B cells in Chronic Obstructive Pulmonary Disease: Moving to Center Stage

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Running title: B cells in COPD
Abstract

Chronic inflammatory responses in the lungs contribute to the development and progression of COPD. Although research studies focused initially on the contributions of the innate immune system to the pathogenesis of COPD, more recent studies have implicated adaptive immune responses in COPD. In particular, studies have demonstrated increases in B cell counts and increases in the number and size of B cell-rich lymphoid follicles in COPD lungs that correlate directly with COPD severity. There are also increases in lung levels of mediators that promote B cell maturation, activation, and survival in COPD patients. B-cell products such as auto-antibodies directed against lung cells, components of cells, and extracellular matrix proteins are also present in COPD lungs. These auto-antibodies may contribute to lung inflammation and injury in COPD patients, in part, by forming immune complexes that activate complement components. Studies of B cell-deficient mice and human COPD patients have linked B cells most strongly to the emphysema phenotype. However, B cells have protective activities during acute exacerbations of COPD by promoting adaptive immune responses that contribute to host defense against pathogens. This review outlines the evidence that links B cells and B cell-rich lymphoid follicles to the pathogenesis of COPD and the mechanisms involved. It also reviews the potential and limitations of B cells as therapeutic targets to slow the progression of human COPD.
Take-home message for clinicians

Although the adaptive immune system has strongly been implicated in the pathogenesis of COPD, most is known about the contributions of T lymphocytes to COPD. However, recent studies of human COPD patients and cigarette smoke-exposed mice have identified B cells and their products as key culprits in the pathogenesis of COPD. Although B cell products may contribute to the autoimmune component of the emphysema phenotype, B cells likely also have beneficial activities in eliminating pathogens from the airways especially during acute exacerbations of COPD. New therapeutic approaches targeting only the harmful activities of B cells may have potential in limiting the progression of human COPD.
1. COPD and Lung Inflammation

Chronic obstructive pulmonary disease (COPD) is projected to become the third most common cause of death by 2020 (78). COPD is characterized by airflow limitation that is not fully reversible. The most common risk factor in developing countries is smoking cigarettes, while inhalation of smoke generated by burning biomass fuels indoors is an important risk factor in developing countries. These exposures induce an abnormal inflammatory response in the lungs that is followed by destruction of alveolar walls, airspace enlargement (emphysema), and small airway remodeling (18; 32; 74).

Mechanisms exist that perpetuate and amplify pulmonary inflammation in COPD lungs as chronic pulmonary inflammation often increases progressively as COPD severity increases and can persist after smoking cessation. Early studies focused on the activities of innate immune cells (25; 26; 31; 62) and then CD8+ and CD4+ T cells in the pathogenesis of COPD (32; 38; 68). More recently, B cells have been linked to COPD as there are increases in the number and size of B cell-rich lymphoid follicles (LFs) in the severe stages of COPD, and the presence of B cell products (auto-antibodies) in COPD blood and lung samples (42; 54; 59). Herein, the biology of B cells and their (controversial) activities in COPD pathogenesis will be reviewed.

2. B Cell Development in Health

B-cell development is a tightly regulated process (51) (Figure 1). Common lymphoid progenitor cells mature through pro- and pre-B cell stages into immature B cells in the bone marrow by rearranging their immunoglobulin (Ig) heavy-chain and light-chain genes into unique B-cell receptors (BCR). This random immunoglobulin (Ig) gene rearrangement generates a highly diverse antibody repertoire including auto-reactive antibodies (auto-antibodies). However, in
health, only weakly or non-self-reactive immature B cells can traffic to the spleen, where they evolve into mature B cells through two transitional stages depending on the strength of BRC signaling. Weak BCR signaling associated with B-cell activating factor (BAFF)- and Notch2-signaling induces marginal zone (MZ) B-cell development. Stronger BCR- and BAFF-signaling drives the development of B cells into follicular B cells. MZ B cells home to the marginal sinus of the spleen, have self-renewal capacity, and respond primarily to blood-borne antigens by rapidly differentiating into Ig-secreting plasma cells (PCs). Most B cells become follicular B cells that re-circulate in the bloodstream (35; 57). However, in humans, MZ B cells also re-circulate and present blood-borne antigens to follicular dendritic cells (FDCs) (17; 35). During inflammation, both MZ and follicular B cells develop into regulatory B cells (B-regs) which produce immunosuppressive cytokines to promote resolution of inflammation and also maintain self-tolerance to prevent auto-immunity (66).

Naïve B cells circulate through the lung (33), but there are two resident B cell populations in the lung. First, long-lived PCs are present in airway submucosa produce and mainly polymeric immunoglobulins (IgA and IgM) that are secreted into the airway lumen (16). Second, memory B cells are generated in peri-bronchial regions during pulmonary infections and rapidly induce secondary immune responses during re-infection (55; 80) (Figure 1). Circulating B cells migrate into pulmonary lymph nodes (LNs) and LFs by exiting through capillary endothelium in pulmonary LNs. When pulmonary inflammation becomes chronic due to persisting antigen exposure and/or tissue injury, activated B cells contribute to LF formation through lymphotoxin signaling (86) (Figure 1).

In LN and LFs, cognate antigens are presented to B cells by FDCs, macrophages, and DCs (86) which induce B-cell differentiation into extra-follicular PCs, germinal center cells, or
early memory B cells depending on the strength of antigen-mediated BCR signaling. High-affinity binding of antigen to B cells induces B-cell proliferation and maturation into PCs expressing high-affinity Igs. Low- to moderate-affinity BCR-antigen interactions cause B cells to undergo a germinal center reaction, which is defined by affinity maturation, somatic hypermutation, and class switch recombination. High-affinity, mutated BCRs are positively selected by interacting with FDCs and T follicular helper cells (Tfh) and become long-lived memory B cells or PCs (35; 41) after co-stimulatory help from CD4+ helper T cells and Tfh. CD40-CD40 ligand (CD40L) interaction induces cytidine deaminase (AID) production by B cells, which is crucial for both somatic hypermutation and class switch recombination. In turn, recognition of antigen-derived peptides presented by major histocompatibility complex class II (MHCII) molecules on B cells coupled with CD40-CD40L interaction, activates T cells causing release of cytokines [e.g., interleukin (IL)-4, IL-10, IL-21, and transforming growth factor (TGF)-β] that control Ig isotype switching (8; 35; 79). This T cell-dependent B-cell activation with associated release of high-affinity Igs occurs not only in the LNs but also in ectopic LFs developing in the chronically inflamed lungs of COPD patients (Figure 1) (4; 13).

T cell-independent B cell activation occurs when microbial products bind to toll-like receptors expressed by B cells or induce BCR cross-linking which stimulates Bruton’s tyrosine kinase-mediated signal transduction (35; 79). T cell-independent B cell activation induces rapid Ig production by extra-follicular PCs and secretion of immune-modulatory cytokines (35; 46). Furthermore, B cells are also activated by myeloid leukocyte release of the tumor necrosis-α (TNF-α) family members, BAFF and A Proliferation-Inducing Ligand (APRIL) (79).

Transcriptional and epigenetic regulation of immune responses in diseases other than COPD is well established (43). An emerging area is the role of non-coding RNAs (ncRNAs) in
regulating the function of adaptive immune cells. Non-coding RNAs regulate lineage differentiation, proliferation, and activation of various types of immune cells including B cells (82). It is likely that dys-regulated expression of specific ncRNAs contributes to the pathogenesis of immunologic diseases, although this possibility has not yet been investigated in COPD (82).

3. B Cells in COPD

Hogg et al. reported that the number of infiltrating B cells and the percentage of small airways containing B cell-rich LFs were both associated with COPD severity (32). Increased B-cell counts were subsequently reported in COPD large airways (28). B cells are also located within LNs and peri-vascular and parenchymal LFs in COPD lungs (77). COPD lungs also contain more memory B cells and PCs than control lungs (12; 87). Increased B-cell counts in human COPD lungs are associated with elevated IgA synthesis. However, the expression of the receptor that translocates polymeric Ig from the airway submucosal space to the airway lumen (pIgR) is down-regulated in COPD airways and expression levels correlate inversely with airway inflammation and remodeling, and COPD progression (27; 58; 63). These results suggest that IgA-mediated mucosal immunity is impaired in COPD and contributes to COPD progression.

**B cells and COPD phenotypes:** B cell-rich LFs have been most strongly linked to the emphysema phenotype and participate in oligoclonal proliferation in emphysematous lung (5; 77). A transcriptomics study reported that B-cell signaling pathways were among the most highly upregulated pathways in patients with high resolution computed tomography-defined emphysema, but did not link B-cell signaling to small airway disease (22). More PCs were reported in lungs of smokers with versus without chronic bronchitis (87). However, COPD patients having frequent exacerbations have impaired expression of genes involved in lymphocyte activation in blood samples (71). Also, delayed adenovirus-specific IgG maturation
responses following an acute exacerbation of COPD (AECOPD) were associated with increased AECOPD recurrence rates, and higher hospitalization and mortality rates when subjects were followed for 2 years (9). Together, these results suggest that B-cell signaling has protective functions during AECOPD. However, additional studies are needed to test this hypothesis.

**Mediators regulating B cell recruitment and activation:** When pulmonary inflammation becomes chronic, activated lymphocytes expressing lymphotoxin-α-β heterotrimer (LTα1β2) interact with the lymphotoxin (LT)β receptor on neighboring stromal cells (20). In turn, these stromal cells express lymphoid chemokines (CC-chemokine ligands [CCL]19, CCL21, CXC-chemokine ligands (CXCL)12 and CXCL13) and adhesion molecules that recruit naïve B- and T-cells, and DCs into the lung (Figure 2). Several other mediators act on B cells to promote the development, organization, and expansion of LFs in COPD lungs and/or COPD severity (Table 1) as outlined below.

**CXCL13:** CXCL13 is the most important inducer of LFs in COPD lungs and binds to CXCR5 expressed on B cells and Tfh cells. FDCs are the main source of CXCL13 in health. However, in COPD lungs, B cells also produce this chemokine thereby creating a positive feedback loop (44). CXCL13 promotes B-cell recruitment into LFs and their compartmentalization within LFs. Neutralization of CXCL13 in cigarette smoke (CS)-exposed mice reduced the number of organized LFs, attenuated airway inflammation, and partially protected mice from alveolar destruction (11). COPD patients have increased sputum CXCL13 protein levels and CXCL13 mRNA transcripts in whole lung samples compared with samples from never-smoker controls (11). These findings suggest that CXCL13 contributes to pulmonary LF organization in human COPD patients as well as CS-exposed mice (Figure 2). However, additional studies are needed to test this hypothesis.
CXCL12 (Stromal cell-derived factor 1): CXCL12 is constitutively expressed in the bone marrow and is essential for B-cell development in the bone marrow by signaling through CXCR4 on B cells (51). CXCL12 expression was reported to be induced in pulmonary LFs in patients with end-stage COPD and CS-exposed mice (65). Furthermore, single nucleotide polymorphisms in the CXCL12 locus have been linked to lung function in a genome-wide association study (85). Thus, it is possible that CXCL12 contributes to late-stage progression of COPD by increasing the proliferation and survival of B cells (Figure 2).

Among the other receptors for CXC chemokines, CXCR3 is expressed by B- and T-lymphocytes, and plasma cells (76). IL-17A, which was reported to be elevated in COPD lungs (65), stimulated the secretion of CXCR3 ligands (CXCL9, CXCL10, and CXCL11) by epithelial cells and thereby promoted the recruitment and further maturation of CXCR3(+) B cells. CXCR3(+) B cells [but not CXCR3(-) B cells] were shown to regulate macrophage polarization in an IgG-dependent manner in other diseases (45). Interestingly, CXCR3-deficient mice had reduced CS-induced acute lung inflammation and especially lower lung CD8+ T cell counts (53) indicating that CXCR3(+) cells promote CS-induced recruitment of CD8+ T cells which are required for emphysema development in mice (47). However, the contributions of CXCR3(+) B cells to these processes were not investigated.

IL-17A: IL-17A levels were reported to be increased in COPD lungs (65). In mice exposed to CS, IL-17A stimulated CXCL12 secretion by stromal cells (24; 65; 83), and also increased neutrophilic lung inflammation and alveolar epithelial cell apoptosis (15). In mice with CS-, lipopolysaccharide- or elastase-induced COPD, there was increased IL-17A release by Th17 cells (24; 65; 83), and neutralizing IL-17A reduced LF formation, lung compliance, and
airway inflammation (83). Thus, IL-17A regulates LF development and contributes to COPD-like lung lesions in CS-exposed mice.

**BAFF:** BAFF is essential for B-cell survival, maturation, and differentiation, and maintenance of LFs. In health, BAFF is produced by epithelial cells, myeloid cells, PMNs, and lymphocytes and binds with highest affinity to the BAFF receptor (BAFFR) (14). BAFF also binds to transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI) and B-cell maturation antigen (BCMA) (14). Administration of BAFFR-Fc (a fusion protein consisting of the extracellular domain of murine BAFFR fused to a linker peptide and the Fc-portion of human IgG1) to CS-exposed mice, blocked BAFF signaling, and thereby prevented the development of both organized and poorly-organized lymphoid aggregates, reduced pulmonary inflammation, and limited alveolar destruction (70). BAFF has been linked to human COPD as COPD patients were reported to have increased BAFF expression in bronchial epithelium and alveolar macrophages, and the number of BAFF-positive macrophages correlated inversely with the severity of airflow obstruction (59). Although B cells do not produce BAFF in health, in severe and very severe COPD, more BAFF-positive B cells were reported in blood and BAL samples from COPD patients versus controls, and more BAFF-positive B cells were present in LFs in COPD lungs (60). Moreover, there was a direct correlation between BAFF-positive B cells in LFs, LF size, and airflow obstruction, and an inverse relationship between BAFF expression in B cells in LFs and B-cell apoptosis (60). Together, these results suggest that BAFF not only promotes the expansion of LFs both in number and size by increasing B-cell survival, but also promotes the progression of COPD (Figure 2). However, additional studies are needed to test this hypothesis.
APRIL: APRIL regulates isotype switching in B cells, and was reported to be upregulated in alveolar epithelial cells in COPD lungs versus control lungs (61). APRIL expression in B cells (and also in alveolar epithelial cells and myeloid leukocytes) in COPD patients with lung cancer was higher than APRIL expression in the same cells in patients with either disease alone (61). Thus, increased APRIL expression by B cells (and possibly other cells) may either alter immune responses to promote the development of lung cancer in COPD patients or be a response to lung cancer developing in the environment of the COPD lung.

4. Autoimmunity in COPD

In 2003, the hypothesis that COPD has an autoimmune component was formulated based upon several observations: 1) not all smokers develop COPD; 2) lung inflammation can persist after smoking cessation; and 3) auto-antibodies develop in some COPD patients (3). There are several similarities between COPD and autoimmune diseases. Both COPD and auto-immune diseases are complex gene-by-environment disorders (30) and smoking cigarettes is also a risk factor for several classic autoimmune diseases (19). Also, in both COPD and autoimmune diseases, there is ongoing recruitment of activated antigen presenting cells, production of cytokines that induce auto-reactive T-cell proliferation, pro-inflammatory memory T-cell responses (36), a positive-loop feedback that induces Th1/Th17 T-cell polarization, and increased expression of mediators that activate B cells such as BAFF and APRIL (10; 59-61; 70). Exacerbations occur in autoimmune diseases (75) and COPD (21) that are associated with increases in tissue B-cell counts (7; 72; 84). Both COPD and autoimmune diseases are associated with increased tissue levels of B-cell activators (e.g., IL-6 and BAFF) that contribute to their pathogenesis (6; 40). As in autoimmune disorders, COPD patients have circulating and
tissue-associated auto-antibodies that have potential to contribute to tissue inflammation and injury (including anti-nuclear, anti-tissue, anti-elastin, anti-carbonyl self protein, and anti-endothelial cell auto-antibodies) (23; 42; 54). Auto-antibody generation in COPD has been linked to increased lung levels of carbonyl-modified self-proteins induced by increased oxidative stress levels, and lung levels of these self-proteins correlated positively with COPD severity (37). COPD is associated with proteolytic degradation of extracellular matrix proteins (Figure 2) which contributes to emphysema development and also generates fragments of extracellular matrix (matrokines) that are chemotactic for inflammatory cells (1; 69). However, injury to extracellular matrix proteins and cellular components of COPD lungs also generates neo-epitopes against which auto-antibodies can be generated.

There are conflicting reports in the literature on whether auto-antibodies contribute to COPD pathogenesis. The notion that auto-antibodies promote lung inflammation and injury in COPD patients is supported by studies showing that: 1) COPD patients had anti-elastin antibodies and Th helper-type 1 responses that correlated positively with emphysema severity (23; 42; 54); 2) the prevalence of anti-nuclear and anti-tissue factor antibodies was higher in COPD patients versus controls (54); and 3) serum anti-tissue and anti-carbonyl antibody levels correlated positively with the severity of airflow obstruction in COPD patients (37; 54). However, other studies have not linked auto-antibody levels to COPD. Wood et al. observed that serum anti-elastin antibody levels were higher in controls than COPD patients, related only to CS exposure, and higher in subjects that smoked less than 10 pack-years (81). Two studies did not show differences in anti-elastin autoantibody levels in COPD patients, smokers without COPD, and healthy subjects (29; 64). A better understanding of the contribution of auto-antibodies and other auto-immune processes to the pathogenesis of COPD is hindered by the fact that COPD
patients are exposed to different environmental factors and infectious agents, and vary substantially in their inheritance of COPD susceptibility genes and the pathologies that develop in their lungs and other organs (2). Also, the number and function of B-regs has yet to be assessed in COPD lungs.

5. Mechanisms By Which B Cells Promote COPD Pathogenesis

B cells contribute to CS-induced pulmonary inflammation and emphysema development in mice as CS-exposed B cell-deficient mice are protected from these lung pathologies (34). This study reported that B cell production of IL-10 was necessary for macrophage activation and the release of matrix metalloproteinases that have been implicated in airspace enlargement (56).

Antibodies produced by PCs form complexes with target antigens, and the resultant immune complexes not only engage B cells and activate Fc-receptor-bearing PMNs and macrophages, but also fix and activate complement (C) components, generating complement products that activate leukocytes (Figure 2). Increased C4d levels confined to the pulmonary small vessels were associated with the presence of auto-antibodies in COPD lungs (37). Chronic bronchitis patients were reported to have sustained activation of C3 and C4 in serum samples, and C4 activation correlated positively with emphysema severity, small airway dysfunction, and rates of pulmonary infections (39). Increased induced sputum levels of C5 activation products that are potent chemoattractants for myeloid leukocytes were reported in COPD patients (48). Moreover, sputum levels of C5 activation products correlated negatively with lung diffusion coefficient linking C5 activation to the emphysema phenotype (48). Together, these results suggest that complement activation promotes the recruitment and activation of leukocytes that
release proteinases and oxidants that injure the lung ECM and alveolar septal cells causing airspace enlargement (Figure 2).

6. Therapies Targeting B cells in COPD

Currently, there is a lack of disease-modifying therapies for COPD, and an unmet need for more effective therapies targeting the inflammatory processes underlying COPD (88). Both the human COPD studies and studies of CS-exposed B cell-deficient mice outlined above support the notion that B cells promote COPD pathogenesis and especially the emphysema phenotype. The complexity of B-cell maturation presents opportunities for therapeutic interventions (Figure 1), as outlined below.

**B Cell Depletion:** Rituximab is a monoclonal antibody (mAb) that targets the CD20 B cell-specific antigen which is expressed on pre-B cells and mature B cells, but not on other cells (including PCs). Rituximab induces apoptosis of human B cells and reduces activated B-cell counts in blood by up to 97% in rheumatoid arthritis (RA) patients (52). Rituximab is approved for the treatment of CD20+ B cell non-Hodgkin’s lymphoma and RA patients that are unresponsive to anti-TNFα therapies. However, a randomized placebo-controlled clinical trial of Rituximab in COPD was terminated early because of increased pulmonary infection rates in the Rituximab arm (13).

**Manipulation of B Cell Survival:** Inhibition of BAFF signaling with anti-BAFF mAb (Belimumab) reduces B-cell counts in tissues in animal models of auto-immunity and RA patients (50). Belimumab has not been tested in animal models of COPD or COPD patients. However, prophylactic and therapeutic administration of a soluble BAFFR-Fc fusion protein to CS-exposed mice reduced pulmonary B-cell counts and prevented CS-induced LF formation (70). Prophylactic (but not therapeutic) administration of BAFFR-Fc attenuated pulmonary
inflammation and emphysema development (70) indicating that early intervention with BAFFR-Fc is needed to modify emphysema progression in CS-exposed mice.

Potential Limitations of B Cell Therapies: COPD is a chronic disorder and thus it is crucial that any new disease-modifying therapy is safe and well-tolerated by patients. A major limitation of B cell-targeted therapies is that the B cells have beneficial as well as deleterious activities in COPD. For example, B cells contribute to adaptive immune responses that kill pathogens. The lung microbiome of COPD patients is altered (and less diverse) than that of smokers without COPD (73) and this may contribute to AECOPD frequency and severity. However, it is not clear how alterations in the lung microbiome are linked to B cell function.

Targeting B cells may suppress IgA production which is essential for maintenance of a microbiome that protects against airway infection with pathogens (67). It is noteworthy in this respect that Rituximab therapy was associated with increased rates of pulmonary infections in COPD patients (13). B cells also have immune-regulatory activities by presenting antigens to T cells and releasing mediators. Innate immune activation of B cells also generates natural cross-reactive antibodies that maintain B-cell memory and protect against autoimmunity (49).

The optimal COPD phenotypes and disease stage(s) for intervention with anti-B cell therapies need to be defined. The results obtained when BAFFR-Fc was tested in CS-exposed mice indicate that only early intervention adequately targets BAFF-BAFFR signaling in emphysema development in mice (70). B cells are unlikely to be involved in the initiation and/or progression of early-stage human emphysema, as greater declines in FEV1 occur in mild COPD patients in whom pulmonary B cell counts are lower and LFs are small and infrequent when compared with those in advanced disease (28; 60). Thus, the current literature suggests that late-stage emphysema is the most likely stage and phenotype to be modified by anti-B cell therapies.
in human COPD patients. Future studies should provide information about different B-cell subsets and their functions and contributions to disease processes in COPD patients. Thus would enable therapies to be developed that target only deleterious B-cell subsets in COPD patients.

Conclusions

Current evidence suggests that B cells contribute to the emphysema COPD phenotype. However, B cells may be protective in the setting of AECOPD which are associated with high morbidity and mortality. There are knowledge gaps on the B-cell subsets that contribute to COPD, the COPD phenotypes to which they contribute (especially large and small airway disease), and the mechanisms involved. Whether auto-antibodies injure the lung or are only an epiphenomenon reflecting lung injury in COPD is not clear. These knowledge gaps could be addressed by future longitudinal clinical studies that will determine the relationships between B cells, B-cell products, the lung microbiome, and clinical outcomes in well-phenotyped COPD patients having a range of COPD severities. However, therapies that target only deleterious B cell subsets or their harmful products have potential as future disease-modifying therapeutics for COPD patients.
Acknowledgements

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Legend to Table 1: Mediators involved in B cell responses in COPD.

Abbreviations used in Table 1:

APRIL: A proliferation inducing ligand

BAFF: B cell activating factor of tumor necrosis factor family

BAFF-R: BAFF receptor

BCMA: B-cell maturation antigen

CD: Cluster of Differentiation

COPD: Chronic obstructive pulmonary disease

CS: Cigarette smoke

CXCL13: C-X-C motif chemokine ligand 13

CXCL12: C-X-C motif chemokine ligand 12

CXCR4: C-X-C motif chemokine ligand receptor 4

CXCR5: C-X-C motif chemokine ligand receptor 5

FDCs: Follicular dendritic cells

IL-17A: Interleukin 17A

LF: Lymphoid follicle

TACI: Transmembrane activator and CAML interactor
**Figure 1: B cell maturation and lymphoid follicle formation:**

1. B cell activation and maturation: B-cell activation is induced when a naïve B cell binds via B-cell receptor (BCR) to either a soluble antigen or to an antigen that is presented by macrophages or dendritic cells (DCs). T helper cells (T<sub>H</sub> cells) assist in the maturation of B cells into plasma cells and memory B cells. B cells then become mature memory B cells expressing CD20, and plasma cells. Only mature B lymphocytes can enter the lymphoid follicles and efficiently participate in the immune response. Within lymphoid follicles, B and T lymphocytes segregate into different areas, but interact by the binding of cluster of differentiation (CD)40 expressed on B cells to CD40 ligand (CD40L) expressed on T cells.

2. Lymphoid follicle (LF) development: In response to environmental insults (e.g., bacteria and bacterial lipopolysaccharide), airway epithelium and macrophages express cytokines that recruit immature B- and T-cells and DCs. When inflammation becomes chronic due to persisting antigen exposure and/or tissue damage, lymphocyte aggregates will give rise to organized lymphoid follicles with separated B- and T-cell areas. B cell activating factor of the TNF family (BAFF), which is expressed by T cells, macrophages, and DCs activates B cells via the BAFFR. Mature lymphoid follicles contain high endothelial venules (HEV), follicular dendritic cells (FDCs) and germinal centers.

3. Production of immunoglobulins by plasma cells. When B cells are activated following antigen binding, they proliferate to form plasma cells which produce antibodies which promote mucosal immunity (IgA) that increases clearance of pathogens.
**Figure 2: B cells and B cell-rich lymphoid follicle expansion in COPD:**

In response to environmental insults (e.g., cigarette smoke and bacteria), airway epithelium and macrophages express cytokines that recruit immature B- and T-cells, and DCs. When inflammation becomes chronic due to persisting antigen exposure and/or tissue injury, activated lymphocytes expressing lymphotoxin-α-β heterotrimer (LTα1β2) interact with the lymphotoxin (LT)β receptor on neighboring stromal cells. Stromal cell stimulation induces the expression of lymphoid chemokines (CC-chemokine ligands [CCL]19 and CCL21, and CXC-chemokine ligands [CXCL]12 and CXCL13) and adhesion molecules that promote additional recruitment of naïve B and T lymphocytes, and DCs. B-cell activating factor of TNF family (BAFF), which is expressed by T cells, macrophages, and DCs (and in advanced COPD by B cells themselves) activates B cells leading to increases in B cell numbers in the lung and an expansion in pulmonary lymphoid follicles both in size and number. Activated B cells release interleukin 10 (IL-10) which activates macrophages to release matrix metalloproteinases-9 (MMP-9) and MMP-12 which degrade the lung extracellular matrix (ECM) proteins leading to emphysema development and the generation of matrix fragments (matrikines) that recruit PMNs into the lungs. Increased production of IL-17A by Th17 cells regulates the formation of LFs and the recruitment of PMNs into the lungs. PMNs release serine proteinases including neutrophil elastase that contribute to loss of alveolar walls.

Activated B cells proliferate and mature into plasma cells. Plasma cells release antibodies to bacteria and/or auto-antigens to lung components including proteolytic degradation products of the ECM and lung cells. Binding of auto-antibodies to their target antigens induces complement activation which recruits and activates inflammatory cells and induces immune
complex-mediated injury to the lung. These processes contribute to the progression of airspace enlargement.

There are two monoclonal antibodies targeting B-cells that potentially could limit COPD progression: 1) Rituximab which targets CD20 but was associated with increased risk of pulmonary infections when tested in COPD patients; 2) Belimumab which targets BAFF but has not been tested in animal models of COPD or human COPD patients. In addition, a BAFFR-Fc fusion protein of the extracellular domain of murine BAFFR fused to a linker peptide and the Fc portion of human IgG1 attenuated emphysema development in CS-induced mice but has not yet been tested in human COPD patients.
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Figure 1

1. B cell activation and maturation

- naive B cell
- antigen presentation
- memory B cells
- B cell selection, class switching, and differentiation

2. LF development

- High endothelial venules
- Follicular dendritic cell
- Pathogens
- Dendritic cell

3. Production of immunoglobulins by plasma cells

- secreted IgA
- IgA
- B cell receptor
- LYMPHOID FOLLICLE
- BAFF
- BAFFR
- CD40
- CD40L
- naive B cell
- Pathogens
- Follicular dendritic cell
- High endothelial venules

Legend:

- B cell
- T cell
- B cell receptor
- Epithelial cell
- Plasma cell
- Immunoglobin/auto-antibody
- Dendritic cell
- Pathogens
- Follicular dendritic cell
- High endothelial venules
Figure 2

- B cell
- T cell
- Plasma cell
- Epithelium
- Neutrophil
- Immunoglobulins/auto-antibodies
- Dendritic cell
- Pathogens
- Follicular dendritic cell
- High endothelial venules
- Alveolar macrophage
- Antigens
- Neutrophil elastase
- Matrix metalloproteinases
<table>
<thead>
<tr>
<th>Name</th>
<th>Cellular sources in the lung</th>
<th>Receptors</th>
<th>Role in COPD</th>
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<tr>
<td>CXCL13</td>
<td>Follicular dendritic cells in health B cells in COPD</td>
<td>CXCR5</td>
<td>Promotes recruitment of B cells into lymphoid follicles and their compartmentalization within lymphoid follicles</td>
</tr>
</tbody>
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| CXCL12  
*(Stromal cell-derived factor 1)* | Constitutively expressed by bone marrow cells. Expressed by B- and T-cells in lymphoid follicles and in alveolar macrophages in COPD lungs | CXCR4 | May promote late-stage progression of COPD by increasing the proliferation, homing, and survival of B cells |
| IL-17A | CD4- and CD8-positive T cells, γδ T cells, NK T cells, neutrophils, and innate lymphoid cells | IL17 receptor | Promotes lymphoid follicle development and contributes to COPD-like lung lesions in CS-exposed mice |
| BAFF | Monocytes, macrophages, follicular dendritic cells, T cells, and alveolar and bronchial epithelial cells. B cells in COPD lungs produce BAFF | TACI, BCMA, BAFF-R | Promotes the expansion of lymphoid follicles both in number and size by increasing B-cell survival |
| APRIL | B cells, alveolar epithelial cells, and myeloid leukocytes | TACI, BCMA | Increases B-cell development and maturation. APRIL expression is upregulated in B cells in COPD patients with lung cancer |