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Hiding in Plain Sight: Time to Unlock Autoimmune Clues in Human CD5+ B Cells by Using NextGen Technology

Rizwan Ahmed, PhD[1], **Zahra Omidian, PhD**[1], **Thomas Donner, MD**[2], **Abdel Rahiam A. Hamad, BVSC, PhD**[1,*]

[1]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

[2]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Abstract

CD5$^+$ B cells expand in many autoimmune diseases, including type 1 diabetes (T1D), rheumatoid arthritis (RA), and systemic lupus erythematosus (SLE). Furthermore, CD5$^+$ B cells contain important subsets: IL-10-producing B-reg cells, FasL-expressing subset, and the majority of pre-naive B cells. In addition, they are major sources of natural autoantibodies, which are polyreactive and autoreactive. Thus, CD5$^+$ B cells are clearly loaded with autoimmune clues that are yet to be unlocked and understood. We hypothesize that human CD5$^+$ B cells are likely to yield enormously important novel information about the role of B cells in autoimmune disease if analyzed using the new technological advances in molecular biology and genomics. Use of high-throughput sequencing of B cell receptors (BCR) of CD5$^+$ B cells could reveal public BCRs associated with autoimmune diseases, whereas transcriptional analysis of CD5$^+$ B cells using single-cell RNA-seq may delineate distinct sublineages and their relationship to conventional B cells. If it turns out that autoimmune repertoires are concentrated in CD5$^+$ B cells, given that CD5$^+$ B cells are clearly identifiable by flow cytometry, therapeutic strategies can be developed to safely remove CD5$^+$ B cells to mitigate ongoing autoimmunity and protect at-risk individuals.

It Is Time to Directly Study Human CD5+ B Cells Instead of Their Murine Surrogates

Unlocking the mystery of human CD5$^+$ B cells is a decades-long quest. It began with the discovery that the pan-T cell marker CD5 is expressed on most human B-type chronic lymphatic leukemia (CLL) cells (Lanier *et al.,* 1981). Analyses over ensuing years, as discussed below, have shown a clear association of CD5$^+$ B cells with autoimmune diseases. However, the use of traditional methods and assays has failed to answer questions as basic as the composition of CD5$^+$ B cells and their relationship to their CD5$^-$ counterparts, let alone their role in disease pathogenesis. Furthermore, it remains unclear whether human CD5$^+$ B

*Corresponding Author: Abdel Rahiam A. Hamad, M.D. (ahamad@jhmi.edu).

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cells represent a distinct sublineage, similar to murine $CD5^+$ B1 cells, or merely represent activated B cells (Baumgarth, 2017). Sophisticated analysis of the immune cells is now doable not only from peripheral blood but also from lymphoid and non-lymphoid organs of humans. Thus, direct analysis of $CD5^+$ B cells from healthy individuals and patients could be very useful in delineating their composition and lineage specification. In addition, it is expected to provide novel information about their roles in the pathogenesis of autoimmune diseases. Generated clues could then be used to design mechanistic studies in mouse models. This clinic-to-the-bench and back-to-the-clinic approach could streamline and limit preclinical studies to those highly relevant to human disease, thereby minimizing disappointments and agony caused by failure to translate otherwise highly successful preclinical studies into successful treatments to the human diseases.

We hypothesize that using the new technological advances in molecular biology and genomics will be key to better understanding of human $CD5^+$ B cells. Use of high-throughput sequencing of B cell receptors (BCR) of $CD5^+$ B cells could reveal public BCRs associated with autoimmune diseases and use of single-cell RNA-seq analysis may delineate distinct sublineages.

Here we briefly discuss described subsets of $CD5^+$ B cells, their association with human autoimmune diseases, and how use of new technologies could lead to better understanding of their composition, lineages, and role in disease pathogenesis.

Subsets of Human $CD5^+$ B Cells

$CD5^+$ B cells are heterogeneous and several subsets have been described to date. The most notable subset of $CD5^+$ B cells is the IL-10-producing B-reg subset (referred to as B10 cells) that downregulates detrimental inflammatory responses through production of IL-10 (Saxena *et al.,* 2014; Yanaba *et al.,* 2008). However, many questions still remain regarding the origin of B10 cells and their mechanism of actions and whether the expression of CD5 is an indicator of specific lineage or merely a sign of activation. Another subset of $CD5^+$ B cells is characterized by constitutive expression of the apoptosis-inducing Fas ligand (Lundy and Klinker, 2014; Saxena *et al.,* 2017). This subset is expanded in type 1 diabetes (T1D) patients (Saxena *et al.,* 2017) and appears to negatively regulate homeostasis of IL-10-producing B-reg cells in the NOD mouse model of T1D (Xiao *et al.,* 2011). Many questions remain unanswered regarding the origin of FasL-expressing B cells and their relationship to conventional and pathophysiological roles. In this regard, Lundy *et al.* (2015) showed that experimental transformation of human B cells by a non-replicative variant of Epstein-Barr virus (EBV) consistently resulted in high expression of functional FasL protein, thereby providing another mechanism for the existence of $FasL^+$ B cells. On the other hand, a prenaive B cell population that exists between transitional and naive B cells in human peripheral blood is reported to account for the most circulating $CD5^+$ B cell population (Lee *et al.,* 2009). Pre-naive $CD5^+$ B cells display some of the functional properties of normal mature naive B cells. Because of this ability, it is proposed that pre-naive B cells pose the risk of causing autoimmunity when inappropriately stimulated (Lee *et al.,* 2009). However, it is notable that since the original description of pre-naive B cells several years ago, there have been no follow-up studies that illuminate their role in host defense or autoimmunity

settings. However, one study by the same group showed that production of IL-10 by pre-naive B cells limits their participation in normal immune responses, but they rather differentiate into autoantibody-secreting plasma cells in lupus patients (Sim *et al.,* 2015). Human transitional T1 B cells, defined by high expression of CD24 and CD38, also express CD5 (Sims *et al.,* 2005) and possess immunoregulatory properties to inhibit differentiation of CD4 T cells and suppress production of tumor necrosis factor (TNF-α) and IFN-γ by CD4 T cells in healthy, but not, lupus patients (Blair *et al.,* 2010). However, CD5 is also upregulated on B cells by BCR stimulation (Youinou *et al.,* 1999), making it difficult to determine whether CD5 expression denotes specific subsets, marks activated B cells, or does both. Use of single-cell RNA-seq will be useful in investigating heterogeneity of $CD5^+$ B cells and their relationship to one another and their identity. On the other hand, analysis of their BCR repertoires using high-throughput immunosequencing will determine whether their expansion in autoimmune diseases is associated with specific clonal and autoantigens.

Association of $CD5^+$ B Cells with Autoimmune Diseases

Different autoimmune diseases are associated with the expansion of $CD5^+$ B cells. Evidence that $CD5^+$ B cells expand in T1D has been described more than two decades ago (De Filippo *et al.,* 1997; Munoz *et al.,* 1995; Schatz *et al.,* 1991; Smerdon *et al.,* 1994). More recently, we linked expansion of $CD5^+$ B cells in T1D subjects to a specific subset that is characterized by constitutive expression of FasL (Saxena *et al.,* 2017). Interestingly, FasL and IL-10-producing subsets appear by flow cytometry to represent two distinct subsets (Hamad *et al.,* 2012; Saxena *et al.,* 2017). However, very little is known about their transcriptional profiles and relationship to one another and to other $CD5^+$ B cells and conventional B cells. Use of deep sequencing and single cell analysis will help answer these questions. Rheumatoid factor-producing B cells are significantly enriched within $CD5^+$ than in $CD5^-$ B cells isolated from the peripheral blood of RA patients (Youinou *et al.,* 1987). There are also increased numbers of $CD5^+$ B cells in spondylarthritis (Cantaert *et al.,* 2012). $CD5^+$ B cells have also been reported to be increased in myasthenia Gravis patients (Araga *et al.,* 1995), and $CD5^+$ B-reg cells have been implicated in suppressing experimental myasthenia gravis by an IL-10-dependent mechanism (Sheng *et al.,* 2014; 2015). In addition, prenaive $CD5^+$ B cells are found in increased proportions in peripheral blood of lupus patients (Lee *et al.,* 2009). Selective expansion of $CD5^+$ B cells in these autoimmune diseases provides identifiable subpopulations of disease-associated and presumably autoreactive cells that are ripe for high-throughput repertoire sequencing and single-cell RNA-seq to understand their sublineages and their roles in driving autoimmunity. If $CD5^+$ B cells turn out to be drivers of autoimmunity, new strategies can be developed to spare normal productive B cells and target depletion to pathogenic subsets, thereby mitigating one of the most serious side effects of pan-B cell depletion immunotherapy.

$CD5^+$ B Cells Are Major Sources of Natural Autoantibodies in Humans

Like murine $CD5^+$ B1 cells, human $CD5^+$ B cells produce polyreactive and autoreactive Abs (Casali and Notkins, 1989). These natural antibodies afford hosts rapid protection immediately following infections and prior to the development of adaptive immune responses. Natural antibodies also facilitate elimination of apoptotic cells thereby

maintaining homeostasis and preventing inflammation. However, due to their polyreactivity and autoreactivity, natural antibodies can recognize self-antigens and promote autoimmune diseases in susceptible individuals. Studies in mice show that they can be positively selected by self-antigens (Hayakawa *et al.,* 1999; Kasaian and Casali, 1993). Transgenic mice expressing a BCR specific for Thy-1 antigen produce specific antibodies only when mice express Thy-1 (Hayakawa *et al.,* 1999). Given that most natural antibodies are considered non-pathogenic, the question of whether $CD5^+$ B cells produce natural antibodies that promote autoimmune diseases in humans remains unanswered. In addition, specificities of BCRs of natural antibodies in humans are not well-understood. Applying new technologies to analyze BCR repertoires of $CD5^+$ B cells could prove useful in elucidating whether $CD5^+$ B cells harbor public BCRs associated with specific autoimmune diseases.

Use of Next-generation Technologies to Unlock Mysteries of $CD5^+$ B Cells

Use of high-throughput sequencing and application of single-cell genomics technologies have revolutionized the study of immune cells. Both types of technology are highly appropriate and needed for breakthrough analysis of $CD5^+$ B cells and their mysteries. Below, we briefly describe how these technologies can advance our understanding of human $CD5^+$ B cells and their role in disease.

Use of high-throughput analysis of $CD5^+$ B cells may lead to identification of elusive public BCRs associated with autoimmunity

High-throughput next-generation sequencing provides a powerful tool to profile TCR and BCR repertoires at the single-cell level (Sherwood *et al.,* 2011). As noted above, $CD5^+$ B cells expand in several autoimmune diseases. Yet there have been, to our knowledge, limited and rather no deliberate attempts to analyze immunoglobulin heavy chain variable regions (IGHV) repertoire of $CD5^+$ B cells in the steady state or patients with major autoimmune diseases using high-throughput deep immunosequencing. Several properties support the ideas that IGHV repertoires of $CD5^+$ B cells, rather than those of conventional B cells, may be enriched for disease-relevant public BCRs. Generation of highly diverse repertoires is critical for equipping the immune system with the ability to recognize and respond to various invading pathogens. The high-level diversity of the immune repertoire of conventional B cells has made it difficult to identify public BCRs that are clonally expanded in autoimmune settings Please cite Hamad et al (Discovery Medicine 2016). One reason could be limiting the analysis to conventional B cells and exclusion of $CD5^+$ B cells. We argue that public BCRs could be particularly enriched within autoreactive $CD5^+$ B cells. BCR repertoire of $CD5^+$ B cells, unlike that of conventional B cells, is generally highly restricted (Hardy and Hayakawa, 2001; Sanz *et al.,* 1989; Schutte *et al.,* 1991). N-region addition and somatic hypermutation are highly restricted among $CD5^+$ B cells (Gu *et al.,* 1990; Hardy *et al.,* 1989; Pennell *et al.,* 1989). Consequently, natural antibodies derived from $CD5^+$ B cells, unlike those derived from conventional B cells, tend to have germline-like sequences with broadly reactive and autoreactive repertoires (Forster *et al.,* 1988; Hayakawa and Hardy, 1988; Hardy *et al.,* 1989; Pennell *et al.,* 1989; Gu *et al.,* 1990). This leads to the focus of autoreactive receptors among $CD5^+$ B cells and might explain their expansion in autoimmune diseases as a result of the recognition of self-antigens. Thus, in

light of the close association of $CD5^+$ B cells with autoreactivity and limited BCR repertoires, $CD5^+$ B cells might be enriched for public BCRs that drive autoimmunity.

Use of single-cell RNA-seq could reveal composition of $CD5^+$ B cells and their lineage relationship to $CD5^-$ B cells

Single-cell RNA-Seq has proven to be an effective method for comprehensive cellular decomposition of heterogeneous immune populations (Jaitin *et al.,* 2014; 2015). $CD5^+$ B cells are found at high frequencies in fetal cord blood and in serous cavities of healthy adult individuals (Berland and Wortis, 2002; Duan and Morel, 2006). In addition, they constitute the majority of pre-naive B cells (Lee *et al.,* 2009). However, conventional B cells were also reported to upregulate CD5 expression upon BCR activation (Youinou *et al.,* 1999). Thus, the relationship between $CD5^+$ and $CD5^-$ B cells is unclear. Another unresolved question is focused on the identification of the human equivalent of the mouse B1 cells (Griffin *et al.,* 2011a; 2011b). Resolution of these questions is of great importance because characterization of $CD5^+$ B cells and understanding of their relationship to conventional $CD5^-$ B cells are prerequisites for understanding the overall role of B cells in host defense and autoimmune diseases.

Concluding Remarks

Recent genomics technological advances have allowed analysis of repertoires of T and B cells and their identities at the single-cell level, generating troves of information that are being mined for understanding roles of various immune cells in host defense, autoimmunity, and cancers. Consequently, different cell types and subtypes are being subjects of new analysis. Given that $CD5^+$ B cells are some of the least understood subpopulations, we expect that there will be high rewards and great returns by subjecting their antigen receptors to high-throughput sequencing and analyzing their transcriptional profiles using single-cell RNA-seq. Such analysis is expected to be done under different platforms by different investigators with interests in different diseases. Generated information could solve problems that have been difficult to solve by subjecting the analysis of conventional B cells to high-throughput sequencing such as identifying public BCRs associated with different autoimmune diseases, which could be concentrated in expanded $CD5^+$ B cells. Likewise, subjecting $CD5^+$ B cells to single RNA-seq is expected to clarify their relationship to conventional B cells, their composition, and roles of their subsets in health and disease.

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