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The CCR6-CCL20 axis in humoral immunity and T-B cell immunobiology

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ABSTRACT

Traditionally, chemokine immunobiology has focused on chemotaxis and the positioning of cells at sites of inflammation and within lymphoid organs. More recently, however, regulation of intricate immune responses has emerged as a function attributed to chemokines and their receptors. One such pair, CCR6 and its chemokine ligand CCL20, has been receiving interest for its potential role in the coordination and regulation of humoral immune responses and in particular, memory responses, at the cellular level. B cells up-regulate CCR6 after activation in secondary lymphoid organs; however, its function is still unclear. In an important insight, the CCR6-CCL20 chemokine axis has been implicated in the regulation of effective humoral responses – disruption of this pair led to an increased number of ineffective T-B cell conjugates and poorer quality antibodies. Interestingly, follicular helper T cells and their precursors also up-regulate CCR6; though, again, the precise purpose of this is yet to be discovered. The chemokine axis in relation to secondary lymphoid organ (SLO) structures will be briefly reviewed as well. With the implication of CCR6 and CCL20 in the pathogenesis of autoantibody-driven autoimmune diseases such as systemic lupus erythematosus, understanding the intricacies of this chemokine pair would be conducive to the development of appropriate, targeted therapeutic strategies.

1. Introduction

A humoral immune response that germinates and is sustained in secondary lymphoid organs (SLO) rely, at least initially, on the molecular interactions of chemokines with their cognate receptors to correctly position cells and enable an interaction with their respective partners. Of the chemokine superfamily, the pivotal roles of the homeostatic chemokine-chemokine receptor pairs, CCL19-CCR7, CCL21-CCR7 and CXCL13-CXCR5 in the overall T-B cell architecture and development of SLOs have been well characterized (Ansel et al., 2000; Forster et al., 1996, 1999) and the regulation of these chemokines by the pro-inflammatory cytokines tumor necrosis factor (TNF) and lymphotoxin (LT)-α has been noted (Ngo et al., 1999). The CXCR5-CXCL13 axis is particularly important in the proper migration and positioning of B cells in SLOs since mice lacking CXCR5 have underdeveloped SLOs, B cells that do not migrate to their correct compartments, and have altered primary follicles and germinal centers (GCs) (Forster et al., 1996). CCR7$^{-/-}$ mice have abnormal distribution of T and B cells in SLOs, have impaired lymphocyte and dendritic cell (DC) migration to SLOs, and have delayed humoral immune responses. (Forster et al., 1999) In addition, CXCR5—but not CCR7—has also been shown to be integral to the overall development of SLOs (Müller et al., 2003).

Optimal formation of B cell follicles is necessary for humoral immune responses and CXCR4 on B cells has been shown to be important for correct B cell compartmentalization in SLOs (Nie et al., 2004). Within the GC reaction, B cells move from dark zone (DZ) to light zone (LZ) utilizing the chemokines of CXCL12 and CXCL13 (Fig. 1) (Barinov et al., 2017). These data demonstrate the importance of chemokine regulation for immune system homeostasis and humoral immune responses.

Since its discovery in the 1990s, the CC chemokine ligand 20 (CCL20) (also known as macrophage inflammatory protein [MIP]-3α, exodus 1, and liver and activation-regulated chemokine [LARC]), has garnered increasing interest in molecular and cellular immunology. At present, CCL20 has only one known receptor, CC chemokine receptor 6 (CCR6) (Baba et al., 1997). The CCR6-CCL20 pair has traditionally held two major roles in immunology: (1) that of a chemokine pair involved in immunological homeostasis and structure; and (2) a driving pair in inflammation and autoimmunity, primarily via the Th17 pathway for

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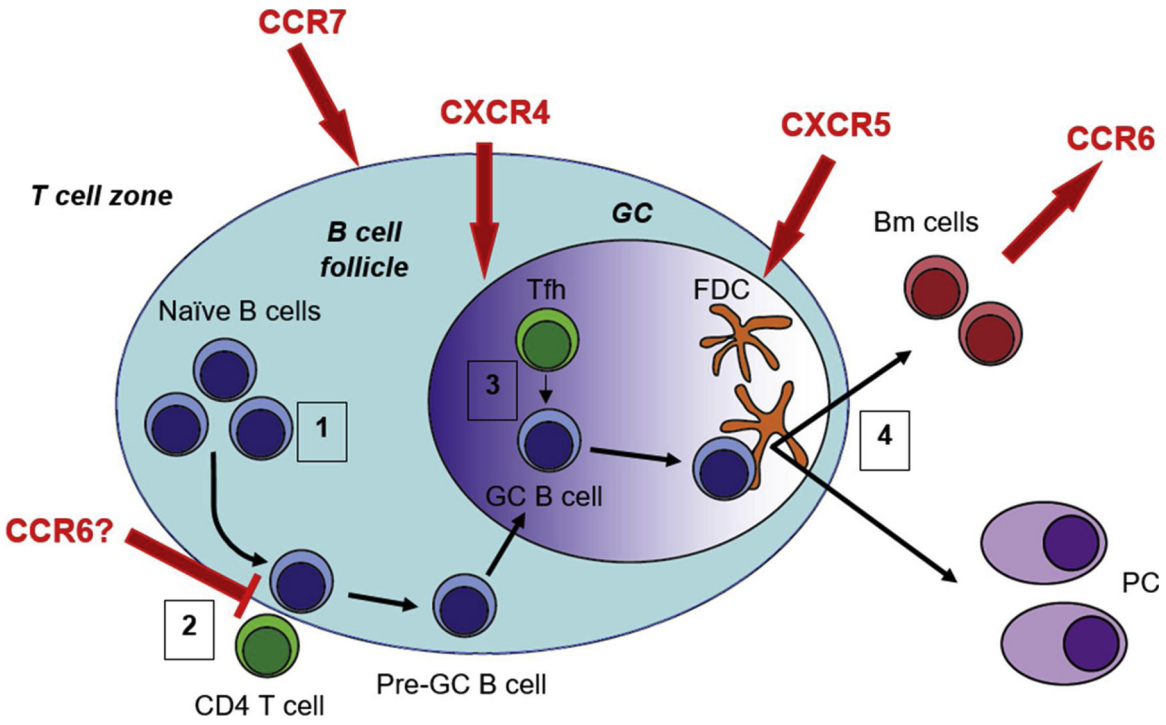

Fig. 1. Chemokines and their receptors coordinate the germinal center reaction. (1) Naïve B cells are arranged in B cell follicles assisted by the CCL19/CCL21-CCR7 chemokine axis. (2) B cells become activated after meeting their cognate CD4 T cell at the T-B cell border. The CCR6-CCL20 axis may play a role in negative regulation of this critical interaction. (3) Following activation, the B cell enters the germinal center (GC) to become a GC B cell and receives critical help from follicular helper (Tfh) cells in the dark zone, for which CXCR4-CXCL12 is an important regulator. Affinity maturation occurs in the light zone after encountering follicular dendritic cells (FDCs) and promoted by the CXCR5-CXCL13 axis. (4) GC B cells then differentiate into either $CCR6^+$ memory B (Bm) cells (for which the CCR6-CCL20 axis is involved in chemotaxis of these cell subtypes) or long-lived plasma cells (PC).

which CCR6 is a signature chemokine receptor to these cells (Singh et al., 2008).

Th17 cells display CCR6 as a phenotypical marker which is augmented by TGF-β1 and IL-6. (Wang et al., 2009) and express the canonical transcription factor of retinoic acid receptor-related orphan receptor-γt (RORγt) (Marks et al., 2009). They secrete various cytokines including CCL20 and IL-17 which are important in propagating inflammation and immunity (Ye et al., 2001). In autoimmune pathology, there is a fine balance between the closely related $Foxp3^+CCR6^+$ regulatory T (Treg) cells, which share the same developmental lineage as Th17 cells (Marks et al., 2009; Komatsu et al., 2014), and the closely-related Th1 cells (Bettelli et al., 2007). Th17 cells have been implicated in various autoimmune diseases including multiple sclerosis (MS), rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) (Bedoya et al., 2013). Interestingly, in human RA, T cells express CCR5 and CXCR3 (Qin et al., 1998). Therefore, the role of CCR6-CCL20 in Th17-driven pathology still needs to be further delineated; nevertheless, it is known that CCR6 is not only a homing receptor for Th17 cells – it has the downstream effect of regulating inflammation as seen in murine colitis models (Wang et al., 2009), and regulating the Th17-Treg balance (Kulkarni et al., 2018).

2. Basic biology of the CCR6-CCL20 axis

CCL20 is an 8 kDa protein whose gene is mapped to 2q33-37 (Hieshima et al., 1997). At a tissue and organ level, CCL20 is expressed strongly at mucosal sites (lung, intestines), liver, thymus, skin, prostate and testis (Hieshima et al., 1997; Louis et al., 2003). Constitutively, murine *Ccl20* expression in SLOs varies from a strong presence in the appendix to undetectable in the spleen and LNs (Hieshima et al., 1997; Hromas et al., 1997). This tissue/organ expression indicates that CCL20 is involved in the maintenance of immunological homeostasis. Immune cells that express CCL20 include Th17 cells, CD8 T cells, neutrophils, macrophages, dendritic cells (DCs), mast cells and endothelial cells (Lee et al., 2013; Lin et al., 2003; Meissner et al., 2003).

As an inflammatory chemokine, CCL20 plays a prominent role in innate immunity. It is up-regulated by transcription factors, such as NF-κB, (Zhao et al., 2014) and induced by the inflammatory cytokines TNF and IL-1β (Fujiie et al., 2001). Upon immune system induction by inflammatory stimuli such as lipopolysaccharide (LPS) and microbes, *Ccl20* is rapidly up-regulated (Lee et al., 2017; Tanaka et al., 1999). This leads to a marked influx of immune cells as can be seen with the up-regulation of splenic CCL20 and the rapid accumulation of leukocytes in the spleen (Paradis et al., 2014).

The chemokine CCL20 has only CCR6 as a confirmed receptor (Baba et al., 1997) despite some controversial observations that this receptor also recognizes β-defensin *in vivo* (Yang et al., 1999). As with all chemokine receptors, CCR6 is a G protein-coupled receptor. It is found on $IL\text{-}17^+CD4^+$ T cells, memory $CD4^+$ and $CD8^+$ T cells. Furthermore, it can be expressed by myeloid and plasmacytoid DCs, Langerhan's cells, neutrophils and various B cell subtypes (Krzysiek et al., 2000; Kucharzik et al., 2002; Li et al., 2017; Yamashiro et al., 2000). Human tissue expression of CCR6 can be seen in spleen, lymph nodes (LNs), intestines and liver (Baba et al., 1997). It binds CCL20 with a *Kd* value of 0.9 nM (Baba et al., 1997). Curiously, the innate, anti-microbial peptide β-defensin, can also bind CCR6 and induce chemotaxis thought to be due to the similarity in secondary and tertiary protein structures to CCL20 (Yang et al., 1999; Lee et al., 2015).

3. CCR6-CCL20 in T-B cell immunobiology

Most B cells display CCR6 prominently; however, freshly isolated human B cells tend not to migrate towards CCL20 despite their CCR6 expression (Liao et al., 1999). Overnight culture abrogates this impairment (Brandes et al., 2000) suggesting either downstream signaling modulation of chemotactic responses, or an initial paralyzed state of *ex*

vivo B cells. Supporting the former explanation, activated B cells show enhanced migration to CCL20 without significant changes in CCR6 protein or mRNA expression, or ligand binding (Liao et al., 2002), highlighting that CCR6 expression does not always infer CCL20 responsivity, and may have other some other role apart from chemotaxis.

As opposed to early (pro- and pre-) B cells, late B cells ($CD19^+CD34^-$, lambda/kappa$^+$; immature and mature B cells) display high amounts of CCR6 protein and mRNA and are able to migrate to CCL20 accordingly (Honczarenko et al., 2006). Immune system activation does not up-regulate CCR6 on pre-B cells, indicating that the up-regulation of CCR6 on late B cells occurs as part of normal B cell development rather than immune system induction (Honczarenko et al., 2006). Follicular and marginal zone naïve B cells display CCR6 prominently (Meissner et al., 2003; Bowman et al., 2000), and upon immunization, *Ccr6* is up-regulated (Reimer et al., 2017). This, in part, functions to assist $CCR6^+$ naïve B cells in circulation to engage in rolling adhesion to endothelial cells and probably assists in the diapedesis of naïve B cells from the circulation into lymphoid organs (Meissner et al., 2003). After B cell receptor (BCR) ligation (antigen capture) or CCL20 exposure, CCR6 is down-regulated, the chemotactic response to CCL20 is abrogated and responsiveness to CCL19 is enhanced, hinting that the CCR6-CCL20 axis may assist with initial antigen contact but is dispensed to allow B cells to migrate to T cell zones to meet cognate T cells (Meissner et al., 2003; Casamayor-Palleja et al., 2002).

Pre-GC B cells markedly up-regulate CCR6 upon immune system induction (Reimer et al., 2017; Schwickert et al., 2011), and this occurs in a CD40-CD40L-dependent manner (Reboldi et al., 2016). The purpose of this up-regulation is not yet known; but it is possible that it plays some role in the critical B cell maturation checkpoint as the involvement of CD40 L indicates that these B cells receive T cell help before entering the GC reaction Schwickert et al., 2011).

CCR6 expression is thought to be lost once B cells develop into GC B cells; yet some studies do find low expression (Reimer et al., 2017; Chappell et al., 2017; Suan et al., 2017) which may be, in part, due to the definition of GC B cells in flow cytometry. Suan et al. (2017) found 5–10% of GC B cells to be $CCR6^+$ which were predominately identified in the LZ. Likewise, Victora et al. (2011) found predominant CCR6 expression in LZ B cells. These cells were shown to be to be the most quiescent cells in the GC which predominately were leaving the GC reaction and transitioning into memory B (Bm) cells that showed evidence of isotype switching. Accordingly, $CXCR4^-$ centrocytes show increased *CCR6*, as well as other chemokine receptors (Caron et al., 2009), whilst $CXCR4^+$ centroblasts down-regulate *CCR6*, with the former transitioning to a genotype akin to naïve B cells (Klein et al., 2003). These changes may facilitate the promotion of key interactions for survival and stabilization of centrocytes before exiting from the GC reaction. Unidentified cells expressing *CCL20* are almost exclusively found in the DZ of GCs (Buri et al., 2004). How they relate to the dynamic expression of CCR6 on the GC B cells is yet to be determined though it is possible they have a role in positioning $CCR6^+$ GC B cells in the DZ. Upon BCR cross-linkage, CCR6 is down-regulated and it is possible this may be a mechanism for absent or low CCR6 on GC B cells as they enter the cell cycle (Krzysiek et al., 2000).

Following exit from the GC, plasmablasts maintain weak expression of CCR6 and CCL20 migratory properties (Wehrli et al., 2001); however, other studies point to negative CCR6 expression (Krzysiek et al., 2000). Terminally differentiated normal or myeloma plasma cells are also considered CCR6-negative; yet, some groups have demonstrated low expression of this receptor (Krzysiek et al., 2000; Nakayama et al., 2003; Trentin et al., 2007).

The differentiation of B cells into long-lived Bm cells for effective secondary immune responses is of chief priority in the generation of humoral immune responses. Bm cells have a higher expression of CCR6 protein and mRNA than naïve B cells and preferentially migrate towards CCL20 (Elgueta et al., 2015). While CCR6 is dispensable for the formation of Bm cells (Suan et al., 2017), the CCR6-CCL20 axis is necessary for the appropriate responses of Bm cells in the secondary immune response. $CCR6^{-/-}$ mice challenged with antigen displayed a reduction of antigen-specific Bm cells in the spleen and bone marrow, indicating impaired migration to effector sites (Elgueta et al., 2015).

Curiously, all post-GC $CCR6^{-/-}$ B cells (including Bm cells) were redistributed from the perifollicular and marginal zone areas to the follicular and GC areas (Elgueta et al., 2015). This observation results in larger GCs in $CCR6^{-/-}$ mice (Lin et al., 2017); however, GC dynamics and the quality of the resulting antibodies are compromised. Bone marrow chimera experiments demonstrate that B cells from a $CCR6^{-/-}$ donor were immunologically less efficient. In these, murine B cells IgG_1 and IgG_2 were substantially reduced after immunization whilst IgM (which is likely from an extrafollicular pathway) was comparable to a WT ($CCR6^{+/+}$) donor (Reimer et al., 2017). With regard to the CG response, $CCR6^{-/-}$ mice displayed faster kinetics and more numerous GC; yet, this seemingly advantageous outcome was offset by a greater percentage of low-specificity antibody-secreting B cells (Wiede et al., 2013). Using flow cytometry, the percentages of DZ and LZ B cells did not differ between $CCR6^{-/-}$ and WT mice except at Day 13 when $CCR6^{-/-}$ DZ B cells were marginally more numerous than WT DZ B cells (Reimer et al., 2017).

In a similar manner, the CCR6-CCL20 axis plays an important role in the delivery of optimal mucosal humoral immunity. As a chemotaxis system, CCR6-CCL20 recruits $CCR6^+$ B cells to the subepithelial domes (SED) of PP to interact with DCs to ultimately promote isotype switching from IgM to IgA (Reboldi et al., 2016; McDonald et al., 2007). Mice deficient in CCR6 subsequently show low serum IgA, have fewer IgA^+ GC B cells (Lin et al., 2017) and correspondingly, show limited IgA response to rotavirus infection (Cook et al., 2000). However, $CCR6^{-/-}$ mice had comparable IgA^+ Bm cells to WT mice; yet, the former were prone to apoptosis indicating poorer survival of these cells (Lin et al., 2017). These data indicate that the chemokine CCR6-CCL20 axis plays an important checkpoint in the GC reaction and the generation of high-specificity antibodies.

A number of CD4 T cell subsets express CCR6 including, most prominently, Th17 T cells, for which CCR6 is a defining characteristic (Singh et al., 2008). CCR6 expression facilitates migration of these cells into effector immune sites (Yamazaki et al., 2008), and has been a phenotypical marker of activated/memory T cells (Ebert and McColl, 2002). Though CCR6 is often attributed to Th17 cells, more recent studies point to low expression of CCR6 on Tfh cells (Lee et al., 2017; Aoki et al., 2011; Velu et al., 2016). This CCR6 expression contributes to the pathogenesis of disease, as $CCR6^+$ Tfh cells can be blocked by anti-CCL20 antibodies from migrating to the liver from the spleen and abrogating the induction of autoimmune hepatitis (Aoki et al., 2011).

In addition, it is possible that $CCR6^+$ Tfh cells represent a Tfh-Th17 overlap as indeed, $CD4^+CXCR5^+$ T cells that were also $CCR6^+$ had B cell-activation capabilities and produced IL-17 A and IL-22 (Morita et al., 2011). Since Tfh cells are traditionally $CCR6^-$ (Liu et al., 2012), further studies are required to elucidate the role and purpose of CCR6 on a small subset of Tfh cells. Furthermore, it needs to be determined if CCR6 assists with positioning or memory responses. This is certainly an area of interest considering both Th17 and Tfh cells have been implicated in the immunopathogenesis of autoimmune diseases.

Of particular interest in the coordination of humoral immune and GC responses is a group of intermediary or pre-Tfh cells, henceforth designated Tfh^{int} cells. These cells are $CXCR5^+Bcl6^{lo}$ (rather than $BCl6^{hi}$ of Tfh cells), prime B cells at the T-B border, and are a necessary transition step to mature into Tfh cells (Liu et al., 2012). As opposed to Tfh cells, these cells intracellularly express large amounts of CCL20 protein and mRNA, and moderate amounts of CCR6 (Lee et al., 2017; Liu et al., 2012). Curiously, CCL20 was found tethered to the surfaces of Tfh^{int} and Tfh cells which was dispensable for the formation of T-B cell conjugates since $CCR6^{-/-}$ mice displayed a higher percentage of T-B cell conjugates (Lee et al., 2017) in agreement with an accelerated and

ineffective GC reaction (Wiede et al., 2013). Formation of long-lived T-B conjugates occurs shortly after immunization (Okada et al., 2005) and occurs at the T-B cell border or the interfollicular zones (Kerfoot et al., 2011). It is possible that the high expression of CCL20 found in these Tfh^{int} cells coupled with the up-regulation of CCR6 on pre-GC B cells helps to create a long-lived, high-quality interaction between these cells thus facilitating the production of high-specificity antibodies. $CCR6^{-/-}$ mice pre-GC B cells express lower levels of Bcl6 protein than WT mice which may lead to poorer sustained cognate T-B cell interactions (Lin et al., 2017).

4. SLO architecture and homeostasis

Finally, it is important to examine the tissue distribution of the CCR6-CCL20 axis in SLOs since this provides the environment in which optimal T-B cell interactions can occur. The CCR6-CCL20 pair is involved in key homeostatic functions in mucosal SLOs, such as Peyer's patches and isolated lymphoid follicles (ILFs), as well as their associated immune functions, and this has been quite well established in the literature (See Williams (2006) for review). The chemokine axis can be found performing a similar homeostatic role in other SLOs; however, its relative impact on humoral immune responses has not yet been established.

CCL20 is found in the epithelial crypt cells of human tonsils and Bm cells are seen to co-localize to this area, suggesting a role for the CCR6-CCL20 axis in positioning these cell (Casamayor-Palleja et al., 2001). *CCL20* is also found in the follicles of tonsils and LNs, and increasing expression is seen in maturing follicles, suggesting a possible role for the CCR6-CCL20 axis in the fine-tuning of humoral immunity and lymphoid architecture (Buri et al., 2004).

In the LNs, fibroblastic reticular cells (FRC) and lymphatic endothelial cells (LEC) stromal cells up-regulate *CCL20* expression after infection (Gregory et al., 2017). CCL20 may also be found at the subcapsular sinus of the LN (Pegu et al., 2007) assisting inflamed cells that up-regulate CCR6 to adhere or migrate to the LN stromal cells (Katakai et al., 2004; Severino et al., 2017). By microarray transcriptome profiling, *Ccl20* was poorly expressed in LN hematopoietic cell lines whilst *CCR6* was highly expressed on follicular B cells, moderately expressed on CD4 T cells and absent in LN stromal cells (Malhotra et al., 2012). Transgenic mice have assisted our understanding of the role of the CCR6-CCL20 pair in SLO homeostasis. In the oncostatin M (OM) transgenic (LckOM) mice, extrathymic T cell development in LNs is stimulated and they act as a primary and secondary lymphoid organ. Curiously, *Ccl20* expression was markedly elevated in the LN of LckOM compared to control B6 LNs, but the primary source being the stromal cells of the thymus. By performing reconstitution experiments with OM^+ or OM^- T-cell-depleted bone marrow in WT mice, the CCR6-CCL20 axis was found to regulate enrichment of T cells in SLOs, thus emphasizing the importance of the CCR6-CCL20 axis in T cell homeostasis (Louis et al., 2003).

$CCR6^{-/-}$ mice did not display any obvious disruption to the splenic structure (Elgueta et al., 2015) or positioning of DCs and polymorphonuclear cells (Paradis et al., 2014) indicating the redundancy of the CCR6-CCL20 axis' in the establishment of the SLO microarchitecture. This is not surprising since the CXCR5-CXCL13 and CCR7-CCL19/CCL21 axes have been shown to be critical for correct compartmentalization of lymphocytes and humoral immune responses via secretion of chemokines via stromal cells (Forster et al., 1996, 1999; den Haan et al., 2012). Though the spleen has similar stromal cells to LNs, there has been no exploration of the CCR6-CCL20 axis and these cells in the literature to date. It can be speculated that if splenic stromal cells do secrete CCL20, they would have the same putative function as those found in LNs, and are, similarly, responsible for recruiting $CCR6^+$ cells to the spleen post immune system induction (Paradis et al., 2014).

In the mucosal lymphoid organs, the CCR6-CCL20 axis is particular important in normal structure and function. $CCR6^{-/-}$ mice have fewer ILFs, Peyer's patches (PP) and membranous epithelial (M) cells compared to WT mice (Bouskra et al., 2008; Lugering et al., 2005). PP are smaller and ILF development is inhibited in $CCR6^{-/-}$ mice and indicating that the CCR6-CCL20 axis is important in mucosal immunity development (Lugering et al., 2010). Interestingly, $CCR6^{-/-}$ mice, whom have defective M cells, were protected from *Yersinia enterocolitica* oral infections, since this microorganism exploits M cells to establish infection (Westphal et al., 2008).

At a cellular level, $CCR6^{-/-}$ mice have reduced DCs and CD4 T cells in PPs; however, the CCR6-CCL20 axis was found to be not directly responsible for migration of these cells to the mucosal tissues and instead may affect their development and function (Cook et al., 2000; Lugering et al., 2005). This notion has been supported when DCs from $CCR6^{-/-}$ mice have been shown to have an intrinsically reduced ability to capture antigens and promoting IgA production in *ex vivo* splenic B cells (McDonald et al., 2017). The Th17 axis is seemingly impaired in $CCR6^{-/-}$ mice as well (Yamazaki et al., 2008).

$CCR6^+$ B cells are also attracted to the follicle-associated epithelium (FAE) of PPs which secretes CCL20 and is necessary for the proper functioning of intestinal lymphoid structures (McDonald et al., 2007; Zhai et al., 2014). However, CCR6-CCL20 signaling is dispensable for their migration to the PPs (Lin et al., 2017). A special class of $CCR6^{hi}CD11c^{int}$ B cells were found to be responsible for proper M cell differentiation. Because there was impaired migration of these cells to the SED, this was thought to be a mechanism for compromised mucosal immunity in $CCR6^{-/-}$ mice (Ebisawa et al., 2011). Beyond the scope of this review, the role of CCR6 in mucosal immunity can be appreciated in this review article (Williams, 2006).

5. Conclusion

The CCR6-CCL20 axis has seen an increasing interest from innate immunity to humoral immunity in the last several years. Rapid up-regulation of CCR6 on B and T cells after activation suggests an important role for the CCR6-CCL20 axis in humoral immunity. Recruitment of $CCR6^+$ DCs to immune effector sites, such as PP, allows contact with lymphocytes for antigen presentation, activation of T and B cells, and initiation of the humoral immune response. In conjunction with other chemokine axes, T and B cells are then positioned in SLOs to facilitate cognate interactions via the CCR6-CCL20's role in immunological homeostasis. The T-B conjugates represent a key phase in the humoral response where crucial contacts occur, and disruption of the CCR6-CCL20 axis has been shown to decrease the efficiency of the GC reaction and potentially support autoimmunity. It could be speculated that the CCR6-CCL20 axis acts as a checkpoint or molecular brake in the T-B cell interactions (Fig. 1). At present, however, the bulk of evidence points to the molecular pair's role in the immunopathogenesis of autoimmunity via the Th17 axis and mucosal immunity.

The strong constitutive expression of CCL20 at mucosal sites suggests that the CCR6-CCL20 pair acts prominently in mucosal immunological homeostasis and indeed, disruption of the axis results in underdeveloped mucosal structures and impaired immunity. That there is little constitutive CCL20 expression in the spleen and LNs is not necessarily an indicator that there is little role of the chemokine axis in SLO homeostasis as the stromal network, which does express CCL20, is overshadowed by the hematopoietic cell lines present. Indeed, the stromal network of SLOs in which molecular signals are provided to antigen-presenting cells and lymphocyte subsets to facilitate leukocyte trafficking and positioning is an evolving area of research that helps provide an understanding for the development of humoral immune responses.

The major problem with studies so far is the reliance on analyses of human peripheral leukocytes which only provide circumstantial evidence for the role of the CCR6-CCL20 axis in coordinating humoral immune responses. Only a few, recent *in vitro* studies have begun to delve into the role of the chemokine partnership in this aspect of

immunity; yet the increasing links with autoimmunity (Ranasinghe and Eri, 2018) help strengthen the suspected importance in T-B cell biology and GC kinetics. Mechanistic studies are required in order to elucidate how CCR6-CCL20 acts as a checkpoint inhibitor for GC reactions. Certainly, these will lead to better understanding of their role in autoimmune pathophysiology and how to modulate this pair to provide appropriate, potential therapeutic options for patients.

Author contributions statement

AYSL drafted the manuscript and contributed to concept design. HK made substantial revisions to the manuscript and contributed to concept design.

Conflict of interest

None to declare.

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