Minireview Article

What a clinical hematologist should know about B cells?

ABSTRACT

B cells are very crucial part of our adaptive immune system. They protect us from various infections by secreting antigen-specific antibodies, which neutralize the infectious agents. It is important for a clinical hematologist to know about the B cell development, function and the diseases developing from the quantitative or qualitative defects in B cells. This simple and short review is focused on the basic understanding and clinical hematologist's perspective of B cells.

INTRODUCTION

The B cells pass through a very interesting journey which starts in the bone marrow where they originate. Here they acquire initial B cell receptors which are their main weapons. The RAG1/2 genes help these B cells in acquiring the initial IgM/IgD positive BCRs. These antigen inexperiencied B cells are then released into the blood stream as naïve B cells. Once exposed to antigen these B cells undergo second phase of maturation in the lymph nodes and spleen where they come in contact with helper T cells and with their help get activated to centroblasts and undergo somatic hypermutation of variable region and class switching of the constant region in the germinal center mediated by activation induced cytidine

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deaminase. In this way the B cells get their weapons (antibodies) fully upgraded according to the antigens to which they were exposed to. These cells exiting the lymph nodes develop into memory B cells or plasma cells which remember the antigen in future and get activated on re-exposure with the same antigen. The memory cells keep circulating in the blood searching the same antigen whereas the plasma cells get settled in the bone marrow. Diseases of the B cells can be either inherited or acquired, or benign or malignant. Inherited defects in genes responsible for B cell development can cause primary immunodeficiency disorders. Production of autoantibodies has been linked to many auto-immune diseases like autoimmune hemolytic anemaia and immune thrombocytopenia. Malignant B cell diseases can result in B cell leukemia, lymphomas or myeloma depending upon the stage of B cell maturation at the time of genetic mutation leading to that malignancy. Various B cell specific and non-specific drugs are available to decrease B cell number or function, which are useful in both benign and malignant B cells diseases. This review article highlights the basics of B cell development and function, and the role B cells play in our day-today life in health and disease. Understanding T and B cell development, which form the main pillar of our immunity, is key to management of various benign and malignant diseases associated with them [1]

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THE IMMUNE SYSTEM

The immune system is an organized army of cells, tissues, and organs within the host that keeps working continuously to defend the body against attack by “foreign” invaders, which are primarily microbes such as bacteria, viruses, parasites, and fungi [2]. The function of the immune system is not only to protect from these foreign invaders but also to keep a check on the abnormal or malignant cells developing within the body. This immune system also fights against the donor tissues when transplanted into host. When the immune system is damaged, it can lead to an number of diseases, including various infections, allergies, autoimmune diseases and cancers [3,4]. The immune system can recognize and remember millions of different antigens, and it can produce cells and antibodies to wipe out each one of them. The immunity mediated by B cells is called adaptive immunity because it gets adapted according to the antigen, i.e., it producs antibodies as per the need of the body against various types of antigens, in contrast to innate immunity which cannot be modified.

B CELL DISCOVERY

The antibodies were discovered first, and later it was found that the source of these antibodies is a B cell [5]. Serum from an immune patient when mixed with a fresh culture of the same type of bacteria resulted in clumping of the bacteria, a process called agglutination. When a different bacterial species was used, the agglutination did not happen. This observation led to the finding that there was something in the serum of immune individuals that could specifically bind to and agglutinate bacteria. The cause of this agglutination was an antibody molecule,

also called an immunoglobulin. The function of B cells was discovered in the 1960s by Max Cooper who demonstrated that antibody production was completely abrogated in irradiated chickens after surgical removal of the Bursa of Fabricius (the primary site of B-cell development in birds) from which the notation 'B' cell was derived.

B CELL DEVELOPMENT

B-cell development takes place in two stages and in two different organ systems: (a) the differentiation of B-cell precursors from an hematopoietic stem cell (HSC) to naïve B cells in the bone marrow, and (b) the maturation of naïve B cells to memory/effector B cells in secondary lymphoid tissues (lymph nodes, mucosa-associated lymphoid tissues and spleen) [6].

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Initial development of B cell begins in the bone marrow where recombination-activating genes (RAG) 1 and 2 mediate the V(D)J rearrangement of the heavy and light chains [7]. Cells with successful VDJH rearrangements develop immunoglobulin heavy chain protein in their cytoplasm. Each light chain must be capable of pairing with the heavy chain already expressed [8]. Complete IgM appears in the cytoplasm followed by its expression on the cell surface as a B cell receptor (BCR) capable of binding antigen. This marks the end of the first stage of B cell differentiation—the cell now migrates to the peripheral lymphoid organs as naïve B cell (Fig.1).

Germinal centers (GCs) are transient (not permanent) structures that are formed in secondary lymphoid organs (spleen and lymph nodes) which work as a factory for producing B cells capable of generating highly efficient antibodies [9]. These are the special structures where immunoglobulin genes undergo changes in their variable (V) regions termed somatic hypermutation (SHM) and and in heavy chain termed class switch recombination (CSR). After initial mutations in dark zone, B cells transit to the light zone, where they exit the cell cycle and are selected, on the basis of the affinity of their mutated BCRs, to differentiate into memory B cells or plasma cells [9–11]. B cells can also re-enter the dark zone for additional cycles of somatic hypermutation and division, in a process known as 'cyclic re-entry' for further increasing their affinity for the antigen termed 'affinity maturation' [12]. Thus, germinal center development requires coordinated signals that dictate the induction of proliferation, exit from the cell cycle, cyclic re-entry and differentiation, as well as the elimination of non-selected B cells by apoptosis.

B CELL RESPONSES TO ANTIGEN

Naïve B cells leaving the bone marrow circulate between secondary lymphoid organs in search of antigen. Following antigen encounter, B cells can undergo two different developmental possibilities [13].

a) Firstly, the B cells may not form germinal center but undergo plasmacytic differentiation, form extrafollicular plasmablasts and then IgM secreting plasma cells (Fig.1). These cells do not have somatically mutated

immunoglobulin genes (i.e., they do not undergo CSR and SHM which take place in germinal center, and not in extrafollicular area) and are short lived but provide a rapid initial response to antigen. So, only IgM is produced and not IgG or IgA.

b) The second developmental possibility is the establishment of a germinal center, a specialized structure within which B cells undergo rounds of proliferation accompanied by SHM and CSR, resulting in the formation of highly efficient memory B cells and plasma cells, which exit the lymph nodes (Fig.1). These plasma cells are long-lived and reside in bone marrow (primarily IgG-secreting plasma cells) or mucosal lamina propria (IgA-secreting plasma cells). Persistent antigen-specific antibody titers derive primarily from long-lived plasma cells. Memory B cells can also seed sites of infection, where they are maintained as tissue-resident memory B cells [14]. Here they are quickly activated after pathogen invasion without the need for antigen transportation to draining lymph nodes, thus shortening the time for plasma cell differentiation and antibody production on secondary exposure.

FATE OF B CELLS ENTERING THE GERMINAL CENTER

In the germinal center, B cells have at least three developmental options: a) to continue further rounds of mutation and selection as germinal center B cells by

re-entering the dark zone (shown by arrows in dark zone in Fig.1) and those which do not undergo affinity maturation are destined to apoptosis, b) to become memory B cells, or c) to become plasma cells ([5]. The germinal center reaches its maximal size within approximately two weeks, after which the structure slowly involutes, and it disappears within several weeks [16].

SOMATIC HYPERMUTATION AND CLASS SWITCH RECOMBINATION

The immunoglobulin is a 'Y' shaped structure. An antibody generated by B cells has two parts- a variable region, which binds to the antigen, and a constant region, which gets attached to the other immune cells for the effector function. There is a unique mechanism present in the germinal center whereby the B cells can modify their variable and constant regions according to the type of antigen. In somatic hypermutation (SHM) the immunoglobulin variable region is changed to make it more effective in attaching to the antigen (more antigen specific), whereas in class switch recombination (CSR) the constant region of immunoglobulin is changed so that the class of antibody is changed from IgM to IgG or IgA, so that it can better attach to the effector cells. The antibody repertoire the B cells get in the primary lymphoid organ (bone marrow) is not sufficient to counter the pathogens in periphery as evidenced by increased infections and mortality in patients who have insufficient ability to change their antibodies by SHM or CSR. This is because the antigen countering mechanisms developed in the marrow by B cells are not antigen-experienced and may not be appropriate for the antigen which attacks the body. So, the B cells have

developed the mechanism to modify and upgrade their antigen-fighting machinery according to the antigen [17]. Usually the changes or mutations in DNA are considered harmful as they can lead to development of cancers but in germinal center the activation-induced cytidine deaminase (AID) voluntarily induces mutations in B cells to generate different types of antibodies which can fight diverse infections [18]. B cell AID expression is induced in GCs where CSR and SHM occur (not in marginal zone or mantle zone). The presence of switched immunoglobulin protein is confirmed by detecting more surface IgG+/IgA+ cells among multiply divided cell [19]. In naïve or memory B cells, AID gene and protein are undetectable, because SHM and CSR do not occur in these cells. These, however, are readily and significantly upregulated in activated B cells (centroblasts in germinal center) induced to undergo SHM or CSR. IgG has a longer half-life than IgM (21 and 5 days, respectively). IgE plays a major role against antihelmintic infection. The initial antibody IgM secreted by a B cell is of low affinity against the specific antigen as it has been produced by the immature B cell without being exposed to the antigen and is thus weak and cannot effectively eradicate the antigen and thus SHM and CSR are essential for antibody to mature [19]. Since B cells have not undergone T cell like training in the thymus in the presence of AIRE gene for differentiating the self from the non-self, these B cells are controlled by the helper T cells which downregulate the B cells to produce antibodies against self-antigens. The class switching into 5 major immunoglobulin classes does not reflect the change in the variable regions which can recognize million of antigens, each with different antigen binding sites (of BCRs).

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THE CHARACTERISTIC FEATURES OF B CELL MEDIATED ADAPTIVE IMUNITY

a) Specificity- The mature immune response is specific to the antigen that produces it (e.g., antibody for measles antigen has no effect on rubella antigen). The specificity develops after rigrous and continuous modification (somatic hypermutaion and class switch recombination) of B cell with the help of CD4 T cells in the germinal center of lymph nodes [20]. The ability of antibodies to bind virtually any non-self surface with exquisite specificity and high affinity is the key to immunity.
b) Tolerance- The immune system is tolerant to all self tissues and intolerant to any non-self tissue. This is one of the basic requirements for survival. The major histocompatibility complex (MHC)-1 presents the proteins within the cells after transporting the MHC 1-peptide complex to the cell surface to present it to the CD8 T cells. The CD8 T cells do not respond if the self proteins are presented but kill the cells if foreign or cancer proteins are presented. The immune response can differentiate between self and non-self so that body tissues are not destroyed. Similarly, if B cells present self-proteins to T cells, then those signals are ignored otherwise B cells will get activated and will produce autoantibodies.
c) Memory- With subsequent exposure to an antigen there is a rapid and strong immune response. The memory B cells remember the past infection because these cells are equipped with the BCRs which were generated as per the previous exposure to the antigen. If the same antigen attacks the

host again then that antigen fits into the BCRs exactally in the same way as it did previously, and this activates the required immune cells.

d) Mutual help- B cells are not activated by most antigens without “help” from helper-T cells. The activation of T cells is an essential first stage in virtually all adaptive immune responses. This is called the “T cell-dependent immune response” (Fig.1). T cells do not recognize micro-organisms directly and are helpless if infective micro-organisms are not presented by antigen presenting cells (e.g., dendritic cells or B cells). With T cell help the B cells undergo germinal center reaction (SHM and CSR) and become specific-antibody secreting cells [1,21].

GENES RESPONSIBLE FOR DIVERSIFYING IMMUNOGLOBULIN REPERTOIRE

Two classes of enzymes are required for diversifying immunoglobulin repertoire: The proteins encoded by RAG1 and RAG2 introduce DNA double-strand breaks and recombine V, D and J segments, and AID deaminates cytosines in the antigen recognition (the variable region) and effector domains (the constant region) thereby enabling somatic hypermutation (SHM) and class switch recombination (CSR) of immunoglobulin genes, respectively.

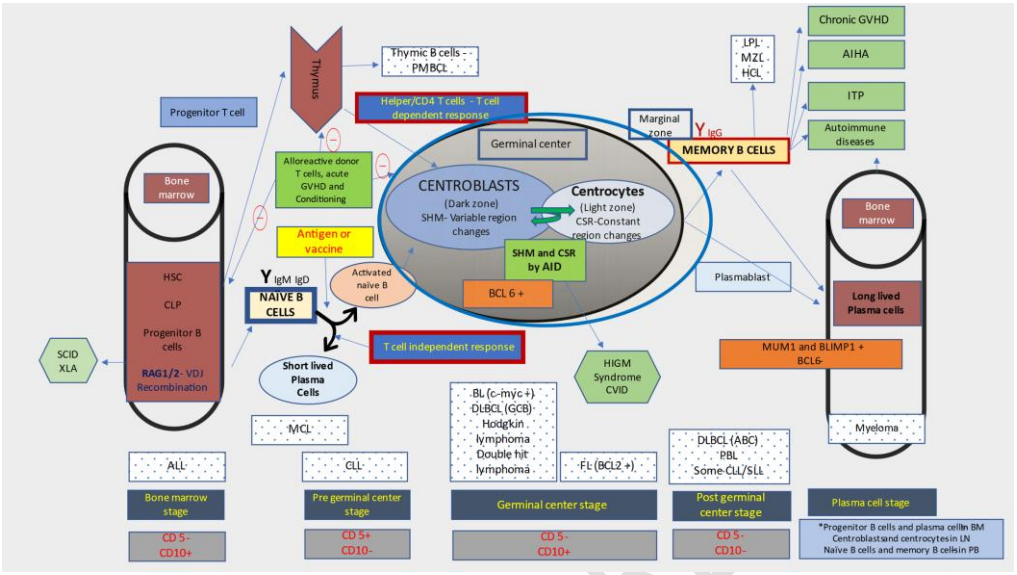

Fig 1: Hematopoietic stem cells

Fig. 1. Left side of the figure shows hematopoietic stem cells (HSCs) giving rise to common lymphoid progenitors (CLP) which in turn give rise to progenitor T and B cells. The progenitor T cells move to thymus for further maturation. The progenitor B cells undergo RAG 1 and 2 induced VDJ recombination resulting in production of IgM+, IgD+ naïve B cells, which have not come in contact with antigen yet. Mutations in RAG or other genes in bone marrow can result in primary immunodeficiency diseases like SCID, XLA. Once outside the marrow, the naïve B cell upon activation with antigen or vaccine can either lead to production of short-lived plasma cells (which produce IgM) or results in formation of germinal center. With the help of T cell, B cell forms centroblast wherein the variable portion of "Y" antibody is molded multiple times such that it can capture the antigen most effectively, termed SHM with affinity maturation, and also change the constant portion of "Y" termed CSR. This results in formation of memory B cells and long-lived plasma cells. The development of B cells can be divided into bone marrow stage, pre-germinal center stage, germinal center stage, post-germinal center stage and plasma cell stage.

The bone marrow, lymph nodes, thymus can be damaged by alloreactive donor T cells in the graft, conditioning agents, acute GVHD and infection. The thymus is necessary for B cell development as it provides CD4 helper T cells which are necessary for T cell dependent B cell development. Defect in T cell help or mutation in AID can lead to defective antibody production as in hyper IgM syndrome (HIGM). MUM 1 and BLIMP 1 are necessary for plasma cell development. Autoantibody production by memory B cells can lead to various autoimmune diseases including ITP, AIHA and chronic GVHD. Various B cell

malignancies are associated with the developmental stage of B cells as shown. B cells present in the thymus can give rise to PMBCL. RAG 1/2 (lymphoblast), BCL6 (centroblast) and BLIMP1 (plasma cell) are shown separately indicating regulation of B cell development at three very important sites by different genes. Hodgkin lymphoma has been shown alongwith other germinal center lymphomas as it originates form centroblasts who have lost all B cell markers.

SCID – Severe combined immunodeficiency , XLA– X Linked agammaglobulinemia, ALL – Aculte lymphoblastic leukemia, MCL – Mantle cell lymphoma, CLL – Chronic lymphocytic leukemia, BL- Burkitt lymphoma, DLBCL – Diffuse large B cell lymphoma, FL – Follicular lymphoma, PBL – Plasmablastic lymphoma, GVHD – Graft vs host disease, SHM – Somatic hypermutation, CSR – Class switch recombination, AID – Activation induced cytidine deaminase, LPL– Lymphoplasmacytic lymphoma, MZL – Marginal zone lymphoma, HCL- Hairy cell leukemia, ITP – Immune thrombocytopenia, AIHA – Auto immune hemolytic anemia, PMBCL – Primary medistinal B cell lymphoma, CVID – Common variable immunodeficiency disorder.

MASTER REGULATOR OF GERMINAL CENTER B CELL REACTION

BCL-6 is the master regulator of the germinal center reaction the site where B cells (centroblasts) undergo SHM and CSR, and is the key oncogene in B cell

lymphoma-genesis [22]. Another major function assigned to BCL-6 is the inhibition of the differentiation of germinal center B cells into memory B cells and plasma cells, so as to allow sufficient time for the B cells to undergo AID mediated SHM and CSR [23].

ROLE OF C-MYC IN B CELL DEVELOPMENT

The transcription factor c-myc is ubiquitously expressed in proliferating cells, where it controls cellular processes necessary for normal cell growth and proliferation, however, its expression is almost completely absent in germinal center B cells [16]. There is the disappearance of c-myc expression at the time of BCL-6 expression in dark zone of germinal center [24], to create a temporary pause till somatic hypermutation by AID takes place. The c-myc gene is necessary for the entry or re-entry of B cells into the dark zone of germinal center [25].

PLASMA CELLS AND ROLE OF BLIMP-1 AND MUM-1 IN PLASMA CELL DIFFERENTIATION

Plasma cells operate as factories to manufacture the required antibody and then secrete those antibodies. Any antibody-secreting cells in the blood en-route to bone marrow are called plasmablasts. Plasma cells are specialized to secrete large amounts of antibodies, about 1000 per second [26]. Plasma cell differentiation

requires the silencing of several transcription factors that are required for B-cell development in the bone marrow or in the germinal center. Complete plasma cell differentiation requires a transcriptional repressor known as BLIMP-1 (B lymphocyte induced maturation protein-1), which actively supresses Pax5 and BCL-6. Therefore, BLIMP-1 is often viewed as a key regulator of plasma cell differentiation [27]. MUM-1 also plays a critical role in downregulating BCL-6 expression, so that germinal center activity can be reduced, and cell can proceed to plasma cell differentiation. Because plasmablasts no longer need to bind or present antigen, the expression of the BCR (surface Ig) and MHC Class II are present at decreased levels on the cell surface and are ultimately absent on the surface of plasma cells [15].

DIFFERENCE BETWEEN NAÏVE B CELL, MEMORY B CELL AND PLASMA CELL

The B cells released from the primary lymphoid tissue (bone marrow) are naïve B cells, whereas the B cells released from secondary lymphoid tissues (lymph nodes and spleen) are memory B cells and plasma cells (Fig.1). Naïve B cells must go through all the processes of germinal center reaction (SHM and CSR for affinity maturation) to get activated and produce high affinity antibodies whereas memory B cells, who have already undergone the steps of affinity maturation in germinal center get activated directly without the need of germinal center reaction or T cells help again and so the response is quick and antigen specific.

The key difference between plasma cells and memory cells is that plasma cells are the final stage of B cell proliferation that produce antibodies while memory B cells are the dormant stage of B cell proliferation that remember antigens and react immediately upon exposure to that antigen second time [28]. These cells produce larger quantities of antibodies against specific pathogens. Memory cells are later stage of B cells generated from naïve B cells after undergoing SHM and CSR in germinal center, and memory B cells can also develop into plasma cells on antigen exposure (Fig.1). Naïve B cells are not antigen specific as they have not encountered antigen and have not undergone affinity maturation in germinal center whereas memory B cells have undergone SHM and CSR and are therefore antigen specific. These cells are responsible for the immunological memory against that specific antigen i.e. they will proliferate and produce same antibodies if again exposed to that antigen, Morphologically, memory B cells resemble naïve B cells. Memory cells live a longer life than naïve B cells.

T CELL DEPENDENT AND T CELL INDEPENDENT ANTIGENS

Activation of B cells occurs through different mechanisms depending on the molecular class of the antigen. Activation of a B cell by a protein antigen requires the B cell to function as an antigen presenting cell, presenting the protein epitopes with MHC II to helper T cells. Because of their dependence on T cells

for activation of B cells, protein antigens are classified as T-dependent antigens. In contrast, polysaccharides, lipopolysaccharides, and other non-protein antigens are considered T-independent antigens because they can activate B cells without antigen processing and presentation to T cells. The T cell-independent response is short-lived and does not result in the production of memory B cells. Thus, it will not result in a secondary response to subsequent exposures to T-independent antigens. Polysaccharide vaccines are composed of long chains of sugar molecules that make up the surface capsule of encapsulated bacteria. The immune response to a pure polysaccharide vaccine is typically T cell independent. Polysaccharide vaccines are poor inducers of immune response compared to protein vaccines. By linking a polysaccharide to a protein (diphtheria toxoid protein is commonly used) the immune response becomes T cell-dependent and immunogenicity is improved [29]. This process is called conjugation and the vaccine as “conjugate vaccines”.

B CELL RECONSTITUTION AFTER STEM CELL TRANSPLANT

The B cell immunity is the slowest to reconstitute and may take up to 2-3 years after allogeneic SCT [30]. B cell reconstitution after stem cell transplant (SCT) can be assessed by a) B cell quantification (CD19 positive cells), b) immunoglobulin assays (IgM, IgG, IgA, IgE levels), and c) by measuring antibody development following vaccination [31]. Complete reconstitution of the B cell compartment includes the recovery of both CD19+CD27- naïve B cells (which represent the functional capacity of the bone marrow where the initial development of B cells takes place) and CD19+CD27+ memory B cells (which represent the functional capacity of secondary lymphoid tissues where further maturation including SHM

and CSR take place). Reconstitution of memory B cells requires CD4+ T cell help (for SHM and CSR), so T cell maturation is important for development of adequate B cell response [32]. Development of memory B cells is very important for fighting with infections. The naive B cell reconstitution is relatively faster compared to memory B cell recovery [33]. Delayed T cell recovery and the reversed CD4/CD8 ratio may also contribute to low circulating B cell numbers following SCT [34]. After SCT, immunoglobulin levels drop, reflecting the absence of immunoglobulin producing B cells. Some immunoglobulin production may persist, probably due to surviving long-lived plasma cells of host origin.

Since naïve B cells recover early but because of lack of memory B cells the appropriate antibody response is not generated for a prolonged time resulting in immunodeficiency post SCT [34]. Lack of CD19+CD27+ memory B cells and decrease in the immunoglobulin levels render these patients susceptible to encapsulated bacteria and viruses [35]. Recovery of memory B cells is critical for development of immunocompetence following SCT. The rapid decline of antibody titers against vaccine-preventable diseases (e.g., tetanus, polio, measles, mumps, rubella) is a manifestation of this B cell deficiency following allogeneic SCT when the recipient is not revaccinated. Vaccination starts 3-6 months after SCT, but the better tools to guide re-vaccination are recovery of the CD4 T cells and ability for class switch recombination of B cells, which might be useful biomarkers to guide the timing of vaccination compared to fixed time point after SCT [36].

SOURCES OF ANTI-MICROBIAL ANTIBODIES POST ALLOGENEIC STEM CELL TRANSPLANT

Following allogeneic SCT, humoral immunity in the recipient recovers from three sources.

a) First, recipient antibody persists with an average half-life of 30–60 days, and some recipient plasma cells persist for years following allogeneic SCT [31]. The persisting recipient antibodies provide protective anti-microbial humoral immunity in the initial few weeks following allogeneic SCT, but these recipient antibodies may be anti-donor and may be detrimental contributring to primary graft rejection (by rejecting the donor stem cells) and prolonged red cell aplasia when donors and recipients are ABO major mismatched (by rejecting the donor erythroid cells, causing delayed engraftment or poor-survival of erythroid cells) [37].
b) Second, donor grafts contain- i) naïve B cells (B cells recently released from bone marrow which have not been exposed to antigen, therefore have not undergone SHM and CSR for antigen specific affinity maturation), and ii) memory B cells (those B cells that have already undergone SHM and CSR in the donor and contribute to adoptive anti-microbial and alloreactive B cells, but these are antigen specific i.e., will respond to those antigens only to which donor has been exposed earlier).
c) Third, the engraftement of donor stem cells will give rise to common lymphoid progenitors which will inturn produce naïve B cells and memory B cells which will be tolerant to recipient and will remain capable of

responding to infectious challenges and vaccinations [31].

Humoral immunity is predominately recipient derived in initial few months after allogeneic SCT, and this persistent recipient derived antimicrobial IgG may benefit RIC allo-SCT patients and contribute to their decreased transplant related mortality [31]. In the absence of revaccination, both autologous and allogeneic transplant recipients lose seroprotection to pathogens they were immunized against during childhood [38]. Although there is some variability in the time to protective titer loss among different transplant groups, loss of pneumococcal, Hemophilus influenza and tetanus titers usually occur by two years post SCT [39].

FACTORS AFFECTING THE RECOVERY OF B CELLS POST STEM CELL TRANSPLANT

a) In early stage after SCT, B cell numbers seem to recover faster when using peripherally harvested stem cells compared to marrow stem cells. After 6 months, no differences have been shown in B cell recovery using marrow compared to peripherally harvested stem cells [40]. Stromal cells (niche of bone marrow and lymph nodes) and T cells (present in lymph nodes) are important for optimal B cell development and function.
b) Anti-thymocyte globulin is a polyclonal immunoglobulin and induces elimination of B cell populations alongwith T cells and results in delayed immune reconstitution.
c) The use of total body irradiation (TBI) is also associated with delayed B cell reconstitution.

d) GVHD is associated with significantly poorer B cell reconstitution, in both function and numbers [36,41]. GVHD itself as well as the associated immunosuppressive therapies contribute to delayed B cell recovery (Fig.1). B cells take part in the pathophysiology of GHVD by acting as antigen presenting cells and by secreting cytokines [36].
e) Alloreactive T cells and germinal center B cells often participate in germinal center reactions to produce pathogenic antibodies against host tissue, which result in chronic GVHD (Fig.1). Although regulatory T cells (Tregs) can inhibit germinal center reactions, Treg numbers are reduced in chronic GVHD [42].
f) Donor and recipient's age has an inverse relationship with total and memory B cell reconstitution [36].

With better understanding of the role of B cells in chronic GVHD pathogenesis, multiple additional strategies have been developed that deplete B cells, reduce their activation via manipulation of BCR-downstream events, or inhibit their migration toward inflammatory sites (Table 1). Therefore, B cells have been targeted with several therapies such as rituximab, bortezomib, ruxolitinib and ibrutinib in patients with GVHD, with promising clinical results [43].

B CELL MALIGNANCIES

Tumors of B cell origin are a heterogeneous group of malignant diseases, and most harbor characteristic genetic alterations [44]. They can involve developing B cells within bone marrow (e.g., precursor B-ALL), mature antigen-experienced B cells in lymph nodes (e.g., diffuse large cell lymphoma), and terminally differentiated plasma cells in bone marrow (e.g., multiple myeloma) (Table 2). Immunoglobulin genes have long been thought to be susceptible to translocation because they undergo DNA damage during gene diversification reactions (VDJ recombination, SHM and CSR) [45]. The enzymes RAG 1 and 2 and AID, which diversify immunoglobulin-encoding genes, are strictly segregated in developing cells during B lymphopoiesis in marrow and peripheral mature B cells in lymph nodes, respectively [46]. Aberrant RAG-mediated VDJ recombination targeting non-immunoglobulin genes causes genetic lesions that may drive clonal evolution of B cell ALL [47]. Immunoglobulin gene rearrangement studies have shown that Hodgkin/Reed-Sternberg (HRS) cells are clonal and derived from germinal center B cells despite the frequent absence of B-cell markers other than Pax5 [48]. Primary mediastinal B cell lymphoma is a specific type of DLBCL originating from B cells present in the thymus [49]. Multiple myeloma is a neoplasm of post–germinal center, terminally differentiated B cells (Fig.1).

Based on genes (e.g., BCL6, MUM1) expressed by diffuse large B cell lymphoma (DLBCL), it has been divided into two main groups which also define its cell of origin. Germinal center B cell-like (GCB)-DLBCL exhibits a transcriptional profile that resembles that of a GCB cell with expression of CD10 and BCL6 and mutated immunoglobulin genes with ongoing somatic hypermutations suggestive of highly proliferative centroblasts [50]. Activated B cell-like (ABC)-DLBCL shows several features of activated B-cells with up-regulation of genes required for plasma cell

differentiation (IRF4/MUM-1), these tumors do not show evidence of ongoing SHM (Fig.1). The Hans algorithm has been the most widely used to differentiate GCB and ABC DLBCLs. In this algorithm three antibodies are used CD10, BCL6 and IRF4/MUM-1 [51]. Cases positive for CD10 or cases positive for BCL6 and negative for IRF4/MUM-1 are classified as GCB phenotype whereas cases that are IRF4/MUM1 positive with or without expression of BCL6 are assigned to the non-GCB subtype [49,52]. Genes encoding BLIMP-1 (required for plasma cell differentiation) are recurrent in ABC DLBCLs but are not present in GCB DLBCLs [53]. The ABC subtype of DLBCL resembles post–germinal center plasmablasts [10, 16]. The tumor arising from further mature B cells would be plasmablastic lymphoma where the B cells are no longer ABC type but of plasmablast type.

HIGH GRADE B CELL LYMPHOMAS

a) High-grade B-cell lymphoma (HGBL) is a group of aggressive, mature B-cell lymphomas which are not classified as diffuse large B-cell lymphoma (DLBCL)-NOS, or as Burkitt lymphoma [48]. There are two categories of HGBLs. The first category, HGBL with c-myc and BCL2 and/or BCL6 rearrangements, i.e. the so-called double-hit and triple-hit lymphomas and the second category, HGBL-NOS, that are high grade but not DLBCL-NOS or Burkitt lymphoma or double hit or triple hit lymphomas [48].
b) Most double-hit lymphomas are also double-expressers, but most double-expressers are not double-hit lymphomas. Where feasible, all cases of DLBCLs/HGBLs should be tested for c-myc rearrangement by FISH and, if

detected, further testing should be performed for BCL2 and BCL6 rearrangements [54]. FISH is a sensitive and specific method to detect MYC rearrangement due to translocation. IHC cannot identify translocation.

c) R-CHOP immunochemotherapy is insufficient for double-hit or triple-hit lymphoma and more intensive therapy, such as R-EPOCH or novel therapy with or without stem cell transplantation should be considered [49,52].

CLASSIFICATION OF B CELL LYMPHOMAS BASED ON CD5, CD10, BCL2 AND BCL6

The pre-germinal center B cells (naïve B cells) are usually CD5 positive. The lymphomas originating from them include mantle cell lymphoma and chronic lymphocytic leukemia (Fig.1). The normal germinal center B cells (centroblastas and centrocytes) are BCL6 and CD10 positive and BCL2 negative. BCL2 and c-myc are suppressed in germinal center by BCL6. If germinal center B cells become positive for BCL2 then they form follicular lymphoma and if they express translocated c-myc then they from Burkitt lymphoma [55]. The other CD10 positive germinal center tumor is DLBCL. DLBCL expresses CD20, CD79a, CD19, CD22 and the B-cell transcription factor PAX5. The post germinal center B cells are memory B cells, and these are negative for CD5, CD10 and BCL6 (Fig.1). The tumors originating from these memory cells are marginal zone lymphomas, Hairy cell leukemia and lymphoplasmacytic lymphomas. CD10 and BCL-6 typically label both normal reactive and neoplastic germinal centers and are negative in small cell/indolent lymphoma like chronic lymphocytic leukemia/small lymphocytic

lymphoma, marginal zone lymphoma, and mantle cell lymphoma. LMO2 and HGAL (human germinal-center-associated lymphoma) are markers of germinal center B cells that may be of use when other germinal center markers are absent or indeterminate [56]. The germinal center B cell high grade lymphomas showing expression of both BCL2 and c-myc are called double expresser lymphomas. As the B cells start maturing towards plasma cells, they start expressing MUM1, CD38 and CD138. The identification of a markedly predominant kappa expressing or lambda expressing B-lineage population (e.g. 90% or greater predominance of one light chain over the other) provides strong support for malignancy. About 95% of mantle cell lymphomas show nuclear labeling for Cyclin D1/BCL-1. Sox11 is an excellent marker of mantle cell lymphoma and remains positive also in cyclin D1-negative cases. Evaluation of annexin A1 can help to exclude hairy cell leukemia, which should stain for this marker, but should not stain marginal zone lymphomas.

POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDER

Post transplant lymphoproliferative disorders (PTLD) are lymphoid or plasmacytic proliferations that develop following immunosuppression in a solid organ or stem cell graft recipient [48]. In contrast to solid organ transplant, PTLD after HSCT is almost exclusively of donor origin and develops during the first 6 months after transplant [57]. This unique feature is a

consequence of the profound T cell–depleting conditioning regimen, leading to lack of EBV-specific T cells and, hence, the rapid growth of an EBV positive clone, even within the first weeks [57]. Because immune reconstitution occurs in the first 6 to 12 months and oral immune suppression can often be stopped at that time, late PTLD is rare after allogeneic SCT. By 6 months following transplantation, the level of cytotoxic T lymphocytes is restored in most patients, and after 12 months, T-cell function is normalized. PTLD arising following solid organ transplants are derived from the postgerminal center host B lymphocytes suggesting a role for chronic B-cell stimulation by the graft and endogenous EBV reactivation [58], as EBV infects and persists in memory B cells. EBV can integrate into normal B-cell program leading to proliferation and transformation of these cells. Normally, these antigens would trigger a T-cell response capable of destruction of most of the EBV-infected B cells. However, this T cell mediated immune defense mechanism is compromised in transplant recipients leading to unlimited B-cell transformation and the evolution of lymphoma [59].

Table 1. Various drugs effective on B cells

Drugs	Mechanism of action
Steroid	It induces apoptosis or programmed cell death in lymphoid cells

Vincristine	It results in the inhibition of microtubule formation in mitotic spindle, resulting in an arrest of dividing cells at the metaphase stage
Daunorubicin	This anthracycline antibiotic damages DNA by intercalating between base pairs resulting in uncoiling of the helix, ultimately inhibiting DNA synthesis and DNA-dependent RNA synthesis
Cyclophosphamide	This alkylating agent alkylates or binds to DNA. Its cytotoxic effect is mainly due to cross-linking of strands of DNA and RNA and inhibits protein synthesis
Asparaginase	It is an enzyme that breaks down asparagine. Unlike normal cells, ALL cells are unable to make their own asparagine
Methotrexate	It competitively inhibits dihydrofolate reductase, an enzyme that participates in the tetrahydrofolate synthesis
Ibrutinib	It is a small molecule that acts as an irreversible potent inhibitor of Burton's tyrosine kinase (akin to X-linked agammaglobulinemia)
Rituximab	It is a chimeric monoclonal antibody targeted against CD20 which is a surface antigen present on B cells. Therefore, it acts by depleting normal as well as

	pathogenic B cells while sparing plasma cells and hematopoietic stem cells as they do not express the CD20 surface antigen
Blinatumumab	It is a BiTE-class (bi-specific T-cell engagers) monoclonal antibody. One arm of this antibody binds CD19, while the other arm binds CD3. By redirecting unstimulated primary human T cells against CD19-positive lymphoma cells, the bispecific CD19/CD3 antibody shows significant cytotoxicity
Inotuzumab	It is humanized anti-CD22 monoclonal antibody
Tisagenlecleucel	It is a CD19-directed genetically modified autologous T cell immunotherapy, a CAR-T cell therapy
Daratumumab	It is a human monoclonal antibody that targets CD38 on plasma cells
Bortezomib	It is a selective inhibitor of the 26S proteasome, preventing the activation of NF-κB
Lenalidomide	It inhibits production of pro inflammatory cytokines TNF-α, IL-1, IL-6, IL-12 and elevates anti-inflammatory cytokine IL-10. It also has antiangiogenic activity
Selinexor	It binds to and inhibits exportin-1 (XPO1). It blocks the transport of proteins involved in cancer-cell growth from

	the nucleus to the cytoplasm.
Polatuzumab vedotin	It is a CD79b specific antibody conjugated to the antineoplastic agent monomethyl auristatin E
Tafasitamab	It is a monoclonal antibody directed at CD19 which is a pan-B cell marker, present on almost all B cells
Belimumab	Fully recombinant human, monoclonal antibody directed against BAFF

Table 2. Cell of origin of B cell malignancies (Fig.1)

Malignancy	Cell of origin/Postulated counterpart
Precursor B cell ALL	Hematopoietic stem cell or a B-cell progenitor
Mantle cell lymphoma	Peripheral B cell of the inner mantle zone (pre-germinal center origin)
Chronic lymphocytic leukemia	An antigen experienced mature CD5+ B cell with mutated or unmutated IGHV genes
DLBCL	Peripheral mature B cells of either germinal center origin (centroblasts, GCB subtype) or germinal center exit/early plasmablastic or post-germinal

	center origin (ABC subtype).
Burkitt lymphoma	A germinal center B cell with translocation of c-myc at band 8q24
Follicular lymphoma	Germinal center B cells (typically both centrocytes and centroblasts/large transformed cells)
Double hit lymphoma -	Cases with c-myc and BCL2 rearrangements originate from mature germinal center B cells
Primary mediastinal B cell lymphoma	B cells present in the thymus
Primary CNS lymphoma	A late germinal center exit B cell arrested in terminal B-cell differentiation that shares genetic characteristics with both activated B cells and germinal center B cells
Splenic marginal zone lymphoma	A marginal-zone B cell that may or may not demonstrate evidence of antigen exposure
Lymphoplasmacytic lymphoma	A post-follicular B cell that differentiates into plasma cells
Hairy cell leukemia	Activated memory B cell
Plasmablastic lymphoma	A plasmablast

Multiple myeloma	Long-lived plasma cells.
Hodgkin lymphoma	A germinal center B cell at the centroblastic stage of differentiation

CONCLUSION

To better understand the pathophysiology of any disease it is important to understand the cellular and non-cellular factors involved in that disease, changes in them leading to that disease and how to manipulate the cell and its environment to control the disease. B cells play a very crucial role in the adaptive immunity and understanding their development helps in understanding how they control infection and what happens when there is under or over functioning of these cells. This short review helps a clinical hematologist is understanding the basics of B cells.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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