCs form in the center of B-cell follicles during T-dependent antibody responses after a series of coordinated movements through lymphoid environments by antigen-activated B and T cells (12). Prior to activation, naive B and T cells are compartmentalized into distinct regions within the spleen and lymph nodes, characterized by B-cell follicles arranged around a central T zone. Both regions also contain distinct reticular networks of stromal cells, among which naive B and T cells move (13, 14). These stromal cells have long processes that present chemokines that help to guide the movements of B and T cells into and throughout their respective compartments. In the B-cell follicle, follicular dendritic cells (FDCs) as well as other follicular stromal cells express CXCL13, which attracts CXCR5-expressing B cells (Fig. 1). T-zone fibroblastic reticular cells (FRCs) express CCL19 and CCL21, which promote migration of CCR7-expressing T cells.

Migration through follicles promotes the encounter of B cells with their cognate antigen (15). Antigens can reach the follicle and be displayed to B cells in several different ways, which depend on the size and form of the antigen and the route of its entry to the body. Small soluble antigens can be drained from skin tissue by the flow of lymph, enter lymph nodes through afferent lymphatics and flow through the subcapsular sinus (SCS) before reaching the medullary sinus. Some small antigens can access the follicle by diffusing through conduits that reach from the SCS to high endothelial venules (HEVs) where B cells enter the lymph node. SCS macrophages can also pick up antigen through a number of cell surface receptors and transfer it to B cells in the follicle. Large particles, on the other hand, may be taken up by dendritic cells (DCs) or other myeloid cells, which can migrate into the lymph node and display recycled antigen to B cells. In addition, B cells themselves can carry antigen in the form of immune complexes on their complement receptors (CRs) or possibly FcγRIIb, and can pass them to FDCs in the center of the follicle. FDCs can bind and display immune complexes for long periods of time for recognition by cognate B cells.

Upon encountering cognate antigen and undergoing activation through the B-cell receptor (BCR), B cells upregulate CCR7, which directs them to the border of the T zone (12). There, the likelihood of interacting with cognate T cells is increased. Antigen-engaged B and T cells undergo stable contacts at the B-T border (16). Recent studies have revealed a previously overlooked stage of B-cell migration early during T-dependent immune responses, where activated B cells migrate to interfollicular (IF) and outer follicle regions after receiving T-cell help, where they can undergo proliferation for 1–2 days before returning to the center of the follicle to initiate GC clustering (12, 17). This outward movement is directed by EBI2, a chemoattractant receptor that is upregulated on activated B cells (18–20). The oxysterol 7α,25-dihydroxycholesterol (7α,25-OHC) has been identified as a ligand for EBI2 and is abundant in lymphoid tissue (21, 22). Activated B cells in IF regions of lymph nodes undergo interactions with cognate T cells (16, 23, 24). In the recent study of Kerfoot *et al.* (24), GC commitment appeared to take place in the IF region as expression of Bcl6, a transcriptional repressor critical to GC function, was first observed in B cells in this region. The authors also observed a trend for B cells in the IF region to

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occasionally move into the center of the follicle, consistent with seeding of the GC by cells from the IF region. Another study utilized a YFP-Bcl6 fusion protein as a reporter for Bcl6 expression to identify activated B cells undergoing GC commitment and differentiation and found Bcl6 expression first among B cells in both IF regions and the outer follicle (25). Exactly what signals activated B cells receive at IF and outer follicle regions are as yet unknown. It will be important to determine how signals in these locations influence commitment to the GC or extrafollicular plasmablast fate.

Movement into the center of the follicle and seeding of the GC

The cues that guide GC B-cell precursors to the center of the follicle are not yet fully understood. Interestingly, the YFP-Bcl6 reporter used to track early B-cell movement and cell-fate commitments seemed to act as a functional hypomorph, and B cells homozygous for YFP-Bcl6 were impaired at forming GC B cells (25). Using transferred MD4 B cells expressing transgenic Ig specific for hen egg lysozyme (HEL), the authors found YFP-Bcl6 homozygous cells to be capable of reaching the GC border but defective at entering GC clusters. This result suggests that Bcl6 upregulation is necessary to confer the ability to migrate into GCs. The activated YFP-Bcl6 homozygous cells failed to downregulate EBI2 at the observed time point during the response, day 3.5. Downregulation of EBI2 takes place in GC B cells and is important for GC participation and GC B-cell positioning in the center of the follicle (18, 20). EBI2 was identified as a Bcl6 target in chromatin immunoprecipitation studies (26, 27), so it is possible that one reason Bcl6-hypomorphic cells couldn't enter GC clusters is the lack of EBI2 downregulation by Bcl6.

Another important determinant in GC positioning and organization is CXCL13. Without CXCL13, B cells still gather into separate rings around T zones but do not form polarized follicular clusters (28). B cells from mice deficient in CXCL13 or its receptor CXCR5 can form GCs, but they are smaller than normal and in the spleen GCs form in the periarteriolar lymphoid sheath (PALS) rather than the follicle, suggesting that CXCR5 function is important for the localization of GC precursors (28, 29). However, in the lymph nodes of CXCR5-deficient mice GCs are still localized to B-cell areas, indicating that additional factors can participate in the proper localization of GC precursor B cells in lymph nodes. In addition, CXCL13 is not known to be focused in the center of the follicle, as it is made broadly by follicular stromal cells (13, 30, 31), so while CXCL13 is important for follicular organization and attraction to the B-cell area, it is likely that additional cues exist to aid in the positioning of the GC B-cell precursors.

In a recent search for cues that may regulate GC B-cell positioning, GC B cells were found to upregulate and express high levels of sphingosine-1-phosphate receptor 2 (S1PR2), a member of the family of sphingosine-1-phosphate (S1P) receptors that includes 5 members (S1PR1-S1PR5) (32). S1P is a lipid signaling molecule that exerts wide effects upon immune cells (33). In particular, S1PR1 has a critical role in B and T-lymphocyte egress from lymphoid organs (34). We found that S1PR2 plays an important role in GC B-cell positioning, as well as in the homeostasis of chronically-stimulated GCs (32). *In vitro* migration assays showed that in the presence of S1P, S1PR2 negatively regulates GC B-cell migration to chemoattractants. Within lymphoid microenvironments, S1PR2 promotes B cell movement to the center of the follicle and confinement within the GC. In the sections below we detail present understanding of S1PR2 and S1P biology as it pertains to GC B cells and attempt to place this information in the context of a current model of GC function.

S1PR2 expression on GC B cells

Among different types of B cells S1PR2 is expressed specifically on GC B cells (32, 35). Little is known about the transcriptional regulation of *S1pr2*, the gene encoding S1PR2.

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Whether its upregulation in GC B cells is part of the BCL6-mediated GC transcriptional program, or whether other factors induce its expression during GC B-cell differentiation, remains an interesting question. As there is not yet a commercially available antibody against surface S1PR2 useful for flow cytometry, it is not known at what point during B-cell activation and GC differentiation S1PR2 appears on the cell surface. However, because S1PR2 inhibits cell migration, its functional activity can be measured by adding S1P to transwells during *in vitro* migration assays. Experiments in which antigen-specific B cells were labeled, transferred, and tracked throughout an immune response showed that after several cell divisions and coincident with upregulation of GC markers such as Fas and GL7, S1P began to inhibit B-cell migration to chemoattractants, suggesting it has an early role in positioning GC B cells (JAG and JGC, unpublished data).

S1PR2 inhibits cell migration

G-protein-coupled receptors (GPCRs) can mediate diverse signaling events through coupling to distinct heterotrimeric G-proteins (36). Unlike S1PR1, which couples to Gαi and promotes directed cell migration, S1PR2 inhibits the migration of various cell types (32, 37–41). Rather than coupling to Gαi, S1PR2 couples to Gα12 and Gα13, two closely related G proteins, and possibly also Gαq, thus providing an explanation for the different outcomes of signaling by the two receptors (42). Gαi signals downstream to induce activation of the Rac GTPase through a mechanism that is not fully understood in lymphocytes but that involves the activation of guanine nucleotide exchange factors (GEFs) specific for Rac such as DOCK2 (43–45). Activation of Rac-GEFs leading to Rac stimulation and cell migration seems to be mediated both through PI3K-dependent and PI3K-independent mechanisms that can involve direct activation by Gβγ subunits (44, 46). Rac-GEFs catalyze the exchange of GDP for GTP and activation of Rac, which can stimulate actin polymerization through WAVE and the Arp2/3 complex (47). In this way, sensing of Gαi-coupled ligands at the front of a migrating cell induces local Rac activation and actin polymerization and movement towards the ligand (Fig. 2).

In contrast to Gαi-coupled signaling, GPCRs that couple to Gα12 and Gα13 such as S1PR2 stimulate Rho activity through Rho-GEFs, including p115RhoGEF (also known as Lsc) (48, 49). Rho has a long-appreciated role in mediating cell body contraction and rear end retraction in migrating cells (50) but has also been shown to antagonize Rac activity through its effector ROCK and the activation of GTPase activating proteins (GAPs) specific for Rac, such as FilGAP (51) or ARHGAP22 (52). GAPs activate the GTP hydrolyzing activity of Rac, accelerating the transition of Rac to its inactive GDP-bound state. Rho-mediated antagonism of Rac has been shown to be important for S1PR2's inhibitory effect on cell migration in several cell types (38, 40). It is likely that the presence of ligands for Gα12- and Gα13-coupled receptors at the leading edge of a migrating cell leads to a local activation of Rho, which can antagonize Rac at this site and allow for Rac activation at a different side of the cell to predominate and promote movement away from the Gα12- and Gα13-coupled ligands (Fig. 2).

S1P production and distribution in the follicle

A sharp gradient of S1P is maintained between circulation and tissues, with much higher levels in lymph (high nanomolar range) and blood (micromolar range) than within tissues (53, 54). Current technologies have not permitted interstitial S1P concentrations within lymphoid tissue to be directly measured. However, S1P causes internalization of S1PR1, and surface levels of this receptor have been used as a proxy for the relative amounts of S1P within a tissue to show that they are likely in the low or sub-nanomolar range (34). Regulation of both S1P production and degradation are critical for proper S1P distribution *in*

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vivo and for immune cell exit from lymphoid organs (55). S1P is produced by sphingosine kinases intracellularly in all cell types, but the cell types important for the generation of secreted S1P in the extracellular space seem to be more specific. Red blood cells are an important source of plasma S1P, which contributes to thymic and splenic egress (53), and non-hematopoietic sources contribute to plasma S1P as well (53,56). Lymphatic endothelial cells produce S1P necessary for exit of lymphocytes from lymph nodes into lymph (57), suggesting that different cell types are important for S1P production in different compartments.

S1P lyase is critical for the maintenance of the S1P gradient between circulation and tissue, as it contributes to S1P degradation and low S1P levels within tissues (55). However, treatment of mice with an inhibitor of S1P lyase did not disrupt S1PR2 function in the GC, suggesting S1P lyase may not be critical for determining the S1P distribution pattern needed to promote GC organization (JAG and JGC, unpublished data). In addition to S1P lyase, sphingosine phosphate phosphatase 1 (Sgpp1) and Sgpp2 and three lipid phosphate phosphatases (LPP1-3) possess S1P-degradative ability. Recent evidence shows that LPP3 expression on endothelial and epithelial cells in the thymus contributes to the maintenance of low S1P levels that permit T-cell exit from the thymus into circulation (58).

How S1P is distributed within B-cell follicles in such a way to exert effects on GC B-cell centering and clustering is not yet fully understood. Staining of S1PR1 in tissue sections has been used to show internalization of the receptor on B cells close to or within S1P-containing lymphatic vessels (59), suggesting that this method could be used as an indirect test of the S1P levels to which B cells are exposed within follicles. In several attempts with this technique, we were not able to detect differences in the ratio of surface to intracellular S1PR1 on B cells in inner and outer regions of the follicle (JAG and JGC, unpublished data). This may in part have been due to background staining with the polyclonal rabbit antibody (Santa Cruz Biotech), as we observed B-cell staining in follicles of mice conditionally lacking S1PR1 from B cells (S1PR1f/- Mb1Cre mice; JAG and JGC, unpublished data). Even with adequate sensitivity, one reason that this technique might fail to detect an S1P gradient in the follicle is that B cells traverse distances of several microns per minute, possibly moving between regions of differing S1P concentration in time frames that are faster than the rate of S1PR1 internalization and recycling. However, several findings imply that S1P is present in a decaying gradient in the follicle (Fig. 1). The first is that S1PR2 overexpression in B cells favors their movement to the center of the follicle (31, 32). S1PR2 engagement by S1P inhibits migration to chemoattractants, so by extension if cells are present in a uniform field of attractant (such as CXCL13) S1PR2-expressing cells are most likely to move in the direction of lowest S1P. Second, the influence of S1PR2 on GC cell distribution in mixed chimeras, to be discussed further below, was lessened when chimera hosts lacked the ability to produce S1P, but not when S1P production was lacking specifically in FDCs (32). Thus, the important source(s) of S1P are stromal cells that are not the FDCs in the center of the follicle, implying that much of the relevant S1P is being produced outside the follicle center.

S1P's half-life in plasma is on the order of 15 min, suggesting tight temporal and spatial control of S1P distribution within tissues would be possible (56). Red blood cells in the plasma do not express S1P-degrading enzymes (53, 60), while B cells in the follicle do and can efficiently degrade S1P *in vitro* (32), suggesting that the S1P half-life in the follicle is likely very short and that B cells could actively maintain low S1P levels. In particular, B cells express higher levels of the ectoenzyme LPP3 than T cells, suggesting they may have a specialized ability to degrade extracellular S1P (32). Further, there is not extensive entry of naive B cells or other cell types into the GC throughout the GC response, suggesting that circulatory S1P would not be carried into the GC and would likely be degraded by B cells in

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the follicle before it reached the GC. As such, it seems reasonable to speculate that the center of the follicle and the GC in particular is a region of low S1P, allowing S1PR2 to contribute to maintaining GC confinement. It will be useful to determine if B cell-specific expression of enzymes with S1P-degrading potential is important for maintaining low levels of S1P in the follicle, and to narrow the source of relevant S1P to a specific subset of stromal cells.

GC organization and FDC polarization

Interactions between GC B cells and FDCs induce the maturation of the latter into GC-associated FDCs, which are characterized by increased expression of a number of molecules including VCAM-1 and FcγRIIb (13, 30). The fully formed GC is divided into distinct zones known as the light zone and the dark zone. The light zone is made of GC B cells called centrocytes, the FDC network, antigen-specific T cells that are termed follicular helper T (Tfh) cells, and tingible body macrophages. In contrast, the dark zone is made up primarily of GC B cells known as centroblasts, as well as some FDC processes and additional poorly defined stromal cells, tingible body macrophages, and small numbers of T cells. The light zone of GCs is positioned towards the marginal sinus of the spleen and the subcapsular sinus of the lymph node, possibly to facilitate the transport of antigen from sites of entry into the lymphoid tissue to the FDC network, where it can be displayed to GC centrocytes (3).

The segregation of light and dark zones is mediated by CXCL13 and CXCL12 (61). Within the GC CXCL13 is detectable on light zone FDCs but not on stromal cells in the dark zone, and its receptor CXCR5 is important for the localization of GC B cells to the light zone. CXCL13 and CXCR5 are also important for the proper polarization of GC-associated FDCs to the light zone. Without CXCL13, the GC FDC network still forms and expresses markers associated with maturation, but it is not positioned distal to the T zone as in a normal GC. This occurs even in mice lacking CXCR5 only in hematopoietic cells, indicating it is likely secondary to mispositioning of the lymphocytes. CXCL12 binds to CXCR4, which is expressed more highly on centroblasts than centrocytes and promotes the movement of centroblasts to the dark zone where CXCL12 is focused within the GC (61). When CXCR4 function is disrupted, either through genetic deficiency in B cells or the use of chemical antagonists, the polarized network of GC-associated FDCs disperses throughout the GC, demonstrating that continued CXCR4 signaling on B cells is needed to maintain FDC polarization (60). Exactly how CXCR4 function enables GC B cells to maintain FDC polarization is not understood. FDC development is dependent upon expression of tumor necrosis factor (TNF) and lymphotoxin (LT)-α1β2 by hematopoietically derived cells, as FDCs are undetectable in mice lacking lymphocytes, LTα, LTβ, or TNF (62). In addition, mice lacking the receptor for LTα1β2, LTβR, which is normally expressed on FDCs, also lack detectable FDCs (9, 63). Mice deficient in any of these factors lack FDCs and additional stromal cell types and exhibit severely impaired GC formation. Naive B cells express LTα1β2 on their surface, and GC B cells further upregulate expression of LTα1β2 (28, 64). This upregulation is thought to be important for the maturation of primary FDCs into GC-associated secondary FDCs. However, when LTα-deficient B cells were transferred into CD40-deficient hosts, so that the GC B cells selectively lacked LTα expression, GC-type FDCs still developed (JAG, CDC Allen and JGC, unpublished data). This suggests that LTα1β2 expressed by naive B cells or by some other GC cell type such as Tfh cells may be sufficient to permit FDC maturation within the GC.

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GC B-cell subsets and movement between zones

In the classical model of GC kinetics and function (2), centroblasts of the dark zone and centrocytes of the light zone each play distinct roles in the development of high affinity antibody-secreting cells. Centroblasts, which localize to the dark zone through higher CXCR4 expression, were proposed to have a large blasting morphology, proliferate rapidly, and actively mutate their antibody variable region genes while expressing low levels of surface BCR. In the model, after dividing centroblasts would re-express surface BCR and move to the light zone where, as smaller, non-dividing centrocytes they would survey for antigen bound to FDCs in the form of immune complexes, present antigen to T cells, and be selected to undergo apoptosis or differentiation or to return to the dark zone for further rounds of mutation and selection.

Recently, two-photon microscopy experiments have enabled analysis of dynamic movements of GC B cells, and have shown that GC B cells migrate extensively throughout each zone and can cross from one zone to the other in either direction (65–67). Though the methods used to label and image the FDC network and to quantify movement between the zones differed slightly, a general conclusion that cells could cross the boundary in either direction was reached. Recently the use of a photoactivatable fluorescent marker permitted the labeling of cells in either the dark zone or the light zone, followed by the tracking of labeled cells as they migrated within the GC or their isolation and analysis (68). This technique allowed for both a more thorough phenotypic analysis of the differences between centrocytes and centroblasts than had been possible previously as well as a method for measuring the movement from one zone to the other over periods of days. Unlike the classical model of large centroblasts and small centrocytes, cells in the dark zone and light zone were roughly the same size and were larger than naive B cells. Interestingly, though, several features of centroblasts and centrocytes were consistent with the classical model, including enrichment of cells in the G2/M phase of the cell cycle in the dark zone. This suggests that the majority of cell division takes place in the dark zone, though at least some cells can be observed dividing within the light zone FDC network (65). Gene expression analysis showed a signature of increased NFκB activation in cells of the light zone (68, 69), suggesting that signals from BCR and CD40 were primarily localized to this region, while genes involved in mitosis were upregulated in the dark zone. When the movement of photolabeled cells was tracked over time, a higher net movement of cells from the dark zone to the light zone was observed, though cells were observed to move in both directions. Similarly, reanalysis of a prior data set showed an overall tendency for movement of cells in the dark zone toward the light zone (70). The sum of these data suggested that important differences between centroblasts and centrocytes do exist, and provide support for a model in which centroblasts divide, move to the light zone, capture antigen in amounts proportional to the affinity of their BCRs and compete to undergo T cell-driven positive selection and differentiation, otherwise failing to get adequate positive signals and undergoing apoptosis and clearance by tingible body macrophages (2, 3, 68).

Segregation of GC B cells from naive B cells

In addition to the chemotactic cues that organize the GC dark and light zone, the GC is segregated from naive B cells in the follicle through a mechanism that has been largely undefined. While CXCR5- and CXCL13-deficient GCs are small, often mislocalized, and have altered FDC polarization (28, 29), they are still clustered. Naive B cells have been shown to briefly enter and survey the GC light zone (67, 71), but few enter the dark zone, and GC B cells are strongly confined within the GC. S1PR2-deficient mice develop GCs with poorly defined boundaries, characterized by mingling between GC B cells and naive B cells at the edge of the GC (32). When Ig transgenic S1PR2-deficient B cells were

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transferred into a wildtype host and induced to participate in a GC response that also involved responding wildtype cells, the S1PR2-deficient cells localized to the GC area but were for the most part excluded from the GC interior, showing marginalization to the perimeter. Two-photon microscopy experiments showed that S1PR2-deficient cells moved extensively around the perimeter of the GC, mingling with naive B cells but rarely migrating into the center (32). As such, S1PR2 seems to promote the centering and clustering of GC B cells, helping confine them to the GC niche and keep them segregated from the surrounding naive B cells. Tracks of the movements and velocities of wildtype versus S1PR2-deficient GC B cells showed that wildtype GC B cells tended to slow down and exhibit sharper turning angles when they encountered the edge of the GC from within, while S1PR2-deficient GC B-cell movement was unaffected regardless of whether the cells were inside or outside the GC border. When considered together with S1PR2's inhibitory effect on migration *in vitro*, this result suggested that GC B cells encountered increased levels of S1P at the GC border, causing them to reduce their velocity and turn, promoting their confinement.

As was observed in the Ig-transgenic B-cell transfer experiments, GCs of mixed bone marrow chimeras containing non-transgenic S1PR2-deficient and wildtype cells showed segregation of GC B cells of the two genotypes, with S1PR2-deficient cells occupying the outer regions along the perimeter of the GC (Fig. 3). Interestingly, when the ratios of S1PR2-deficient to wildtype cells were low so that S1PR2-deficient cells made up only a small proportion of the GC, they were highly marginalized to the perimeter. When the ratio favored the S1PR2-deficient cells so that they made up a large proportion of the GC, they started to encroach upon the interior of the GC but still avoided the center of the GC and particularly the region containing the FDC network (Fig. 3). Visualization of S1PR2-deficient non-transgenic B cells at the GC boundary was less clear than in the Ig-transgenic transfer system because of the high frequency of naive B cells that share the congenic marker with the GC cells. However, comparison of the distributions of S1PR2-deficient non-transgenic GC B cells (Fig. 3) and Ig-transgenic GC B cells (32) suggested a lesser bias of the non-transgenic cells toward the outer light zone. The basis for this difference in distribution is not clear but may indicate that the Ig transgene has an additional effect that favors cell accumulation proximal to the light zone.

S1PR2 seems to cooperate with other factors involved in GC localization and clustering to promote GC B-cell clustering. Chimeric mice lacking both CXCR5 (through CXCL13 deficiency in bone marrow chimera hosts) and S1PR2 function had severely disorganized GCs, with GC B cells often loosely spread throughout B-cell areas rather than clustered into structures (32), suggesting that S1PR2 function is particularly important in the absence of the follicular organizing cue CXCL13. GCs were similarly disrupted when S1PR2 deficiency was combined with antagonism of LTα1β2 to disrupt stromal cell networks, suggesting that FDCs and S1PR2 cooperate to promote GC B-cell clustering. LTα1β2 antagonism reduces CXCL13 expression (64), so it is not clear if the cooperative effect seen is solely dependent on the reduction of CXCL13 in the treated mice or whether FDCs cooperate with S1P through an independent mechanism. Recent studies in a mouse model that allows selective short term FDC ablation have established a direct role for FDCs in GC B-cell clustering (31). When FDCs were ablated after GC formation, GC B-cell numbers were greatly reduced after two days. When GC B-cell survival was rescued with a Bcl2 transgene, GC B cells persisted in the spleen and peripheral lymph nodes after FDC ablation but were dispersed throughout B-cell areas. Mesenteric lymph node GCs were more resistant to dispersal, but when FDC ablation was combined with S1PR2 deficiency in hematopoietic cells, GC B cells dispersed even in mesenteric lymph nodes (31). These studies provide further evidence that S1PR2 and FDC cooperate in promoting GC B-cell retention and implicate an as yet unknown function for FDC in GC clustering. This could be through the

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production of a chemoattractant for GC B cells, the degradation of an attractant for follicular B cells, or the production of a factor that repels follicular B cells.

T-cell movement into the GC

T-cell help is critical for GC progression and the development of high affinity antibody responses, and T cells must also access the follicle and GC to provide the appropriate signals to GC B cells (7). Like activated B cells, these GC-associated Tfh cells must undergo coordinated movements in order to migrate into the GC. Upon activation, $CD4^+$ T cells downregulate CCR7, contributing to their movement to the border between the B zone and the T zone (72, 73). The upregulation of CXCR5 is a hallmark of Tfh cells, and promotes their movement into the follicle, which is required prior to Tfh entry into the GC (73–75). Both changes in receptor expression are important for Tfh function, as CXCR5-deficient T cells are less efficient at promoting GC responses than wildtype T cells (73–75), and enforced expression of CCR7 in T cells reduces GC responses (73). However, CCR7 and CXCR5 levels do not fully account for Tfh positioning in the GC. In the absence of CXCR5, some T cells are still able to access the GC, but rather than accumulating in the light zone where CXCL13 predominates, they are spread throughout the GC without obvious polarization. This observation suggests that some T cells are able to enter the GC in a CXCR5-independent manner. In addition, access to the follicle may not be sufficient to ensure access to the GC. SAP-deficient T cells, which are deficient in maintaining long-term stable interactions with B cells, can enter the follicle after activation but only rarely enter the GC (76, 77), suggesting that further signals acquired during B-T interactions are required for GC entry. Bcl6 expression by Tfhcells is a requirement that has been revealed as critical for their differentiation and function (78–80), but how Bcl6 supports migration into the GC is not yet known. $CD4^+$ T cells express EBI2 and migrate to its ligand 7α,25-OHC (20–22). Whether downregulation of EBI2, a process that is important for GC B-cell localization and differentiation (18, 20), is important for GC-Tfh function has not been determined. In addition, microarray data shows that GC-Tfhcells express higher levels of S1PR2 than naive $CD4^+$ T cells (25, 78). It will be important to determine whether S1PR2 plays a similar migration inhibition and GC confinement function in GC-Tfhcells as it does in GC B cells.

A role for S1PR2 in GC homeostasis

In addition to S1PR2's contribution to GC B-cell positioning, a role was found for S1PR2 in regulating GC B-cell survival. S1PR2-deficient cells have a growth advantage over wildtype cells within the GCs of mucosal tissues, and in mixed bone marrow chimeras, S1PR2-deficient cells eventually dominate the GC B-cell populations of mesenteric lymph nodes and Peyer's patches (32). When S1PR2-deficient mice are allowed to age for more than a year, large GC outgrowths often form within the mesenteric lymph node, leading to an expansion of the lymph node and a loss of lymphoid architecture. By two years of age, roughly half of S1PR2-deficient mice develop tumors that may be classified as diffuse large B-cell lymphoma (DLBCL) that contain mutations suggestive of GC origin (81). Interestingly, the growth advantage of S1PR2-deficient cells seemed to be specific to GCs that are chronically stimulated by antigens from microbial flora, as there was no detectable outgrowth of S1PR2-deficient cells in spleens or peripheral lymph nodes after acute immunizations. In addition, when GC B cells with BCRs specific for a hapten were tracked over time after immunization with a hapten-carrier conjugate, S1PR2-deficient cells responding to the hapten showed no discernable advantage even in mesenteric lymph nodes. However, when mixed chimeras were left unimmunized, S1PR2-deficient cells slowly outgrew wildtype cells in the small GC populations that formed in the spleen, likely a consequence of low-level chronic stimulation. Thus, S1PR2 deficiency seems to lead to a

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growth advantage in GCs driven by constant stimulation rather than by competition for a limited amount of antigen.

Signaling downstream of S1PR2 leading to Akt inhibition

Contributing to S1PR2's effects on GC homeostasis, in GC B cells S1PR2 signaling dampens activation of the prosurvival kinase Akt, one of the major effectors of the PI3K pathway (32) (Fig. 4). Increased Akt activation is sufficient to cause an outgrowth of GC B cells and resistance to apoptosis. The mechanism by which S1PR2 signaling leads to dampened Akt activation is not yet clearly understood. Downstream effectors Gα12 and Gα13, as well as p115RhoGEF, were also needed for dampening Akt activation, and inhibition of the Rho effector kinase ROCK partially recapitulated the effect, so it is likely that Rho activation is involved in S1PR2-mediated suppression of PI3K signaling. S1PR2 has been proposed to associate with the lipid phosphatase PTEN, which can dephosphorylate PI3K's product phosphatidylinositol (3,4,5)-trisphosphate (PIP3) (82), and Rho and ROCK can activate PTEN (83), but inhibitor experiments did not reveal a role for PTEN in S1PR2-mediated Akt inhibition in GC B cells (32). A second possibility is that the relevant PI3K signaling and Akt activation is downstream of Rac, as Rac has been shown to activate PI3K in several contexts (84–86), and the GC environment contains chemokines which signal through Gαi-coupled GPCRs that stimulate Rac. In this case, S1PR2-mediated Rho activation could lead to Rac antagonism and dampening of Akt activation. A third possibility is that S1PR2 signaling via Gα12 and Gα13 activates adenylate cyclase (87) and inhibits Akt through cAMP-dependent protein kinase A (88). This mechanism has been implicated in S1PR2-mediated attenuation of Akt in macrophages (89). It will be interesting to further determine how S1PR2 signaling is integrated into the various inputs controlling Akt activation in GC B cells and whether one or more of these mechanisms contribute to Akt inhibition.

GC B-cell homeostasis and spatial restriction of S1PR2 signaling

The outgrowth of S1PR2-deficient GC B cells was coupled to increased activation of Akt and increased resistance to apoptosis (32). However, while the outgrowths were largely restricted to chronic mucosal GCs, the increased AKT activation was seen in GC B cells from any tissue analyzed, including from acutely induced GCs in the spleen (32). An explanation for this apparent discrepancy between outgrowth and AKT activation may be that measurements of cell numbers precisely record the *in vivo* state, whereas assessments of pAKT levels and apoptosis reflect the state of the cells following isolation and analysis. When lymphoid tissues are converted into a cell suspension, differences in S1P concentrations across tissue microenvironments are lost. Given our evidence that S1P is present in higher concentrations outside than inside GCs, it is likely that cells from the GC interior are exposed to elevated amounts of the lipid during tissue preparation. This increased exposure may lead to an elevation of S1PR2 signaling in some cells during isolation and thus a reduction in pAKT levels. Indeed, despite efforts to keep the cells ice-cold throughout the isolation procedure, we observed higher pAKT levels in wildtype GC B cells when the S1PR2 antagonist was added at the time of tissue preparation (32); if S1PR2 signaling was inactive in the ice-cold cells, the antagonist should not have had an effect. The apoptosis assays involve incubating cells *ex vivo* for 40 min to 3 h at 37°C. Any increase in S1P exposure that occurred during cell isolation thus has the *in vitro* incubation period to manifest itself. Given that GC B cells undergo extremely rapid apoptosis when removed from their supportive *in vivo* environment, differences in prosurvival factor activity may be prominently revealed during even brief incubation periods. In Fig. 5, we illustrate the model of S1PR2-mediated GC B-cell growth regulation we previously proposed (32) that takes each of these factors into consideration. In this model, S1PR2 signaling antagonizes Akt

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activation and transiently reduces GC B-cell viability as cells are exposed to elevated amounts of S1P at or beyond the GC border. In combination with the many other checks and balances acting on GC B cells, this proapoptotic effect would be small. But in S1PR2-deficient cells, the cumulative influence of a reduced proapoptotic signal may manifest itself over time in chronically stimulated GCs.

One way in which a small prosurvival influence might reveal itself over time is by protecting cells from accumulating genotoxic stress due to off-target mutational activity of AID. The decision of a GC B cell to undergo cell death could take place, for instance, after recognition of double strand breaks by ATM or ATR and activation of a p53-dependent apoptotic response. AID-deficient GC B cells have a small intrinsic survival advantage, likely due to the lack of accumulated mutations (90). Over the lifetime of the organism, resistance to this form of homeostatic regulation would be anticipated to have significant consequences in mucosal tissues. The seemingly sudden loss of GC homeostasis in aged S1PR2-deficient mice with frequent development of lymphoma suggests that secondary oncogenic hits could be taking place in the GC B cells, possibly due to increased resistance to apoptosis upon the acquisition of DNA breaks and genotoxic stress.

Unique signals acting in chronic GCs of mucosal tissues

The inductive signals leading to GC formation and persistence in mucosal tissues are not entirely understood, but some systems have indicated that mucosal tissues can support GC formation with reduced dependence on specific antigen recognition. In a mouse model where the BCR was replaced with the Epstein-Barr virus (EBV)-encoded latent membrane protein (LMP) 2A that transmits constitutive BCR-like signals, B cells were able to form GCs in mucosal tissues but not at other sites (91, 92). This striking result suggested that antigen recognition via the BCR was not essential for GC formation in tissues chronically exposed to microbial antigens. From these experiments, it seems possible that when GCs are driven by signals other than affinity for specific antigen, the conditions regulating survival and selection could be different than in newly generated GCs in which GC B cells are competing for antigen and undergoing affinity-mediated competition.

Consistent with this idea, CD19-deficient mice, which lack induced GCs in spleen and peripheral lymph nodes, contain GCs within Peyer's patches (93). CD19 acts as a stimulatory co-receptor of the BCR signaling complex. The defect in splenic GC formation in CD19-deficient mice is thought to be due primarily to a lack of recruitment and activation of PI3K downstream of BCR stimulation, because PTEN deficiency rescues the ability of CD19-deficient mice to form splenic GCs upon immunization (94). In addition, transgenic mice expressing a form of CD19 containing mutations affecting PI3K recruitment exhibit defective GC maturation in peripheral tissues (95). Increasing evidence is accumulating that PI3K signaling is required for GC formation. Mice deficient in or containing a catalytically inactive form of p110δ (96–98), a catalytic subunit of PI3K, are unable to form GCs even in mucosal tissues. The requirement for p110δ activity may be specific to T cells for GC formation (99), but several pieces of evidence suggest that redundant contributions of p110 isoforms could be critical in B cells. Both p110δ and p110α can associate with CD19 upon B-cell stimulation (100), p110α activation rescues B-cell survival after BCR deletion (101), phenotypes in PTEN-deficient B cells are only partially reversed upon p110δ deletion (102), and a loss of both p110δ and p110α completely blocks B-cell development (103). Taken together, these observations suggest that PI3K activity is likely required for GC formation and/or persistence, functioning downstream of the BCR and CD19 coreceptor, but that in conditions of chronic stimulation there may be signals leading to sufficient PI3K activation that are independent of CD19.

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One possibility for why S1PR2's effects on PI3K signaling preferentially affect GCs stimulated by chronic exposure to microbial antigens is that PI3K signaling downstream of the BCR could be modulated separately from the PI3K signaling regulated by S1PR2 (Fig. 6). This could mean that S1PR2's growth-control effect is not revealed in situations in which BCR-driven PI3K signaling is the determining factor in GC B -cell selection but is more important in the context of non-limiting amounts of microbial stimulation. PI3K signaling downstream of the BCR utilizes the catalytic subunits p110δ and p110α, but the subunits p110γ and p110β signal downstream of GPCRs (104), though in some cases p110δ can be activated by GPCRs as well (105).

PI3K signaling in GC B-cell selection and persistence

The PI3K product PIP3, a membrane-bound signaling lipid, attracts molecules that transmit signals downstream to effector proteins. One such PIP3-binding protein is DOCK8. Loss of function DOCK8 mutations impede GC persistence and development of high affinity antibody responses, possibly due to impaired integrin activation and immunological synapse organization (106). DOCK8 is a member of the DOCK family, whose members contain a DOCK homology region 1 domain (DHR1), which recruits them to PIP3, and have Rho-Rac family GTP-exchange factor activity. Another PIP3-binding protein, Bam32, which is a pleckstrin homology domain adapter protein, was also found to be important for BCR-induced integrin adhesion and spreading as well as the formation of stable conjugates with T cells (107), and Bam32 deficiency resulted in a failure of GCs to persist after formation and a reduction in affinity maturation (108). Bam32-deficient GC B cells were more susceptible to apoptosis. Thus, signaling through the products of PI3K activation is necessary for GC B-cell persistence, but exactly which features downstream of DOCK8 and Bam32 are critical for GC B cells is not known. Goodnow and colleagues (109) have proposed a model in which DOCK8 is important for the recruitment and activation of the integrin LFA-1, which in turn could lead to PI3K activation through outside-in signals mediated by the integrin. In this way, moderate levels of BCR signaling due to binding of small amounts of antigen displayed on FDCs would cause PI3K activation through CD19 and recruitment of DOCK8 and Bam32 to PIP3. This would be followed by subsequent integrin activation and further PI3K signaling, increasing the expression of pro-survival molecules as GC B cells survey for antigen and undergo AID-driven antibody gene diversification. Conversely, binding of the BCR to a widely present self-antigen may cause massive internalization of BCR, resulting in a failure of the cell to receive the tonic or antigen-driven BCR signals necessary for continued survival and to die through negative selection. As such, the model supposes that a primary driving force behind affinity-driven selection in the GC is through continued, intermediate levels of BCR signaling that drive enough Akt activation to allow survival of cells with affinity for foreign antigen but not self-antigen. How this BCR-induced PI3K signaling and subsequent Akt activation relates to the Akt regulated by S1PR2 signaling is an important question. Unlike Gα12-Gα13-coupled S1PR2, GPCRs that couple to Gαi can stimulate PI3K activity through the Gβγ subunit (110–112). CXCR4 in particular has been shown to promote Akt activation in lymphocytes (113, 114). Due to its upregulation in GC B cells and the presence of CXCL12 within the GC (61), CXCR4 is likely to promote Akt signaling in GC B cells. In addition, signals through CD40 (115, 116) and the IL-21 receptor can also stimulate Akt activation in B cells (117, 118). Due to the known roles of these molecules in GC B cells, it is possible they also contribute to Akt activation within the cell. TLR signaling can also lead to PI3K activation (119–121), something that might occur within GC B cells in chronically stimulated mucosal GCs (Fig. 6). How and when these other inputs into PI3K signaling play a relevant role in GC B-cell survival and selection will need to be incorporated into the model of GC function.

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The role of S1PR2 in innate immune cells

In addition to its role in GC B cells, S1PR2 has functions in a number of other immune cell types. One feature that is apparent in several settings is S1PR2's inhibitory regulation of migration, and how this property contributes to the proper positioning of the cell type expressing the receptor. Another interesting feature is that while GC B cells strongly downregulate S1PR1, some cell types coexpress S1PR1 and S1PR2, creating a situation in which a balance of relative signals through the two receptors, which can lead to opposite effects, is important in determining the migratory and positional capabilities of the cell. In a thioglycollate-induced model of peritonitis, macrophages were found to express S1PR1 and S1PR2 (89). S1PR2 inhibited the velocity of macrophage migration towards chemoattractants and dampened Akt activation. S1PR2 deficiency also resulted in increased numbers of macrophages homing to the peritoneum during peritonitis. Exactly how S1PR2 stimulation blocks macrophage recruitment was not fully explained, but these results suggest that S1P in the peritoneum itself or S1P in the vasculature traversed by the macrophages inhibits movement of macrophages into the peritoneum. The authors had previously measured S1P levels in peritoneal exudates to be approximately 20 nM (122), which is much lower than the concentration in plasma or lymph but is consistent with levels known to inhibit cell migration (32, 89). S1PR2 has also been proposed to promote the homing into and retention of macrophages in the arterial wall as well as the uptake of oxidized low density lipoproteins (LDLs) and the promotion of atherosclerotic plaques (123, 124). Two groups found that in the atherosclerosis model of apoE-deficient mice on a high cholesterol diet, S1PR2 deficiency provided protection against the formation of atherosclerotic plaques. Fewer macrophages infiltrated the atherosclerotic lesions in S1PR2-deficient mice and there was reduced expression of proinflammatory cytokines (123). As well as inhibiting macrophage migration, S1PR2 was found to stimulate signaling through Rho and ROCK and to inhibit Akt activation. The authors proposed that S1PR2 signaling helps to promote the transendothelial migration or retention of macrophages in arterial walls, increasing the numbers of macrophages within atherosclerotic plaques and promoting plaque development through inflammatory cytokine production and foam cell formation. Osteoclast precursors (OPs) express both S1PR1 and S1PR2 (125). Interestingly, low concentrations of S1P promoted the directional migration of OPs, while high concentrations inhibited migration or even promoted chemorepulsion, suggesting that an interplay of signaling between the two S1P receptors regulates S1P's effects on OP migration. S1PR2-deficient mice had greater bone density, and pharmacological inhibition of S1PR2 was able to promote OP mobilization into the blood and reduce the severity of an osteoporosis model. Thus, the authors proposed that S1PR2 promotes retention of OPs within the bone by inhibiting their migration towards S1P in circulation, contributing to the maintenance of proper bone turnover.

S1PR2 is involved in mast cell responses, including degranulation, chemokine secretion, and anaphylaxis (126, 127). S1PR2-deficient mast cells exhibit reduced degranulation after stimulation (126), and pharmacological inhibition or siRNA-mediated knockdown of S1PR2 inhibits mast cell degranulation and the secretion of pro-inflammatory mediators CCL2, IL-6, and TNF (127). S1PR2 antagonism or genetic deficiency decreases the severity of histamine release and hypothermia during a mouse model of anaphylaxis. S1PR2's effect seemed to be at the level of mast cell degranulation, because when the authors bypassed this step by treating mice with exogenous histamine, S1PR2 deficiency failed to reduce anaphylaxis (127). How S1PR2 signaling regulates these processes in mast cells is not understood, but mast cell degranulation is known to involve Rho-dependent formation of microtubules necessary for the translocation of granules (128), so it is likely that S1PR2 signaling contributes to the necessary activation of Rho.

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Concluding remarks

How S1P levels are regulated within complex environments of the body and how signaling through S1P receptors like S1PR2 affects cellular processes are expanding fields with many implications. Characterizing the role of S1PR2 in GC B cells has been useful not only for understanding GC organization and function but also for how S1P levels may be controlled in a microenvironment-specific manner to closely regulate the positioning and homeostasis of a population of cells. An important question still to be assessed is how S1PR2 directed movement and signaling affects the ability of GC B cells to encounter antigen and undergo affinity maturation. Although the response to one model antigen was not affected by S1PR2 deficiency, it seems likely that there will be conditions where the normal movement of GC B cells through the GC interior will be critical for successful affinity maturation. Finally, given the high frequency of GC B-cell outgrowths and GC-derived lymphoma in aged S1PR2-deficient mice, it will be important to assess whether this pathway is disrupted in human cases of GC B cell-like diffuse large B-cell lymphoma.

Acknowledgments

We thank Oliver Bannard, Jagan Muppidi, and Tim Schmidt for comments on the manuscript. JGC is an Investigator of the Howard Hughes Medical Institute. Work discussed in this review was supported by NIH grant R01 AI45073.

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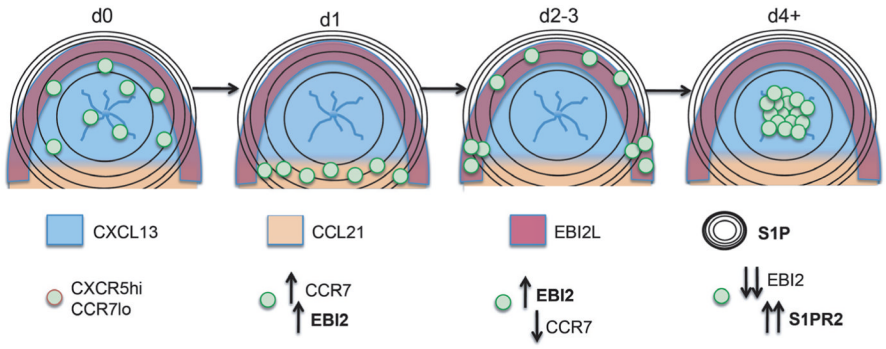

Fig. 1. Coordinated changes in migratory receptor expression during B-cell activation and GC formation

The diagram depicts the distribution of migratory cues important for guiding the movement of B cells before and after encountering antigen. CXCL13 is expressed throughout the follicle. A gradient of CCL21 extends from the T zone into the proximal area of the follicle. EBI2 ligand (EBI2L) is suggested to be present at high levels at outer follicle and interfollicular regions (it is also likely present at the B-T interface but is not depicted here for simplicity). S1P (represented by the contours) is proposed to exist in a decaying gradient with the lowest level over the FDC network (blue lines) at the center of the follicle. Naive B cells at d0 are evenly distributed throughout the follicle. After activation, CCR7 and EBI2 are upregulated, with the result that cells localize to the border of the follicle and T zone at d1. By d2-3, activated B cells have downregulated CCR7 and further upregulated EBI2 function, migrating to interfollicular and outer follicle regions. By d4, GC precursors downregulate EBI2 and upregulate S1PR2, directing their movement into the FDC network in the follicle center and allowing for GC formation.

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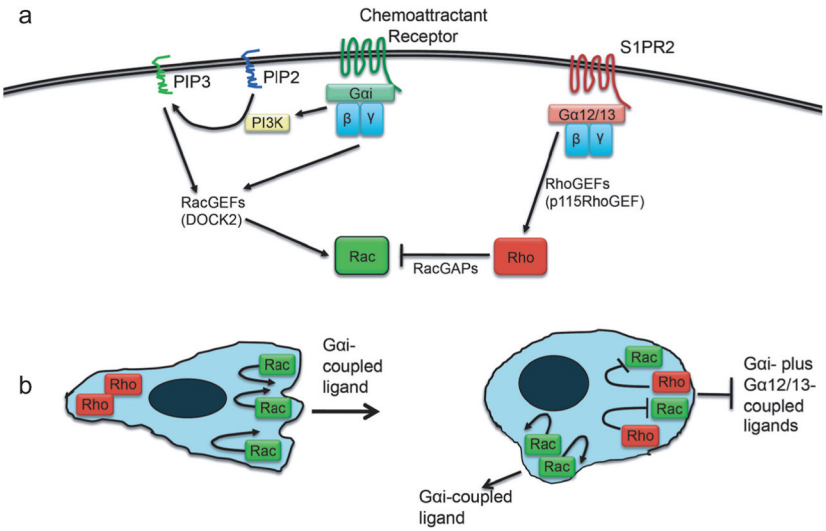

Fig. 2. Model for S1PR2 signaling leading to migration inhibition

(A) Signaling through Gαi-coupled chemoattractant receptors leads to activation of Rac GTPase through PI3K-dependent and –independent pathways. Rac can stimulate actin polymerization at the leading edge of a migrating cell. S1PR2 couples to Gα12 and Gα13, which signal through RhoGEFs such as p115RhoGEF to activate Rho. Rho can in turn inhibit Rac activation. (B) On the left, a cell sensing only a Gαi-coupled ligand will experience signaling leading to Rac activation at the leading edge, resulting in actin polymerization and movement toward the ligand, with constraint of Rho to the trailing edge. On the right, a cell sensing both Gαi and Gα12-Gα13-coupled ligands at one edge will signal through Gα12 or Gα13 to activate Rho, resulting in local inhibition of Rac activation. This will allow the cell to move away from the Gα12-Gα13-coupled ligand, in response to a Gαi-coupled ligand acting on another region of the cell.

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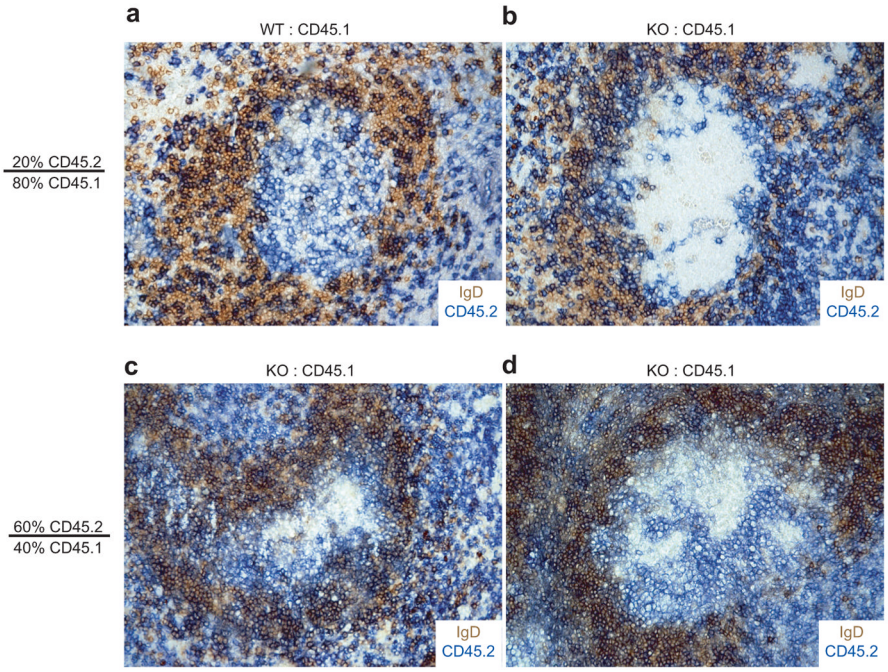

Fig. 3. Positioning of S1PR2-deficient GC B cells within mixed GCs

Images show staining of CD45.2-expressing cells (blue) in spleens from mixed bone marrow chimeras made with 20% CD45.2 and 80% CD45.1 (A,B) or 60% CD45.2 and 40% CD45.1 (C,D) donor cells. Brown staining of IgD outlines the follicle and GC boundary. (A) Wildtype CD45.2 cells are distributed evenly throughout the GC. (B) S1PR2-deficient (KO) CD45.2 GC B cells are marginalized to the perimeter of the GC when they make up a minority of the GC population. (C,D) Examples of S1PR2-deficient (KO) CD45.2 GC B cell positioning when they make up a majority of the GC B cell population. While the light zone-dark zone polarity of the GCs in a–c was not determined, in d the T zone is in the lower right of the image. The enrichment of KO GC B cells towards the T zone (likely in the dark zone) evident in d was seen in a small number of GCs that contained a predominance of KO cells.

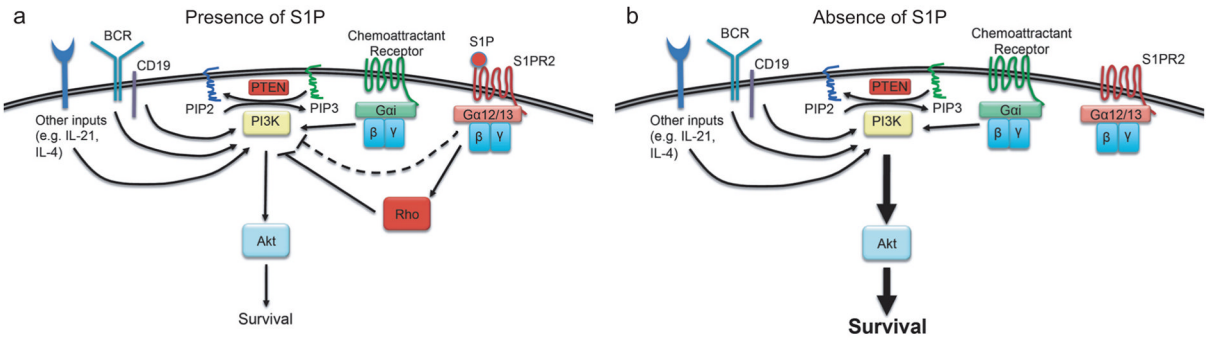

Fig. 4. Model for the influence of S1PR2 signaling on Akt activation in GC B cells

(A) In the presence of S1P, which is thought to be higher at the GC boundary than in the GC interior, S1PR2 signals downstream to dampen PIP3 generation and Akt activation, through Rho-dependent and possibly also Rho-dependent mechanisms. This inhibition of Akt activation counters the pro-survival Akt-activating signals downstream of several inputs, likely including the BCR complex and CD19, Gαi-coupled chemoattractant receptors, and other inputs such as IL-21 and IL-4 receptors. (B) In the absence of S1P, as may be the case in the GC interior, S1PR2 signaling does not occur, and without its negative input GC B cells have elevated pro-survival Akt activity. Wild-type GC B cells may move between states a and b whereas S1PR2-deficient GC B cells would be constitutively in state b.

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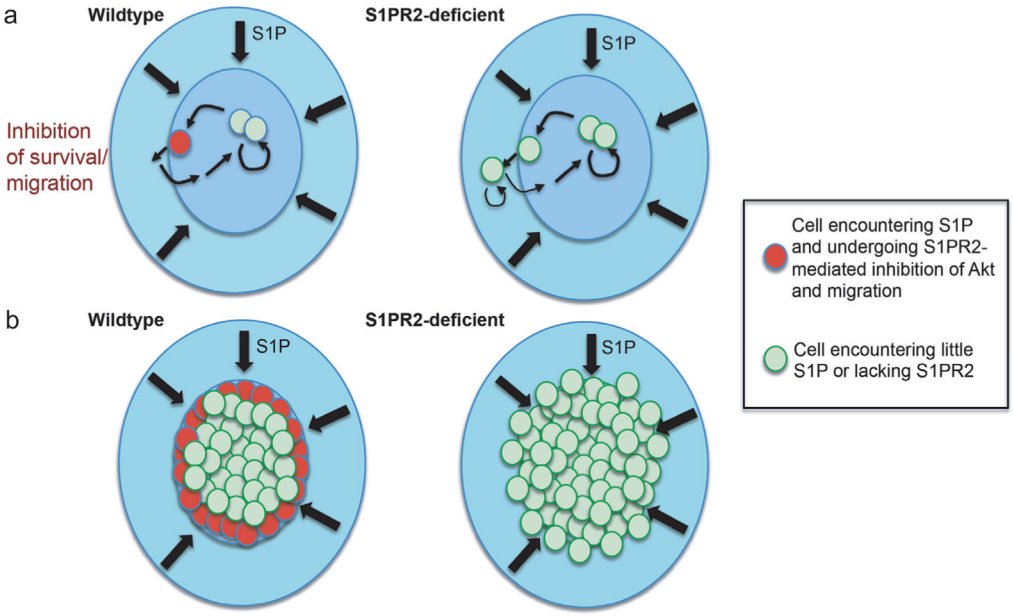

Fig. 5. Model for the spatial restriction of S1PR2 signaling in GC B cells and its outcomes

(A) A gradient of S1P is suggested to result in the highest concentrations of S1P outside the GC and at the boundary. Higher levels of S1P at the GC boundary signal through S1PR2 on wild-type GC B cells at the boundary to inhibit their migration and survival, while GC B cells in the interior do not encounter significant amounts of S1P. S1PR2-deficient GC B cells are resistant to this regulation, resulting in higher movement at the GC border, a less defined GC boundary and an increased likelihood of survival. (B) GC B cells exposed to S1P at the boundary experience dampening of Akt activation, decreasing survival and promoting GC homeostasis. S1PR2-deficient GC B cells do not experience this regulation, eventually resulting in a loss of homeostasis and lymphoma development in S1PR2-deficient mice as they age.

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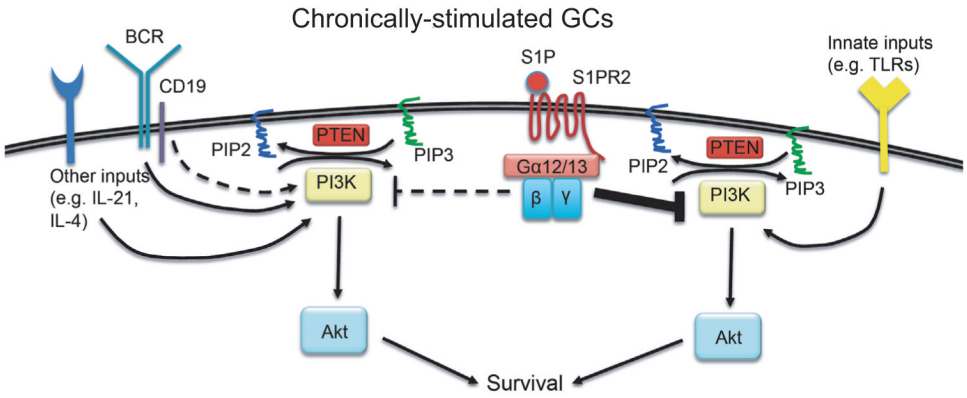

Fig. 6. Speculative model for S1PR2's influence on chronically stimulated GC homeostasis

Chronically stimulated GCs are continuously exposed to microbial components such as TLR-ligands, which can lead to Akt activation upon signaling through their respective TLRs. It is possible that context-dependent signaling events controlling Akt activation result in a greater influence of S1PR2 signaling on the Akt activation downstream of innate signals such as TLR ligands than the Akt activation downstream of BCR signaling. In this way, S1PR2 could have a greater effect on the control of Akt activation in GCs chronically stimulated by microbial determinants than on GCs driven by acute immunization.