

B cell Biology: An Overview

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Abstract In this review we summarize recent insights into the development of human B cells primarily by studying immunodeficiencies. Development and differentiation of B cells can be considered as a paradigm for many other developmental processes in cell biology. However, it differs from the development of many other cell types by phases of extremely rapid cell division and by defined series of somatic recombination and mutation events required to assemble and refine the B cell antigen receptors. Both somatic DNA alteration and proliferation phases take place in defined sites but in different organs. Thus, cell migration and timely arrival at defined sites are additional features of B cell development. By comparing experimental mouse models with insights gained from studying defined genetic defects leading to primary immunodeficiencies and hypogammaglobulinemia, we address important features that are characteristic for human B cells. We also summarize recent advances made by developing improved in vitro and in vivo systems allowing the development of human B cells from hematopoietic stem cells. Combined with genetic and functional studies of immunodeficiencies, these models will contribute not only to a better understanding of disease affecting the B lymphocyte compartment, but also to designing better and safer novel B cell-targeted therapies in autoimmunity and allergy.

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Introduction

In humans, B cells develop from hematopoietic stem cells (HSC) in the bone marrow (BM). Although the developmental process follows an intrinsic program, close contacts between B cell precursors and BM stroma cells providing growth factors and cytokines are critical for correct positioning, survival and differentiation. BM stroma cells including mesenchymal cells, osteoblasts and endothelial cells form special niches where HSC and B cell precursors reside. Adhesion molecules and integrins intensify the interactions among stroma cells, HSC and B cell precursors, allowing self-renewal and differentiation.

Phenotypically, the main stages of early B cell development, the pro-B, pre-B and immature B cells, are characterized by the expression of specific cell surface markers and by successive rearrangements of the immunoglobulin (Ig) heavy (H) and light (L) gene segments [1]. Successful rearrangement of the H-chain gene segments in pre-B cells allows the expression of the pre-B cell receptor (pre-BCR) composed by the μ chain together with the surrogate L-chain components V-preB (CD179A) and lambda-5 (CD179B) and the signal-transducing subunits Ig- α (CD79A) and Ig- β (CD79B). Pre-BCR expression initiates progression into the pre-B cell stages and induces L-chain rearrangement. Having rearranged the Ig-kappa and/or Ig-lambda L chains, the B lymphocyte precursors have reached the final stage of antigen-independent development. These immature B cells express surface IgM. Because they may strongly bind to self-antigens, they are tested for autoreactivity. Cells expressing BCRs with strong autoreactivity are counterselected by either clonal deletion or secondary L-chain rearrangements, but all other B cells (now

termed transitional B cells) are able to migrate from the BM to the spleen. There, they complete maturation by developing into either follicular or marginal zone (MZ) B cells. MZ B cells carry BCRs that bind preferentially to blood-borne antigens like cell wall components of bacteria. Combined with Toll-like receptor (TLR) signals induced by recognition of pathogen-associated molecular patterns (PAMPS), MZ B cells rapidly develop into IgM-secreting plasma cells and form a first line defense against pathogens that reach the spleen. Activation of B cells residing in B cell follicles (“follicular B cells”) requires the transport of antigens into the B cell follicles of secondary lymphoid organs. Upon antigen recognition and activation, follicular B cells present “their” antigens on MHC II to T-helper cells to receive additional activation signals provided by T-helper cells in the form of CD40L and cytokines. These cognate B-T-interactions promote the differentiation of activated follicular B cells into rapidly dividing blasts forming germinal centers (GC). Interactions with GC stroma cells and follicular T-helper (Tfh) cells allow GC B cells to undergo immunoglobulin class switch recombination (CSR) to change the specificities of their BCRs by somatic hypermutation (SHM) and to develop into memory B cells expressing surface IgG, IgE or IgA or into long-lived plasma cells secreting class-switched immunoglobulins.

Transcription Factors in B Cell Development

Ikaros as a Paradigm for Multiple Layers of Gene Regulation by Zinc (Zn)-Finger Transcription Factors

Members of the Ikaros transcription factor family regulate the specification of HSCs to the lymphoid lineage. Ikaros zinc finger proteins induce transcription of genes implicated in pre-BCR signaling, cell cycle regulation and VDJ rearrangement [2]. In mice, the deletion of the *Ikaros* gene (*Ikzf1*) impairs the development of common lymphocyte progenitors (CLPs) leading to a block at early stages of B-cell development and delaying differentiation of thymocytes [3, 4]. Together with the transcription factor EBF, Ikaros regulates lambda-5 expression and thus pre-BCR function. During L-chain rearrangement, Aiolos, another member of the Ikaros family, is involved in the downregulation of lambda-5 expression in pre-B cells. As Aiolos expression is regulated by pre-BCR signals transmitted via the adapter protein B cell linker (BLNK/SLP65), Ikaros and Aiolos form a chain of transcriptional regulators linked to a feedback loop guiding the development of pro-B cells to immature B lymphocytes [5]. In addition, both transcription factors play an important role in regulating proliferation and clonal expansion during the pre-B cell phase as they repress c-Myc expression and downregulate cyclin D3 [6]. Due to this activity, dominant-negative mutations in *IKZF1* and in *IKZF3* (Aiolos) promote the development of acute lymphoblastic leukemia

(ALL) [7, 8]. Ikaros binds to its DNA recognition sites with the two central of its four zinc-fingers, whereas the first and fourth Zn-fingers modulate the specificity of binding to particular sites in regulatory regions [9, 10]. Deletion of the first Zn-fingers caused a selective arrest of B cell development at the stage of pre-BII cells, whereas removal of the fourth Zn-finger increased their numbers and leads in the combination with BCR-ABL with the development of aggressive pre-B ALL malignancies [11]. Thus, differential Ikaros binding to regulatory regions of particular gene subsets required either during pre-B cell differentiation or proliferation seems to be catalyzed by the modulating function of the first and fourth Zn-finger of Ikaros. DNA binding of different combinations of zinc fingers may change the conformation of the bound protein, thus allowing target-dependent interactions with other factors. One of these factors regulated by Ikaros activity is the Mi-2 β nucleosome-remodeling and histone-deacetylase (NuRD) complex [12, 13]. Having both chromatin-opening and chromatin-closing enzymatic activities, Ikaros, an integral component of the NuRD complex in lymphocytes, connects the Mi-2 β complex to genes active in lymphoid differentiation. Hence, loss of Ikaros function inhibits lymphocyte maturation, induces transcription of genes encoding factors involved in cell growth and promotes progression to a leukemic state [13].

Besides Ikaros family members, there are additional transcription factors with DNA binding domains containing four or more Zn-fingers like BLIMP-1 (4 Zn-fingers), Bcl-6 and Gfi1 (6 Zn-fingers), or Miz-1 and CTCF (11 Zn-fingers), regulating critical steps in B-cell development [14–19]. Thus, the specialized functions of Ikaros Zn-fingers may serve as a paradigm for the different modes of Zn-finger transcription factors in regulating B-cell development.

Commitment to the B cell lineage in CLPs is initiated by E12/E47 (E2A) and early B-cell factor 1 (EBF1) [20, 21, 22]. In *Tcf3* (E2A) or *Ebf1* knockout mice, B cells cannot develop as precursors fail to reach the pro-B cell stage [23, 24]. *Ebf1* transcription is regulated by c-Myc and Runx1 [25, 26]. In a cooperative manner, E2A and EBF1 induce the expression of a set of B lymphocyte lineage-determining genes including *Cd79a*, *Cd79b*, *Cd179a*, *Cd179b*, *Pax5* and *Foxo1* [27–29]. FOXO-1 expression is needed to activate recombination activating gene (*Rag*) 1 and *Rag2* transcription required for H- and L-chain gene rearrangement [30]. PAX5 fixes precursor cells to the B cell lineage as precursor B cells from *Pax5*^{-/-} mice can still develop into many different hematopoietic lineages ranging from T cells to osteoblasts [31–34]. Apart from initiating transcription of *CD19* and *BLNK* (SLP65), PAX5 represses the *Notch1* and *MCSF-R* genes, thus preventing T cell and myeloid cell development [33, 35]. Hence, E2A, EBF1, FOXO-1 and PAX5 regulate the most critical steps in early B cell development including VDJ recombination and the expression of pre-BCR components and downstream adapter proteins including CD19 and SLP65.

As the activity of many of these transcription factors is vital for embryonic development, almost all of these insights have been gained by studying mouse models in which the corresponding genes were deleted by gene targeting in either B cell precursors or hematopoietic cells using cell type-specific loxP/Cre site-specific recombination systems. Because the expression patterns of *PAX5*, *IZKF1* and *EBF1* in human B cell precursor cells are similar to those found in murine cells [36–38], it is assumed that these factors have very similar functions during human B cell development. However, as the development of human precursor B cells occurs independently of IL-7-induced proliferation signals [39–41], proliferation, pre-BCR-dependent regulation of cell division and differentiation very likely differ between both species. This is underlined by the fact that mutations in *BLNK* and *BTK* almost completely abrogate B cell development in affected patients [42–45].

In Vivo and in Vitro Models for Human B Cell Development

To study the development of human B cells experimentally in a similar way as it can be done in mice, several in vivo and in vitro models have been developed. Xenotransplantation of human CD34⁺ HSCs by intravenous or intrahepatic routes into adult or newborn NOD/SCID (NSG) or Rag2^{-/-}IL-2rg^{-/-} (common γ -chain) mice allows analyzing the development of human lymphocytes and functional immune responses [46–52]. These humanized mice have been used as models to study viral infections including HIV and influenza [53–56]. Besides differentiation into lymphocytes, engrafted CD34⁺ stem cells were also shown to develop into myeloid lineage cells, macrophages and platelets [57–59, 60•].

In addition to the in vivo model of humanized mice, several in vitro culture systems were developed allowing the differentiation of human CD34⁺ HSC into B and T cells [61–67] including feeder cell-free conditions [60•]. Allowing for the precise control of growth and differentiation, in vitro systems provided strong evidence that human immature B cells can develop from lymphocyte progenitors independent from IL-7, FLT3L and other exogenously added cytokines by autonomous replication of precursor B cells [60•]. Thus, the in vitro B cell differentiation systems confirmed the requirements for human B cell development obtained from studying primary immunodeficiencies [39, 40]. As the cells can be accessed and manipulated easily, in vitro differentiation systems combined with in vivo analyses of humanized mice provide ideal tools to study the function of genes critical for human B cell differentiation.

Selection and Survival

The majority of newly generated IgM⁺ immature B cells developing in the BM recognize self-antigens [68]. Cells

expressing self-reactive IgM of high affinity are negatively selected, can undergo secondary rearrangements of L chains (“receptor editing”) [69, 70] and—if unsuccessful—are eliminated by clonal deletion [71•, 72–74]. Nevertheless, all transitional B cells migrating from the BM to the spleen seem to have some degree of self-reactivity that is detectable by the downregulation of surface IgM and by the activation of target genes downstream of the BCR signaling cascade [75•].

Circulating transitional B cells recognizing self-antigens with high affinity rapidly internalize their BCRs and do not express L-selectin (CD62L), thus being unable to home to the lymph nodes. They also fail to induce the B cell-activating factor receptor (BAFFR) mediating survival in the periphery [76–80]. BAFFR, a member of the tumor necrosis factor receptor superfamily binds a TNF-like ligand named BAFF (B cell-activating factor of the tumor-necrosis factor family). BAFF binding induces essential survival functions that are crucial for the transition from immature to transitional and mature B cells. BAFF binding to BAFFR activates several signaling cascades including the non-canonical NF κ B pathway [81, 82] inducing pro-survival functions and via AKT/PKC β protein synthesis [83]. Deletion of the BAFFR gene in mice [84, 85] and in humans [86] arrests B cell development at the stage of immature B cells because essential survival-promoting signals are missing. As a consequence, the numbers of mature B cells are much lower than in controls. BAFFR-deficient humans suffer from hypogammaglobulinemia and do not mount B cell responses against T-independent antigens such as pneumococcal cell wall polysaccharides. In addition to the BAFFR deletion, several missense mutations are found in the human population including a frequent SNP changing the proline residue at position 21 into arginine (P21R). This mutation locates in the pre-ligand assembly domain of the extracellular part of BAFFR and disturbs ligand-independent BAFFR clustering [87]. Because BAFFR clustering strongly enhances BAFF binding, B cells expressing the P21R BAFFR variant respond only poorly to synergistic activation by BAFFR, TLR9 and BCR signals. As the P21R-encoding SNP is found significantly more frequently among CVID patients than in healthy control populations, disturbed BAFFR function predisposes to CVID [87]. The essential role of BAFFR and of NF- κ B as a key transcription factor in B cell activation is further underlined by the severely compromised function of the immune system and B cell responses in immunodeficient patients carrying mutations in *CARD11* [88, 89•]. Interacting with MALT1 and BCL-10, CARD11 is a positive regulator of NF- κ B activity and of BAFFR expression, thus linking BCR signaling and BAFFR-dependent B cell survival. Cross-feeding between BAFFR and BCR signals is also demonstrated by BAFFR-dependent phosphorylation of SYK followed by the transmission of the activating signals via ERK, PI3kinase and the phosphorylation of the BCR signaling subunit Ig- α into the BCR-dependent signaling cascade [90•].

Thus, the crosstalk between BCR and BAFFR is an essential part of the molecular mechanisms required for the maturation and survival of B cells.

Antigen-Induced, T-Cell-Dependent Development of Memory B Cells and Plasma Cells

Long-lived plasma cells (PCs) and memory B cells expressing high affinity, pathogen-specific antibodies develop after infections, and as they may persist for decades they confer protection against the recurrence of the pathogens [91]. Both cell types are formed in germinal centers (GCs) of secondary lymphoid organs during T-dependent immune responses [92]. GCs are distinct regions in secondary lymphoid organs and are mainly composed of dividing B cells, follicular Tfh cells, follicular dendritic cells (FDCs) and dark zone stroma cells [93].

The GC Reaction

Activation of B cells residing in follicles of the spleen, lymph nodes and tonsils starts with antigen transport into B cell follicles. Microanatomically, the GC is defined by a dark zone, where activated B cells (centroblasts) proliferate, where they change the affinity of their immunoglobulins by somatic hypermutation and the constant regions by class-switch recombination, and by a light zone, where post-mitotic B cells (centrocytes) expressing immunoglobulins with the highest antigen-specific affinities [94] are positively selected into the pool of memory B cells and plasma cells [95]. GC B cells are extremely fast-dividing cells with an estimated cycle time between 6 and 12 h [96, 97], thereby displacing follicular B cells, which form the mantle zone surrounding the GC. Somatic hypermutation (SHM) of Ig-V regions as well as immunoglobulin class switch recombination is catalyzed by activation-induced deaminase (AID) expressed upon antigen-mediated BCR activation in proliferating B cells of the dark zone [98, 99]. Patients lacking AID therefore suffer from hyper-IgM syndrome and primary immunodeficiency, as they do not develop memory B cells and long-lived plasma cells [99].

The well-defined architecture of GCs, shuttling between the dark and the light zone, exit from GCs and re-entry of memory and plasma cells into the circulation strongly depends on differential expression patterns and signaling of chemokine receptors. Bound to the surface of subcapsular macrophages, antigens are presented as immune complexes to non-cognate B cells that carry them into B cell follicles and GCs, where they are recognized by antigen-specific B cells [100]. Then, activated B cells downregulate sphingosine-1-phosphate (S1P) receptor 1 (S1P1) expression to block their egress [60•] and migrate to the outer region of B cell follicles where

they can form stable pairs with antigen-specific T-helper cells [101]. EBI2 (GPR184), a G protein-coupled receptor that is expressed by mature B cells and downregulated by GC B cells, regulates the movement to the outer rim of the B cell follicle [102]. EBI2 binds to $7\alpha,25$ -dihydroxycholesterol ($7\alpha,25$ -OHC) [103] produced by enzymes (CH25H- and CYP7B1) expressed by stroma cells in the outer follicle [104]. Migration towards the T cell zone is promoted by CCR7 and allows CD40/CD40L interactions that lead to the downregulation of CCR7 and the distribution of activated B cells along the B zone-T zone boundary [105]. Co-stimulated by activating signals including CD40L and interleukins like IL-4 and IL-21 provided by T-helper cells, activated B cells then move together with their T-cell company and in a CXCR5-dependent manner toward CXCL13 producing follicular dendritic cells (FDCs) in the follicle center where they form a nascent GC.

In the GC, the proliferation of centroblasts follows an intrinsic, cell autonomous program, but the residence time and their shuttling between the dark and light zone depends on CXCR4 that is expressed by centroblasts and downregulated by centrocytes. As centroblasts express high levels of CXCR4, they are attracted by CXCL12-positive stroma cells of the dark zone, where they can proliferate and change their specificity and effector functions by SHM and CSR. Therefore, the separation of GCs into dark and light zones allows successive rounds of mutation and selection [106••].

In the GC, follicular dendritic cells (FDCs) concentrate opsonized antigens in primary follicles. Genetic ablation of FDCs leads to the disorganization of GCs followed by their disappearance and B cell death, indicating that FDCs, similar to dark zone stroma cells, are critical for GC organization and architecture [107].

In addition to EBI2 and “classical” chemokine receptors including CCR7, CXCR4 and CXCR5, the structurally related sphingosine-1-phosphate receptors (S1P receptors) play an important role in regulating the distribution of B cells in follicles, retention in GCs and egress from secondary lymphoid organs [108]. Stored in erythrocytes [109] and platelets [110], and produced by vascular endothelial cells [111–113], S1P concentrations are high in blood and lymph but low in lymphoid tissues, where it is degraded by different secreted enzymes [114]. Attracted by high S1P concentrations in vessels, resting lymphocytes expressing S1P-receptor 1 (S1P1) leave primary and secondary lymphoid organs into the circulation [115]. Human B cell subsets have a characteristic pattern of S1P-receptor expression. Non-cycling and recirculating mature, follicular and marginal zone as well as memory B cells express S1P1 and S1P4, but very low levels of S1P2, whereas cycling non-recirculating GC B cells and extrafollicular plasma cells express high levels of S1P2 and S1P4 [116•]. Similar to mice, in which S1P2 expression by GC B cells inhibits chemokine receptor-induced migration

and egress from GCs [117], S1P2 signaling overrides S1P1-induced B cell migration in humans [116•]. Interestingly, mutations in three genes linked to primary immunodeficiencies, *LRBA*, *DOCK8* and *WASP*, prevent S1P-induced migration, indicating that some of the clinical manifestations found in these deficiencies may result from impaired responses to S1P-receptor signaling [116•].

As pointed out above, centroblasts leave the dark zone after several rounds of proliferation and migrate towards the light zone where they interact with FDCs presenting antigen in the form of immune complexes [118, 119]. B cells expressing BCRs with the highest affinity to antigen are able to capture the highest amounts of antigen resulting in high numbers of peptide MHC-class II complexes presented to Tfh cells [120]. As Tfh cells provide pro-survival signals, these B cells have an advantage in competing with others for the limited number of Tfh cells [121, 122]. B cells with lower affinity BCRs do not receive T cell help and undergo apoptosis, a process that is enhanced by activation of Fas (CD95) [123], the death-inducing receptor that binds CD95L, which is expressed by FDCs [124].

Following positive selection, B cells may either re-enter the dark zone again or leave the GC as memory or plasma cells. The fate decision between memory and plasma cell development depends on the residence time in the GC and on the number of shuttling rounds between proliferation in the dark zone and selection in the light zone as GC B cells with high BCR affinity are selected into the plasma cell compartment [106•, 125]. Hence, memory B cells have less SHM in immunoglobulin variable regions when compared with GC B cells [123]. Moreover, patients carrying mutations in genes affecting the function of proteins involved in the capture of immune complexes such as CD21 [126] or in enhancing BCR signaling such as CD19 [127] or CD81 [128] still have memory B cells but many fewer plasma cells and suffer therefore from hypogammaglobulinemia.

Several rounds of proliferation are needed in activated B cells to finally reach the stage of plasmablasts or memory B cells [129]. As most post-GC plasma cells derive from high-affinity germinal center (GC) B cells [125], signals with the potential to modify B cell proliferation such as IL21R activation may change plasma cell fate-determining decisions initially imprinted by BCR signals. Histone deacetylases (HDAC) are a group of highly conserved enzymes involved in regulating posttranslational protein modifications by removing acetyl groups not only from histones, but also many other cellular proteins involved in proliferation and differentiation [130–132]. Pharmacological inhibition of HDAC activity in human follicular B cells [133] as well as conditional deletion of HDAC1 and HDAC2 in mouse follicular B cells [134] blocked cell cycle progression and subsequently plasma cell differentiation. Thus, HDACs are promising candidates for promoting cell cycle progression

and linked to differentiation in B cells. Interestingly, HDAC inhibition blocked proliferation of activated human follicular B cells, but did not change the level of expression of plasma cell fate-determining genes including BLIMP1, XBP1, IRF4, BCL6 and PAX5 [133], indicating that induction of fate-determining genes can occur independently of B cell proliferation.

The Plasma Cell Program

Commitment to the plasma cell lineage is initiated in GC B cells and is mediated by antagonizing sets of transcription factors [135]. The first set of transcription factors, the “B-lineage factors,” includes PAX5 and EBF1. Both are successively activated in CLP and pro-B cells. They induce B-lineage commitment and maintain the gene expression program of mature B cells [22•, 24, 31, 33, 136, 137]. The other set, the “plasma cell lineage factors,” includes BLIMP-1, IRF-4 and XBP-1 and induces the “plasma cell program” [14, 138–146]. As the B-lineage factors repress the expression of plasma cell lineage factors, expression and activity of B-lineage factors has to be shut down to start the plasma cell program. BCL-6 is another transcription factor that plays a central role in the differentiation of resting B cells into memory and plasma cells. It is expressed at high levels by GC B cells [147, 148] and in *Bcl6*^{-/-} mice GC, and plasma cells secreting high-affinity antibodies do not develop [15, 149]. BCL6 prevents premature differentiation into plasma cells by repressing genes encoding factors that are part of the BCR-dependent signaling cascades [150] as well as genes such as Blimp-1 and IRF4 inducing the plasma cell lineage [151, 152, 153•]. Moreover, BCL-6-dependent repression of *Bcl2* makes GC B cells more susceptible to apoptosis and facilitates the elimination of cells expressing antibodies with low affinities against the cognate antigen [154]. At the same time, repression of *p53* lowers the sensitivity against DNA damage induced by SHM and CSR and prevents *p53*-induced shutdown of cell division [16]. Thus, BCL6 functions as the central regulator of the GC reaction.

Encoded by the *PRDM1* gene, BLIMP-1 acts as a master regulator for plasma cells. In *Prdm1*^{-/-} mice, plasma cells do not develop [142, 143] and deletion or ablation of Blimp-1 expression in mature plasma cells induces apoptosis [155]. BLIMP1 silences the B cell lineage and the GC program by repressing PAX5 [140] and BCL-6 [141] expression. Thus, BLIMP-1 activity allows GC B cells to exit the cell cycle [138] and to shut down B lineage-specific genes encoding components of the BCR and its downstream signaling cascade. At the same time, *XBP1* expression is upregulated, and the splicing of XBP1 mRNA changes expression from transcripts encoding the short to transcripts encoding the long isoform [156]. XBP1 controls late events on the road to plasma cell formation and acts as a regulator of the unfolded

protein response [157, 158] required for preparing the antibody secreting cells for the ER stress due to extensive immunoglobulin synthesis [144]. In addition to BLIMP-1, IRF4 is essential for plasma cell development [145] as demonstrated by low serum antibody levels in *Irf4*^{-/-} mice [159]. IRF4 expression is gradually induced in activated B cells, and because *Irf4*^{-/-} B cells fail to express BLIMP-1, IRF4 is required to upregulate BLIMP-1 expression [160].

T-helper cell-dependent signals are critical in regulating the expression of these transcription factors. Stimulation of CD40 through CD40L expressed by T-helper cells activates NF- κ B [161] and serves as an initial step of the GC reaction for antigen-primed B cells [162, 163]. NF- κ B induces IRF4 [164], which leads to inhibition of BCL6 [164] and upregulation of BLIMP-1 [160]. In humans, mutations in CD40 [165] or in downstream signaling molecules such as IKK γ [166] prevent the formation of switched memory B cells and of plasma cells secreting IgG, IgA or IgE generated during T-cell-dependent immune responses. In cooperation with CD40-dependent signals, the GC reaction and the development of long-lived plasma cells requires the activation interleukin-receptor induced activation of STAT proteins. Signals mediated through BCR or CD40 increase IL-21R expression on B cells [167]. IL-21 binding to IL-21R augments CD40-dependent activation [168], strongly induces proliferation and class-switch recombination, and promotes differentiation of B cells into plasma cells [169]. In combination with CD40, IL-21R activation induces AID expression resulting in class switch recombination, preferentially to IgG [170]. Therefore, GCs can form in *Il21*^{-/-} and *Il21r*^{-/-} mice, but antigen-specific IgG titers are reduced. In humans, IL-21R deficiency results in a primary immunodeficiency syndrome characterized by disturbed IL-21-dependent proliferation and CSR by B cells, cytokine production by T cells and reduced NK cell cytotoxicity [171]. IL-21 binding to the IL-21R directly transmits signals via the JAK/STAT pathway as demonstrated by the lack of STAT1, STAT3 and STAT5 phosphorylation in B cells of *Il21*^{-/-} mice [171]. STAT5 activation contributes to downregulation of BCL6 [172], whereas STAT3 induces BLIMP-1, thus preparing GC B cells for plasma cell differentiation [67, 173]. Hence, patients lacking STAT3 are immunodeficient because of impaired formation of functional plasma cells and switched memory B cells [174]. However, mutations in *STAT3* are also found in patients suffering from the hyper-IgE syndrome [175–177], indicating that the balance between signals induced by activating different cytokine receptors and by phosphorylating different STAT molecules as central downstream signaling components regulates the development of plasma cells.

Conclusions

B cell development is a tightly regulated process. At the single cell level, the orchestrated action of transcription factors plays an essential role in determining the fate of the B cells. At the tissue level, location and migration of B cells will determine their developmental pathway, specificity and longevity. These two perspectives allow understanding both B cells and their interaction with other immune cells and enlightening their essential role in protective immunity against pathogens. Primary immunodeficiencies are an experiment of nature allowing the understanding of B cell biology in humans and reveal fundamental differences to rodents B cell biology. A precise understanding of human B cell biology constitutes a fundamental step for the understanding of the pathogenesis of B cell-mediated autoimmune and immunodeficiency diseases and B cell tumorigenesis and for designing new therapeutic approaches by targeted therapies.

Compliance with Ethics Guidelines

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- Of major importance

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