

REVIEW

B lymphocytes in inflammatory airway diseases

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Clinical & Experimental Allergy

Summary

B lymphocytes are key players in all facets of adaptive immune responses and are responsible for the production of IgE antibodies, initiators of allergic hypersensitivity reactions. Recent evidence indicates that B cells may be a crucial player in allergic and inflammatory airway pathology, directly populating upper and lower airway tissues. This review examines human and animal studies that directly demonstrated the presence of B lymphocytes in airway tissues and elaborates on their function as antibody-secreting cells, antigen-presenting cells and producers of inflammatory and regulatory cytokines. B lymphocytes appear to contribute to multiple facets of immune homeostasis in inflammatory diseases of the upper and lower airways.

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Cite this as: J.-P. Drolet, H. Frangie, J. Guay, O. Hajoui, Q. Hamid and B. D. Mazer, *Clinical & Experimental Allergy*, 2010 (40) 841–849.

Introduction

Antibodies play a crucial role in the defence against pathogens. They circulate in the blood and act as surveillance molecules in mucosal defence. Conversely, antibodies are well recognized to be involved in pathology. Auto-antibodies can directly initiate destructive inflammation in multiple conditions, and IgE mediates the initiation of allergic diseases such as asthma, allergic rhinitis (AR) and food- or venom-induced anaphylaxis. Although IgE and other antibodies are associated with allergic respiratory diseases, controversy exists regarding the primary location of IgE production. While it is commonly taught that allergic asthma is characterized by a pulmonary infiltrate consisting of neutrophils, dendritic cells, mast cells, eosinophils and T lymphocytes, B lymphocytes and plasma cells (PCs) are rarely mentioned [1]. This review will address the significant body of evidence that antibody-producing B lymphocytes are present in airway mucosa. Additionally, antibody secretion may only be one facet of the role B cells play in the airways. B cells play a role as antigen-presenting cells. They also secrete both inflammatory and regulatory cytokines; the role of B cells as regulators of pathobiology in asthma, allergic rhinosinusitis and chronic obstructive pulmonary

disease (COPD) are underexplored. We will address some of the emerging evidence for the multiple functions of B cells with examples from human disease and animal models of airway pathology.

B lymphocyte organization in the airways

B lymphocytes are a heterogeneous group of cells that originate in bone marrow and are key players in adaptive immunity. Like T lymphocytes, B cells undergo various stages of maturation. Initial maturation in the bone marrow is independent of antigen exposure, but is dependent on key stromal factors and cytokines such as Flt-3 ligand and IL-7 [2, 3]. Although human newborns with severe combined immune deficiency lacking functional IL-7 receptor have B lymphocytes, exposure of human cord blood or bone marrow B cells to IL-7 increases B cell production by over 60-fold [4]. B cells leave the bone marrow as immature, naïve cells and migrate to the spleen where they complete their maturation into surface IgM- and surface IgD-positive naïve B cells [5, 6]. B cells go through a transitional phase [7], circulate and differentiate in lymph nodes (LNs) in contact with antigens and T lymphocytes, and take on the characteristics of mature germinal centre cells (e.g. co-expressing CD38 and other

surface molecules or CD27⁺ memory cells). PCs, which are a final stage in B cell development, do not express a surface antigen receptor, but have very high levels of CD38 expression [8].

Bronchial lymph nodes

In humans, B lymphocytes that populate LNs embedded in the bronchial tree are a source of Igs in the airways [9]. The primary LNs of the lung follow the trachea and large bronchi [10]. There are multiple paratracheal LNs, tracheobronchial nodes at the bifurcation of the right and left mainstem bronchi and at the carina, hilar nodes and intrapulmonary LNs. These have classical LN architecture, with a capsule, high endothelial venules and lymphoid vessels to facilitate blood supply and antigen presentation. Therefore, it is believed that the airway adaptive immune response mimics that of other peripheral lymphoid tissues, although descriptive and dynamic infectious challenge models are lacking.

Passage of antigens to the airways is predominantly via inhalation, rather than via the circulation. Presentation of antigens to T cells within the lung interstitial LNs is facilitated by a network of dendritic cells that line the conducting airways and the interstitium [11]. Inhaled antigens primarily traffic to draining LNs, and not to other regional LNs. Using a rat model and intratracheal antigen exposure, Lehmann *et al.* [12] demonstrated increases in T and B cells within draining LNs 6 h after antigen instillation. This increased over 24–48 h, with little change in lymphocyte populations found in the non-draining LNs. The increase in lymphocytes was associated with lymphocyte extravasation into the bronchial alveolar space via the alveoli [12]. Hutchison *et al.* [13], using intravital microscopy demonstrated multiple waves of T cells increasing in both the lung parenchyma and draining LNs for 1–5 days following antigen exposure. Similar studies have not been performed examining B cells; however, the implication is that the primary responses to antigen in the lung are related to infiltration of cells to the crucial lung compartments. Recently, Luger *et al.* [14] described the appearance of PCs in the lungs of mice that were sensitized and challenged to ovalbumin (OVA). Their work, which focused specifically on long-lived PCs, suggested that PCs appear in the lung and are sustained there as long as antigen is present. Termination of antigen inhalation leads to a rapid depletion of lung PCs, but OVA-specific PCs are retained in the bone marrow and spleens of sensitized animals. This implies that mice are capable of producing long-lasting IgE PCs, which is contrary to opinions regarding IgE-producing cells [8].

Interestingly, large blood-borne pathogens >20 µm such as helminthes are 'filtered' by the lung vasculature and presented to T cells in the mediastinal LNs [15]. This

implies that the respiratory immune system may play a unique role in defence of large blood-borne pathogens.

In the spleen and LNs, B cells, which have also encountered and processed foreign antigens, present antigens to CD4⁺ helper T cells on the edges of B cell follicles. This is an MHC-restricted process, using Class II MHC–T cell receptor interaction [16]. Depending on the cytokines released in the environment, B cells will produce particular Ig isotypes. Classically, the main isotype produced in the lung is IgA, which is under the control of TGF-β, IL-5 and IL-10. However, IgG and IgE are both detectable in the lung. IgE synthesis is under the control of IL-4 and IL-13, IgG can be induced by IL-10, IFN-γ and IL-4 [17]. We have shown that IL-9 can augment IgE production in association with Ig class switching [18,19]; IL-21 can also perform this function [18]. This paradigm is clearly active in the spleen and other secondary lymphoid tissues, but begs the question of whether other cells can drive this process in inflamed tissues such as the airways. For example, TGF-β is produced in large amounts by fibroblasts and epithelium [20], and mast cells in lung tissue are a potent source of IL-4 and IL-13. B cells themselves can secrete cytokines following antigen or T cell activation and contribute to the process of isotype switching [21–23]. More importantly, antigen receptor signalling by memory B cells may be so 'wired' as to bypass the need for T cell help [24]. Thus, a key question regarding airway B cells and the generation of local PCs relates to the requirement for T–B interaction in the development of antibody-secreting cells, especially IgE PCs.

Bronchial-associated lymphoid tissue

In addition to the organized lymphoid tissue that is present in the paratracheal and bronchial regions, there are areas of lymphoid aggregates that reside within the subepithelial layers of the bronchi. These collections of lymphocytes, known as bronchial-associated lymphoid tissue (BALT), are the bronchial equivalent of the gut-associated lymphoid tissue (GALT) such as Peyer's patches. BALT is defined as an aggregate of lymphoid tissue with a follicle-like structure that is demarcated by an overlying epithelium and is fed by high endothelial venules similar to other lymphoid tissues [25]. However, the role of BALT is less well defined than that of the GALT. The main reason why the role of BALT in normal defences remains controversial is the fact that in normal human adult lungs, there is little if any BALT seen as compared with other species where BALT is constitutional. Up to 40% of children have BALT [26], but it is less common in healthy adults [27]; however, it has been observed in patients who have died of pulmonary infection, severe smokers or chronic illness such as rheumatoid arthritis [10, 28].

In many species, BALT is made up of a network of follicles of B cells associated with intraepithelial cells known as M cells. These cells arise from the respiratory

epithelium from basal stem cells, and are specialized cells capable of phagocytosis [9]. Most of the understanding on BALT and nasal-associated lymphoid tissues (NALTs) is from work carried out on species such as rat, mouse and chicken; extensive studies in human have been difficult to perform. Although it is clear that BALT exists in humans in pathological states, the overall contribution to immune responses must be better defined [9].

The relative contribution of BALT to Ig production is unknown, although IgA- and IgM-bearing cells have been found in mouse [29] and in human studies [9]. Our laboratory determined the presence of Ig-producing B cells in lung tissue by measuring IgG1, IgG4 and IgA constant region mRNA from bronchial biopsy samples of normal individuals and mild and severe asthmatics. We detected Ig constant region transcripts for $\gamma 1$, $\gamma 4$ and α , which indicated that there were B lymphocytes capable of producing IgG subclasses and IgA in the lung parenchyma of normal subjects (Fig. 1). Interestingly, constant region mRNA transcripts in severe asthmatics were considerably more difficult to detect, in spite of B cells being clearly present in lung biopsies from subjects with severe asthma (Figs 1 and 2b). While there is little data on the relative contribution of Igs from lung parenchyma vs. the bronchial lymphoid tissues, this suggests that antibody production in the lung is part of the mechanism of pathogen recognition in healthy individuals. An exception may be with regard to IgE production, where IgE⁺ PCs appear to be more abundant in the upper airways relative to the lymphoid tissue in humans [30], but more data are required to determine whether this is so in the lungs.

Airway mucosa

We evaluated B lymphocyte infiltrates in sinus biopsies that were harvested from individuals undergoing surgery

for a wide range of sinus pathologies. The staining of tissue B cells in sinus tissues revealed both collections of cells that resembled follicles, as well as smaller groups of cells near the lamina propria and subepithelial layer of the mucosa (Fig. 2a). These cells were predominantly CD38⁺, suggesting mature B cells and PCs populating the sinuses. There were also CD27⁺ cells that may represent memory cells. Thus it appears that B lymphocytes of mature and memory phenotypes can play a role in antigen recognition and rapid responses to inhaled antigens and allergens. Similar CD20⁺ B cell follicles may be seen in biopsy specimens from severe asthma subjects (Fig. 2) as well as from individuals with COPD [31, 32]. As M cells were not systematically stained in these sections, it is not clear if these follicles reflect variants of BALT or NALT.

Is allergy a disease of local or systemic immunoglobulin E production?

The airway mucosa appears to harbour IgE-producing cells in a disproportionate manner compared with other isotypes [30, 33]. This appears to be antigen specific and is a feature of allergic individuals more than non-atopics [33]. The presence of allergen-specific IgE in the nasal mucosa, in the absence of peripheral IgE, was initially observed using nasal challenges in the 1970s [34]. However, these and other studies did not differentiate between IgE that was produced directly in the nasal mucosa as opposed to local lymphoid tissue. Direct observation of the biology of IgE production in the upper airway has been confirmed by extensive *in situ* study of nasal tissue cultured as explants. Cameron et al. [35, 36] and Smurthwaite et al. [37] cultured small biopsies from individuals with and without AR in the presence and absence of the relevant antigens. This isolated tissue then was assayed for the production of IgE. Only antigen-

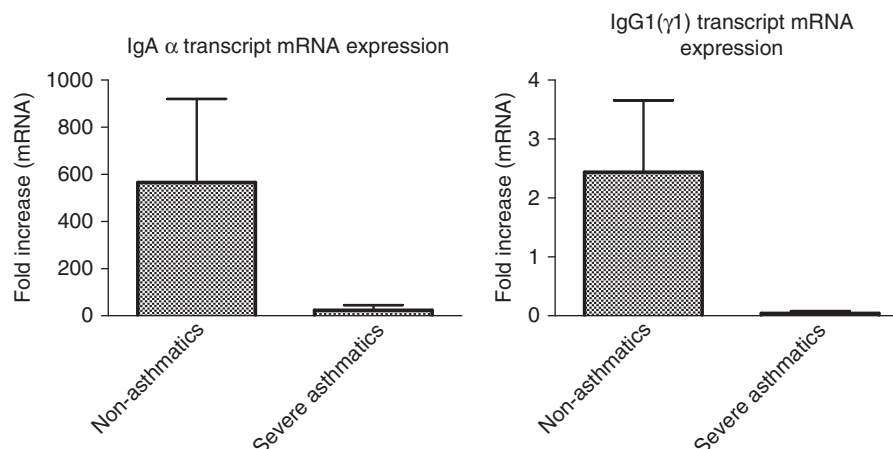


Fig. 1. Ig gene transcripts (for IgA, left panel and IgG, right panel) in biopsy specimens from bronchial biopsies taken from normal control subjects and severe asthmatic subjects ($n = 3$ of each). Note that there is a marked decrease in immune globulin constant region transcripts in severe asthma, compared with normal lungs.

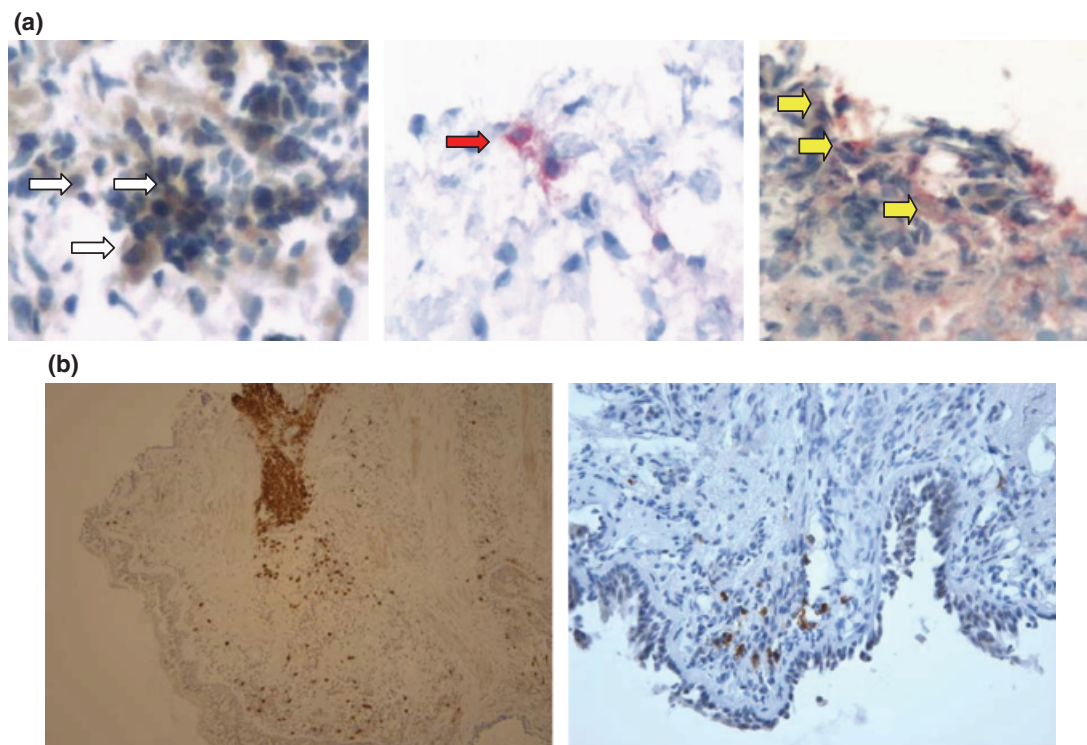


Fig. 2. (a) Immunohistochemical staining of sinus tissue, stained for CD20 (brown, both panels) and CD38 (red, right panel). B cell infiltrate in sinuses includes mature $CD20^{+}$ $CD38^{+}$ B cells and plasma cells. (b) Immunohistochemical staining of bronchial biopsies from severe asthmatics revealing large numbers of $CD20^{+}$ B lymphocytes. The left panel demonstrates $CD20^{+}$ B cells organized in a follicular structure, and the right panel reveals subepithelial infiltration of B cells into lung tissues.

stimulated nasal tissue from allergic individuals produced IgE. This suggested that, in the absence of a systemic circulation and without the LN environment, locally resident B cells could secrete IgE if properly triggered. Subsequent studies evaluated B cells in the nasal mucosa undergoing class switch recombination to IgE in the absence of the lymphoid environment. Cameron *et al.* [38] and Takhar *et al.* [30] detected crucial components of isotype class switching in locally stimulated B lymphocytes. This included enzymes such as activation-induced cytidine deaminase (AID), as well as constant region and switch circle transcripts representing all Ig isotypes, demonstrated *in situ*, as well as by RT-PCR. The increase in Ig transcripts was found in the allergen-stimulated nasal tissue in both studies, underlining the observation that IgE production could be specifically controlled by extra-nodal processes and was a feature of the upper airway mucosa. The rapid production of IgE following antigen exposure may reflect the presence of memory B cells on which antigen receptor signalling by sIgG⁺ and sIgE⁺ B cells is amplified compared with naïve, sIgM⁺ cells, and thus may be capable of rapid secretion of antibody [24]. Additionally, B cells in antigen-stimulated nasal explants stain strongly for IL-13, suggesting an autoregulatory pathway for IgE production [39]. Thus, the appearance of IgE in the nasal mucosa is, at least in

part, a function of the local secretion of IgE by resident B lymphocytes in the mucosa.

Although more difficult to study, the local production of IgE in asthma has also been evaluated. Takhar *et al.* [40], using biopsy specimens from four groups of 10 patients, evaluated the presence of various IgE transcripts as well as the crucial enzyme for isotype switching AID in bronchial biopsies of allergic asthmatics, non-allergic asthmatics, atopic subjects without asthma and non-allergic controls. Evidence of local IgE class switching and expression of epsilon transcripts was detected not only in atopic asthmatics, as hypothesized, but also in the non-atopic asthmatics, who had normal IgE levels and negative skin prick testing. In contrast, only three of seven non-asthmatic subjects with AR exhibited IgE transcripts in bronchial biopsies and only one of 10 normal controls [40]. Although this work needs further confirmation, it suggests that the Th2 inflammation that is characteristic of asthma highly influences the development of local infiltrating B cells in the lung, leading to IgE synthesis, irrespective of skin test positivity.

Animal models of allergic airways disease

Murine models of allergic airways disease have demonstrated that B lymphocytes are present in the lungs of

sensitized animals. Chvatchko et al. [41] were the first to detect prominent B cell follicles in the lungs of sensitized animals. Three groups of 8-week-old female BALB/c mice were first sensitized systemically and then intratracheally challenged with OVA or saline control. Histological and immunohistochemical evaluation showed formation of germinal centres with follicular dendritic cells (FDCs) in the BALT of OVA-sensitized and -challenged mice. In the adjacent tissues, OVA-specific PCs produced specific IgG, IgA and IgE anti-OVA-antibodies. *In vitro* studies on isolated lung cells used the ELISPOT technique to quantify the anti-OVA PCs. Anti-OVA forming cells were only detected in OVA/OVA-treated mice (IgM, IgG1 and IgA), which was confirmed using ELISA. This led investigators to question the role of B lymphocytes and IgE in mouse models of allergic airway disease.

Comparisons of allergic airways disease phenotypes using B cell-deficient mice underlined several important concepts regarding the role of B lymphocytes and the use of murine models of asthma. Hamelmann et al. [42] sensitized 8–12-week-old B10.BR female mice and B cell-deficient $\mu\text{M}^-/-$ mice with nebulized OVA or PBS. Some B cell-deficient mice received anti-IgE neutralizing antibody while control mice received anti-2, -4, -6-trinitrophenyl IgE. As expected, anti-OVA IgE and IgG levels were only increased in B cell-sufficient mice. In both normal and B cell-deficient mice, T cell responses induced by OVA were similar; levels of IL-4 and IL-5 from *in vitro* cultured T cells were significantly increased after airway sensitization. However, airway hyperresponsiveness, determined by measuring tracheal smooth muscle contraction, was increased in normal mice but not in B cell-deficient mice. Addition of OVA-specific IgE to B cell-deficient mice restored airway reactivity. In contrast, when similar groups of mice were systemically sensitized, there was little difference between wild-type and B cell-deficient mice. This includes experiments performed using C57BL/6 wild-type and C57BL/6-Igh-6tm1Cgn (B cell-deficient) mice [43], as well as B cell-deficient mice on the B10.BR background [44,45]. Differences in the extent of airway inflammation, eosinophil recruitment and IgE responses were indicated as reasons for the variability in requirement for B lymphocytes and or IgE in murine models of allergic airways disease.

Chronic allergen exposure models have been used to further dissect the role of B lymphocytes. Using B cell-sufficient and -deficient mice, Lindell et al. [46] determined that antigen presentation by MHC-II expressing B cells drives T cells to produce IL-4 and IL-5. This study used cockroach antigen and a variety of sensitization and challenge protocols to maintain pulmonary reactivity. Much of the salient work was carried out *in vitro* on lung homogenates and co-culture with cockroach antigen, demonstrating that B cells indeed could present antigen to sensitize T lymphocytes, and that expression of co-stimulatory molecules CD40, CD80 and CD86 was

necessary. B cell-sufficient mice were more likely than deficient mice to secrete high levels of IL-4 and IL-5 into the airways, and mucous production was also influenced by B lymphocytes. In another model using both acute and chronic cockroach antigen challenge [47], the same group evaluated defects in the B1a (CD5^+) subset of mucosal B cells. The mice used included CBA/J and CBA/CaHN-btk $(-/-)$ xid/J (Xid); Xid mice have several immune impairments including a deficiency in B cell activation and the B1a subtypes. Both control and Xid mice were able to mount equally high IgE responses, but the Xid mice exhibited much higher levels of IL-4, IL-5, IL-10, IL-13, IFN- γ and RANTES, suggesting that the CD5^+ mucosal B cell population was responsible in regulating both Th1- and Th2-type inflammatory responses. Although lung hyper-reactivity, measured by plethysmography, was similar in wild-type and Xid animals, histological analysis revealed a more prominent inflammatory infiltrate in the Xid mice. Most significantly, the CD5^+ B cell subset expressed the highest levels of FasL among all lung lymphocytes, and the amount of apoptosis observed in lungs of CD5^- deficient Xid mice was significantly lower than in the wild-type. This suggests that CD5^+ B1a cells played a regulatory role in pulmonary inflammation, and that they are required for regulation of both Th1- and Th2-mediated responses to allergens. Finally, in a study of local inhalation tolerance following chronic exposure to OVA, Singh et al. [48] determined that B cells played an essential role in maintaining tolerance. C57BL/6J mice, wild-type BALB/c mice and congenic BALB/c B cell-deficient JhD $(-/-)$ mice were used. B cells (CD19^+ cells) from hilar LNs of mice that were made tolerant via chronic OVA inhalation were transferred into OVA-sensitized mice challenged with OVA by aerosol. The adoptive transfer decreased BAL leucocytes and eosinophils, reduced peribronchial and perivascular cellular infiltration (lung inflammation) and attenuated methacholine responsiveness in the recipient mice, compared with other mice that received vehicle alone or hilar LN B cells from OVA-sensitized mice.

PCs are a consistent finding in the lungs of mice sensitized to allergens. The maintenance of PCs in the airways is under tight control, and survival is generally in so-called PC niches. The presence of PC niches is likely controlled both by local factors such as neurotrophins including NGF [49] and T cell co-stimulatory molecules [50]. Indeed, Luger et al.'s [14] demonstration that the presence of antigen was required to maintain PCs in pulmonary tissues may relate to the need for the proper inflammatory environment to create such niches.

These studies underline important facets of the role that B lymphocytes can play in immune regulation. The fact that the B cells are required for processes beyond antibody production, such as antibody presentation, and regulatory cytokine production, is in keeping with recent work that highlights regulatory B cell subsets in autoimmune

diseases [51–54]. They also underline the care that must be taken in interpreting data depending on the type of model used.

Chronic obstructive pulmonary disease

The paradigms of pathogenesis for COPD have been evolving, with recent studies delineating the inflammation associated with the destructive lesions of COPD and chronic bronchitis. This has led to intense investigation into the immunologic regulation of pulmonary parenchymal destruction [32]. Although the majority of the focus has been on the role of T lymphocytes, characterization of B lymphocytes infiltrating the lungs in subjects with COPD is emerging. At present, a functional role of B lymphocytes in COPD has not been defined.

Hogg et al. [31] examined lung specimens after stratifying patients according to GOLD stages 0–4. They examined specimens for PMNs, macrophages, CD4 and CD8⁺ T cells, B cells and lymphoid follicles. Their analysis of 159 patients showed strong association between GOLD stage, progression of COPD and the percentage of airways containing lymphoid follicles as well as the extent of occlusion of the lumen, mucous, inflammatory exudate, total wall thickness and the thickness of each of the wall compartments. Lymphoid follicles containing B cells were markedly elevated (25–30% of airways) in stages 3–4 compared with stages 0–1–2 (<5%). The same observation was made for the percentage of airways containing any B cells regardless of the presence of follicles. Interestingly, the correlation was not present for CD8⁺ and much weaker for CD4⁺ T cells. The authors hypothesized that the lymphoid follicles reflect an adaptive immune response in response to the microbial colonization and infection known to occur in the later stages of COPD.

After Hogg showed accumulation of B cells in small airways of patients with GOLD stage 3 and 4 COPD, Gosman et al. [55] evaluated B cells in bronchial biopsies of large airway in patients with COPD. Specimens from flexible bronchoscopy of 114 patients aged 45–75 with COPD and 28 control subjects with equivalent smoking history but no airway obstruction or asthma were evaluated. B cells were counted in the subepithelial areas adjacent to each bronchial section. In contrast to Hogg et al. [31], few lymphoid aggregates were seen (7% of both patients and controls). However, B cell staining was significantly higher in patients with COPD compared with controls. Among the COPD patients, the count tended to be higher in ex-smokers than in current smokers. The number of B cells did not correlate with age, gender or pack-years but significantly higher numbers of B cells were seen in stage 3 vs. stage 2 and in stage 2 vs. controls. Greater numbers of B cells also tended to correlate with

lower post-bronchodilator FEV₁/FVC ratio and post-bronchodilator FEV₁. Although the distribution of B cells was different from the Hogg study (which included more severe patients and focused on small airways), the same increase in B cells was seen in the subepithelium of large airways of patients with COPD and correlated with the severity of disease.

In light of this work, more thorough characterization of B cells in the lung parenchyma of individuals with COPD was carried out by van der Stradte et al. [56]. Using specimens from eight individuals with COPD, they detected B cell follicles with most (80%) in the parenchyma and not adjacent to the epithelium (unlike classic BALT). They consisted of CD27⁺ memory B cells bearing IgM and CD40 on their surface. There was also presence of FDCs and the activation marker Ki-67 within the follicles. Scant CD4⁺ and CD8⁺ T cells were found in the periphery of the follicles as well as CD138⁺ PCs in the near vicinity of the follicles. Sequence analysis of the Ig rearrangement showed clonality in seven of eight patients but also ongoing mutations.

These follicles harboured several features (such as structural organization, FDCs, Ki-67 and ongoing mutations in clonally related B cells) compatible with germinal centres. These features suggested an antigen-driven selection process implicated in COPD. Interestingly, these lymphoid follicles did not harbour the features of BALT (not adjacent to the epithelium, no M cells). Similar features of these follicles were reproduced in mice exposed to cigarette smoke up to 4 months. Additionally, significantly higher levels of IL-1b, IL-4, IL-13, IL-6 and KC (mouse IL-8) were found after 4 months of smoking [56].

The role of B cells in the pulmonary infiltrate of individuals with COPD remains unclear. They potentially respond to chronic antigenic stimulation by infectious antigens, or as hypothesized by van der Stradte, respond to antigens in cigarette smoke in uninfected mice.

Histologically, inflammation, hypertrophy of mucus-secreting glands and goblet cell hyperplasia are major characteristics of the airways of smokers with chronic bronchitis. Hypersecretion of mucus may be secondary to release of pro-inflammatory cytokines including IL-4, which also induces differentiation of B lymphocytes. Zhu et al. [57] evaluated the IL-4 mRNA-expressing cells in lung tissues from smokers with chronic bronchitis. They studied lung resection specimens from lung cancer patients from normal, non-asthmatic, non-smokers (AS), individuals with clinical chronic bronchitis but normal lung function (CB) and those with clinical chronic bronchitis and persistent irreversible airway obstruction (CBAO). They performed immunohistochemistry and *in situ* hybridization on unaffected tissue to determine the cellular expression of IL-4. Patients with CB had greater amounts of PCs in both glandular and subepithelial compartments compared with AS and CBAO. Among the CB patients, there were more PCs in glandular

compartments compared with the subepithelium. Unlike PCs, B lymphocytes were mainly seen in lymphoid aggregates and were quite sparse in the glandular and subepithelial areas. PCs were the predominant cell type associated with submucosal glands and were more than two to six times more numerous than CD4⁺, CD8⁺ and mast cells in the CB and CBAO groups. The number of PCs showed a strongly positive correlation with the number of cells expressing IL-4 mRNA in both CB and CBAO. The total number of cells expressing IL-4 protein also correlated strongly with the number of PCs infiltrating both the submucosal glands and subepithelial areas in CB. Similar correlations were observed with IL-5 mRNA while no association was found between IL-4 and IL-5 and CD4⁺ cells or mast cells. A total of 67% and 62% of PCs expressed IL-4 and IL-5, respectively, whereas only 1–3% of either CD4 or mast cells expressed either cytokine mRNA. Double immunofluorescence staining confirmed that approximately 40% of PCs produced and stored IL-4 protein. Double immunofluorescence staining also indicated that B lymphocytes (CD20) and monocytes/macrophages (CD68) can also contribute to the production of IL-4. These intriguing findings, especially IL-4 production by PCs, need further confirmation.

Thus, B lymphocytes are an important part of the pathologic findings of COPDs, contributing to the local inflammation both by Ig production and cytokine production. The full range of cytokines that are produced by B cells in COPD clearly must be elucidated.

Conclusions

The B cell is perhaps the forgotten cell in the evolution of our concepts of asthma and COPD as predominantly inflammatory conditions. The evidence that CD19⁺ CD20⁺ B cells, as well as PCs, populate both allergic airways and those affected by emphysematous changes is extremely strong. There remain major questions regarding the follicular B cell collections and infiltrating B cells. It will be important to define their role in either initiating or contributing to the on-going inflammatory process. For example, what is their role in antigen presentation? We have tantalizing data regarding Th2-type cytokine production, but what about other inflammatory or regulatory cytokines? B cells are known to be an important source of IL-10, which may be how they act as regulatory cells in the studies of Lucaks. This needs further exploration. What are the mechanisms that B cells home and track to the lungs? What are the molecules that control homing, organization and activation of these cells in the airway parenchyma? Although there are data regarding airway chemokines and their effects on B cells, these do not seem sufficient to explain the specific recruitment of B cells in pulmonary conditions. It is also unknown if the local Ig responses to allergens are specific for allergic triggers, representing

primarily IgE, or represent a more generalized response, covering other isotypes such as IgA and IgG. For example, studies revealing isotype specific responses to staphylococci in the upper airway suggest that pathogens are also potentially important triggers of Th2-type B cell responses. There are numerous pathophysiologic and mechanistic questions that must be answered to properly comprehend the role of B cells in airway inflammatory diseases.

Acknowledgements

Supported by a grant from Canadian Institutes for Health Research, the Strauss Family Foundation, the T. Costello Memorial Fund and the Montreal Children's Hospital Research Institute. Drs Mazer and Hamid are supported by the Chercheur National Programme of the FRSQ. The Meakins-Christie Laboratories-MUHC-RI are supported in part by a Center Grant from the FRSQ.

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