Chapter 9

Gene Expression during the Activation of Human B Cells

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Abstract

Human B lymphocytes not only play a critical role in the humoral immunity to generate antibodies, but also are equally important to cellular immunity as B lymphocytes can present antigens to T lymphocytes and can release a range of potential immune-regulating cytokines after stimulations. Human immunoglobulin class switch recombination (CSR) in activated B cells is an essential process in the humoral immunity and the process is complicated and tightly controlled by many regulators. The recent genomic and genetic approaches in CSR identified novel genes that were actively involved in the process. Understanding the roles of the novel genes in CSR will bring new insights into the mechanisms of the process and new potential therapeutic targets for immunoglobulin-related disorders such as allergic asthma and autoimmune diseases.

Keywords: B cells, immunoglobulin class switch recombination, gene expression, regulation

1. Introduction

Human lymphocytes include T lymphocytes, B lymphocytes and natural killer cells. T lymphocytes are majorly responsible for cell-mediated immunity. B lymphocytes (cells) play the critical roles in the humoral immunity to activate immune system by secreting antibodies. B lymphocytes are also equally critical to cellular immunity as they can also present antigens to T lymphocytes and can also release a range of potential immune-regulating cytokines [1, 2].

The "B" from B cells came from the name of the bursa of Fabricius, a lymphoid organ in birds, where B cells mature. It was first discovered by Chang and Glick [3]. B cells mature in the bone marrow in mammals. B cells express B cell receptors (BCRs) on their cell membrane and BCRs allow the cell to bind to a specific antigen and initiate an antibody response. Each B cell carries

a unique receptor for antigen that is composed of the membrane-band form of its antibody. After antigen recognition by the membrane-bound receptor, the B cells can proliferate to increase their numbers and differentiate to secrete their antigen-specific antibodies.

There are three principle classes of B cells in humans according to their ontogeny and anatomic localization: B1 cells arise from fetal liver precursors and are enriched in mucosal tissues and the pleural and peritoneal cavities. B2 cells arise from bone marrow–derived precursors and are enriched in secondary lymphoid organs [4]. Marginal zone (MZ) and follicular (FO) B cells are differentiated from B2 cells in human spleen and lymph nodes [2]. B cells of each linage have distinct and overlapping functions in recognizing antigens via T-independent and T-dependent pathways to produce rapid IgM or long-lasting IgG antibody response [2]. Cytokines play a key role in the commitment of naïve B cells to B effector 1 (Be-1) and B effector 2 (Be-2) lineage. Be-2 differentiation is dependent on the engagement of IL4α on B cells [5], while Be-1 cell development is dependent on the activation of the transcription factor T-bet and the IFNγR on B cells [6].

The process of human B cell development is very complicated and is controlled by many transcription factors [7]. Human B cells are generated in bone marrow from progenitor cells that are committed to the B cell linages (pro-B cells). Each pro-B cell undergoes independent rearrangement of diverse variable (V), diversity (D) and joining (J) gene segments of the immunoglobulin heavy (H)-chain locus [8]. Rearrangement of the H-chain locus creates in each B cell a variable exon with a unique upstream of the immunoglobulin constant region exons and drives the expression of H-chain protein and then proliferate and differentiate to commence immunoglobulin light (L)-chain gene recombination. When a B cell expresses L-chain protein, it pairs with the previously arranged H chain and is expressed as membrane immunoglobulin on the cell surface [9]. Human immune system can generate a diversity of specific antibodies in response to antigen stimulation. This process is of fundamental importance to acquired immunity. The human constant H-chain genes are on chromosome 14 containing Cμ, Cδ and two repeated clusters each having two Cγ genes and Cε genes (Cγ3, Cγ1, pseudo-ε, Cα1 and Cγ2, Cγ4, Cε, Cα2).

In this chapter, I will briefly introduce the roles of germinal centers (GCs) and the steps of immunoglobulin class switch recombination (CSR) in human B cells in GCs. I will discuss the potential functional roles of the newly identified genes from the results of our experiments for global transcript profiling in CSR. I will also discuss the future direction of the researches on CSR in human B cells.

2. Germinal centers and immunoglobulin CSR

Germinal centers (GCs) are the sites within secondary lymphoid organs such as lymph nodes and the spleen where mature B cells can proliferate, differentiate and mutate their antibody genes and switch the class of their antibodies (e.g., from IgM to IgE) during a normal immune response to antigens [10]. In the GCs, naïve B cells can have clonal expansion, somatic hypermutation, affinity maturation, development of B cell memory and long-life plasma cells [11–13]. B cell activation is initiated in the follicle in GCs when it encounters specific antigen [14], and then the B cells are relocated to the periphery of the follicle [15]. The inter-follicular zone in GCs is the site where B cell and T follicular helper cell differential initiates [16]. They

develop dynamically after the activation of follicular B cells by T-dependent antigen. B cells in GCs proliferate and can class-switch the BCR constant region from IgM/IgD to IgG, IgA and IgE (discussed later). Additionally, the IgV region genes of B cells in GCs can undergo somatic hypermutation to change the affinity of the encoded BCR for its cognate antigens, allowing subsequent antigen-driven selection and clonal expansion of high-affinity B cells [17]. Human B cells in GCs, in vitro–activated naïve B cells, and those with specific and rapid recall responses to previously encountered antigen express cell-surface CD27. B cells with CD27 expression correlated with greater cell sizes, proliferative capacity, antigen presentation capacity and differentiation into antibody secreting cells [18–20].

In order to generate antibodies, two somatic DNA recombination events of the genetic elements take place in B cells. Firstly, V(D)J recombination generates the functional variable regions of the Ig heavy-chain (IgH) and light-chain genes. Initiation of V(D)J recombination requires the products of recombination activating genes (RAG) 1 and 2 [21, 22]. Lymphoid-specific expression of RAG 1 and 2 limits V(D)J recombination to B and T lymphocytes. Following activation, mature B cells can undergo CSR, linking the IgH variable regions with one of the downstream CH genes, changing the effector function of the antibody [23]. CSR and the other main diversification event, somatic hypermutation (SHM), are both dependent on activation-induced cytidine deaminase (AID), a protein expressed only in activated germinal center B cells [24]. The basic steps of CSR include creating double-strand DNA breaks (DSBs) for CSR and joining donor and acceptor S regions. Class switch recombination occurs between switch (S) regions located upstream of each of the CH regions except Cδ and results in a change from IgM and IgD expression in naïve B cells to express one of the downstream isotypes such as IgG subclasses, IgA and IgE. AID plays a critical role in the vertebrate adaptive immune response [24, 25]. It initiates the conversion of several dC bases to dU bases in each S region, dU bases are then excised by uracil DNA glycosylase (UNG), and the resulting abasic sites are nicked by apurinic/apyrimidic endonuclease (APE), creating single strand breaks (SSBs), that can spontaneously form DSBs if they are near each other on opposite DNA strands. After formation of the DSB in the donor and acceptor S regions, the S regions are recombined by ubiquitous proteins that perform nonhomologous end-joining (NHEJ) [26]. VDJ recombination and early B cell development takes place in the bone morrow. Immature B cells expressing IgM on the surface migrate to peripheral lymphoid tissue in the spleen, lymph nodes and gut-associated lymphoid tissue. CSR and SHM happen in the germinal centers of secondary lymphoid tissues but also in germinal center–like structures in local (nonlymphoid) tissues [27]. CSR is induced by both T lymphocyte–dependent (TD) antigens and T lymphocyte–independent (TI) antigens. TD antigen stimulation can be mimicked in vitro by culturing B cells in the presence of anti-CD40 antibodies along with specific cytokines. IL-4 and anti-CD40 induce isotype switching to IgG1 and IgE [28]. Chromatin structure also contributes to the regulation of CSR. Ig heavy-chain constant genes and 3-regulatory regions are in an active chromatin conformation (acetylated H3 and H4 and lysine 4 trimethylation H3) in unstimulated human B cells, and these modifications can spread to the S region after cytokine stimulation [29]. AID is exclusively expressed in the germinal centers [21]. The basic AID-mediated mechanisms of CSR are quite well studied and defined, but the global regulation of the CSR, accompanying networks of AID and other well-known regulators, remains relatively unclear.

3. The global gene regulation of CSR in human B cells

In vitro, IL4 and anti-CD40 signals can mimic signals from T cell in GCs to induce a strong activation of NF-κB leading human B cells to a proliferative burst and CSR to IgE and IgG [30]. These costimulation signals were applied in the naïve B cells isolated from healthy tonsils and profiled the transcripts at 6 time points for 12 days (0, 12, 36, 72, 120 and 288 h). More than one thousand genes were observed to have significantly differentiated expression after IL4 and anti-CD40 stimulation [31]. The significantly differentiated genes can be formed in 4 cluster groups including 13 temporal profiles. Each cluster contains many new genes that were not known to have roles in CSR before.

3.1. Cluster A group

Cluster A group represented the gene expression on (Cluster A1) or off (Cluster A2) in naïve human B cells after IL4 and anti-CD40 stimulation.

3.1.1. Cluster A1

Cluster A1 was immediately upregulated after IL4 and anti-CD40 cosignal stimulation and the expressions did not change during the course of 12 days for the experiments. The cluster contains 153 genes. The analysis of transcription factor–binding sites for the cluster showed that genes from this cluster were enriched to transcription factors BACH1 and BACH2. BACH1 and BACH2 promote B cell development by repressing the myeloid program [32]. They belong to the basic region–leucine zipper family and are transcription repressors binding to Maf-recognition elements (MAREs) [33]. BACH2 has critical roles in both acquired immunity and innate immunity, including immunoglobulin CSR, the somatic hypermutation of immunoglobulin-encoding genes [34, 35]. BACH2 expression is activated by E2A, Foxo1 and Pax5 in pro-B cells. BACH2 may have a role in early B cell development [36]. BACH1 structure is closely related to BACH2, but its role in B cell development and hematopoiesis largely remains unclear [33]. BACH2 expression frequently preceded that of Ebf1 and Pax5 in the common lymphoid progenitors (CLPs). BACH factors directly repressed various myeloid genes in CLPs and this repression restricted the fate of CLPs to the B cell lineage [32].

In this cluster, chemokine genes *CCL22* and *CCL17* were the most significantly differentiated genes during naïve B cell activation after IL4 and anti-CD40 signal stimulation. CCL22 and CCL17 are both ligands for the chemokine receptor CCR4. *CCR4* gene was also showed in Cluster C6 to have a transient induction after IL4 and anti-CD40 signal stimulation. All three transcripts were within the top 20 differentially expressed genes during the activation of immunoglobulin class switching in human activated B cells. CCL22 and CCL17 are NF-kappa B (NF-kB) target genes, indicating a central role for the NF-kB pathway in the activation of CSR stimulated by IL4 and anti-CD40. The top differentially expressed genes also contained another NF-kB target gene, the TNF receptor-associated factor (*TRAF1*) [37], which was also profiled in Cluster A1. There were many clinical reports that indicated both chemokines might be involved in human immunoglobulin class switching. A significantly higher increase in CCL17, CCL22 and IL-4 serum levels in grass pollen–exposed subjects was observed [38]. Sensitized children with allergic symptoms showed higher CCL17 and CCL22 levels and

higher ratios between these Th2-associated chemokines and the Th1-associated chemokine CXCL10 than nonsensitized children without allergic symptoms [39]. Using human dendritic cells (DCs), in vitro exposure to house dust mite (HDM) of DCs from HDM-allergic patients but not healthy controls caused CCL17 and CCL22 release that resulted in chemoattraction of polarized human Th2 cells in a CCR4-dependent way [40]. Both CCL22 and CCL17 have been suggested as biomarkers for disease activity in atopic dermatitis (AD), and raised cord blood (CB) levels of CCL22 predict subsequent allergic sensitization, while raised CCL17 in GC predicts the later development of allergic symptoms, including asthma. Consistent with these observations, allergen exposure in sensitized individuals leads to a dynamic increase in CCL17 and CCL22 [38]. High-affinity neutral ligands have been developed for CCL22 and CCL17 and attenuate levels of CCL22, CCL17 and IgE in a mouse model of atopic dermatitis as well as improve skin inflammatory symptoms [31]. *CCL17* was also shown to have exon retention during B cell activation [31]. All the evidence indicated both chemokines and their receptor CCR4 play important roles in immunoglobulin class switching.

3.1.2. Cluster A2

Cluster A2 was immediately downregulated after IL4 and anti-CD40 cosignal stimulation and the expressions did not change during the course of 12 days of CSR. Cluster A2 contains 83 genes that present downregulating genes during B cell activation. These genes expressed significantly lower in activated B cells than in naïve B cells during the time course of IL4 and anti-CD40 stimulation. The analysis of gene ontology indicated the genes in this cluster were involved with immune system process.

FOSB and *FOS* were the most significantly downregulated genes in Cluster A2 during CSR in naïve B cells after IL4 and anti-CD40 stimulation. *FOS* genes encode leucine zipper proteins that can dimerize with proteins of the JUN family and form transcription factor complex activating protein-1 (AP-1) [41]. The FOS family consists of 4 members: FOS, FOSB, FOSL1 and FOSL2. Activating protein-1 (AP-1) is a dimeric transcription factor composed of Jun, FOS or activating transcription factor (ATF) subunits that bind to a common DNA site, the AP-1–binding site [42]. The different AP-1 factors may regulate different target genes and thus execute distinct biological functions [43]. In addition to regulation by heterodimerization among Jun, FOS and ATF proteins, AP-1 activity is regulated through interactions with specific protein kinases and a variety of transcriptional coactivators [44–46]. Nitrogen oxide (NO) is the radical inhibiting IgE/Ag-induced IL-4, IL-6 and TNF production. It inhibits phosphorylation of phospholipase Cγ1 and the AP-1 transcription factor protein c-Jun. NO further completely abrogated IgE/Ag-induced DNA-binding activity of the nuclear AP-1 proteins FOS and Jun to regulate allergic inflammation [47]. FOS-interacting protein (FIP) is a transcription factor that binds to c-FOS. The aggregation of the mast cell's high-affinity receptor for IgE induced the synthesis of FIP and increased its DNA-binding activity. Moreover, downregulation of the isoenzyme protein kinase C-β (PKC-β) resulted in profound inhibition of FIP-FOS DNA-binding activity [48].

3.2. Cluster B group

Cluster B group showed gradually sustained induction during CSR in B cells. Cluster B1 is the most interesting cluster that sustained induction earlier than Cluster B2.

3.2.1. Cluster B1

Cluster B1 was the first group to show gradually sustained expression after IL4 and anti-CD40 cosignal stimulation. Cluster B1 contains 126 genes and the analysis of gene ontology showed that genes in this cluster were majorly involved in the cellular amine metabolic process. The analysis of transcription factor–binding sites indicated the genes in this cluster were enriched to transcription factors RSRFC4 and STAT. Both transcription factors were involved with allergic and airway epithelia inflammations [49, 50]. RSRF-binding sites were found in the regulatory sequences of a number of growth factor–inducible and muscle-specific genes [51]. It was showed that engagement of the B cell antigen receptor could activate STAT through Lyn in a JAK-independent pathway [52].

There were several well-known genes to regulate B cell differentiation in germinal center including *AICDA* [24], *IRF4* [53], *XBP1* [54], *BATF3* [55] and *NFIL3* [56] in this cluster. The cluster showed other genes exhibiting synchronic, coordinated expression with the well-documented regulation genes. *IL17RB* and *BHLHE40 genes* were the most significantly differentiated in the cluster. *IL17RB* encodes a cytokine receptor that specifically binds to IL17B and IL17E but does not bind to IL17 and IL17C. IL17RB has been shown to mediate the activation of NF-κB [57]. *IL17RB* showed highly synchronic expression with *AICDA* in the cluster. IL17RB abundance has previously been shown to increase upon allergen challenge in patients with seasonal allergic rhinitis [58], IgE [59] and asthma [60]. The result indicated that the increase in IL17RB formed an early component of the transcriptional cascade that initiated the germinal center response in B cells. *BHLHE40* encodes a basic helix-loop-helix protein expressed in various tissues and is an environmentally inducible moderator of circadian rhythms and cellular differentiation. *BHLHE40* was profiled at its core of the B1 Cluster. BHLHE40 was recently shown to operate as a master regulator of germinal center activities, modulating the expression of more than 100 target genes [61]. Circadian oscillations in symptom severity are a prominent feature of atopic diseases including atopic dermatitis, asthma, chronic urticarial and allergic rhinitis [62–64]. The variation in IgE/mast cell allergic reactions was recently demonstrated to depend on the circadian clock in mice [65]. Mice deficient for the BHLHE40 ortholog display a variety of immune features including abnormal IgG1 and IgE levels and defective elimination of activated B cells, as well as exhibiting circadian rhythm phenomena [66]. Like *BHLHE40*, *NFIL3* in this cluster also participates in signaling pathways relating to the circadian clock [67], and together, these data suggest there may be a circadian component to class switch recombination and that this may be of relevance to time-of-day phenomena in IgE-driven diseases.

3.2.2. Cluster B2

The genes in Cluster B2 were also gradually sustained induction but they come later in time than Cluster B1 during CSR in human B cell. The cluster contains 112 genes. The most significantly differentiated genes were *EPHB1* and *TNFSF4*.

Erythropoietin-producing hepatocellular carcinoma (Eph) receptors are a subfamily of receptor tyrosine kinases (RTKs) [68, 69]. The receptors and their ligands, the ephrins, mediate numerous developmental processes, particularly in the nervous system [70]. Tyrosine phosphorylation of EphB1 requires presentation of ephrin-B1 in either clustered or membrane-attached forms [71]. Eph receptors and ephrin ligands have been shown to be differentially expressed on leucocytes. Ephrin-B3 binds to B lymphocytes, most likely via a nonclassical

receptor, and induces migration of the memory B cell subpopulation [72]. *NFSF4* encodes a cytokine of the tumor necrosis factor (TNF) ligand family. The encoded protein functions in T cell and antigen-presenting cell (APC) interact and mediate adhesion of activated T cells to endothelial cells. The tumor necrosis factor ligand superfamily member 4 gene (TNFSF4, OX40L), which encodes for the costimulatory molecule OX40 ligand, has been identified as a susceptibility gene for systemic lupus erythematosus (SLE) in multiple studies [73, 74].

3.3. Cluster C group

Cluster C group has six profiling clusters to show transient induction during CSR in B cells according to the time they were inducted.

3.3.1. Cluster C1

Cluster C1 was the first group to be inducted transcendently during CSR. It has 79 genes and the analysis of gene ontology indicated the genes in this cluster were involved in ribonucleoprotein complex biogenesis. Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a large family of RNA-binding proteins that are important for multiple aspects of nucleic acid metabolism [75]. *TFEC* and *RRP12* were the most significantly introduced genes in this cluster.

Transcription factor EC (TFEC) acts as a repressor or an activator. TFEC works as a transcriptional repressor on minimal promoter containing element F in an E-box sequence-specific manner [76]. It can act as a transcriptional transactivator on the proximal promoter region of the tartrate-resistant acid phosphatase (TRAP) E-box containing promoter. It also acts as a transcriptional repressor on minimal promoter containing mu E3 enhancer sequence [77]. Gain-of-function assays indicated that TFEC was capable of expanding hematopoietic stem cells–derived hematopoiesis. TFEC mutants were showed to reduce hematopoiesis in the caudal hematopoietic tissue, leading to anemia. It mediated these changes by increasing the expression of several cytokines in caudal endothelial cells [78]. Ribosomal RNA Processing 12 Homolog (RRP12) is a protein that may have a function to bind to poly(A) RNA. Rrp12 and the exportin Crm1 participate in late assembly events in the nucleolus during 40S ribosomal subunit biogenesis [79], but there is little knowledge of TFEC and RR12 regulating B cell growth.

3.3.2. Cluster C2

Cluster C2 was the second group to be inducted transcendently during CSR in human B cells and this cluster contains 112 genes. *LMNB2* and *B4GALT5* were the most significantly introduced in this cluster.

LMNB2 encodes a B-type nuclear lamin. The nuclear lamina consists of a two-dimensional matrix of proteins located next to the inner nuclear membrane. Lamin proteins are thought to be involved in nuclear stability, chromatin structure and gene expression. B-type lamins play a role in DNA replication, the formation of the mitotic spindle, chromatin organization and regulation of gene expression [80]. *B4GALT5* encodes one of seven beta-1,4-galactosyltransferase. It is the type II membrane-bound glycoproteins that appear to have exclusive specificity for the donor substrate UDP-galactose; B4GALT5 was found to have a change in a statin-induced experiment in gene expression in EBV-transformed and native B cells [81].

3.3.3. Cluster C3

Cluster C3 was the third group to be inducted transcendently during CSR in B cells and the cluster has 105 genes. The analysis of gene ontology indicated the genes in this cluster were involved with DNA metabolic process. *UHRF1* and *CHEK1* were the most significantly introduced in this group.

UHRF1 gene encodes a member of a subfamily of RING-finger–type E3 ubiquitin ligases. The protein binds to specific DNA sequences and recruits a histone deacetylase to regulate gene expression. Its expression peaks at late G1 phase and continues during G2 and M phases of the cell cycle. Colonization of germ-free mice with gut microbiota showed increasing expression of Uhrf1 in Treg cells. Uhrf1 deficiency resulted in de-repression of the gene (Cdkn1a) [77]. *CHEK1* encodes a protein belonging to the Ser/Thr protein kinase family. It is required for checkpoint-mediated cell cycle arrest in response to DNA damage or the presence of unreplicated DNA. Activated CHEK1 can phosphorylate and modulate the activity of a number of proteins including p53, providing a link between sensing of DNA damage and p53 checkpoint activity. BCL6 can directly bind to a DNA consensus element in the CHEK1 promoter and repress its expression in normal and malignant B cell [82].

3.3.4. Cluster C4

Cluster C4 was the fourth group to be inducted transcendently during CSR and it has 151 genes. The analysis of gene ontology showed that the genes in this cluster were majorly involved in the M phase. The analysis of transcription factor enrichment indicated the genes in this cluster were enriched to nuclear transcription factor Y (NF-Y). NF-Y in eukaryotes consists of three different subunits, NF-YA, NF-YB and NF-YC, which are all necessary for the formation of NF-Y complexes and binding to CCAAT boxes in promoters of their target genes. Recent studies demonstrated novel contributions of NF-Y to apoptosis and apoptosis-induced proliferation and in photoreceptor cell differentiation during the development of the Drosophila compound eye [83].

KIF14 and *PRC1* were the most significantly differential expression genes in the cluster. *KIF14* encodes a member of the kinesin-3 superfamily of microtubule motor proteins. These proteins are involved in numerous processes including vesicle transport, chromosome segregation, mitotic spindle formation and cytokinesis. Knockdown of this gene results in failed cytokinesis with endoreplication. This gene was identified as a likely oncogene in breast, lung and ovarian cancers, as well as in retinoblastomas and gliomas [84]. Protein regulator of cytokinesis 1 (*PRC1*) gene is a crucial regulator of cytokinesis [85]. Its suppression may result in mitotic failure and its involvement in various cancers [86]. PRC1 is a key regulator of cytokinesis that cross-links antiparallel microtubules. Multiple mitotic kinesins and microtubule-associated proteins (MAPs) act in concert to direct cytokinesis [87]. The MAP and microtubule-bundling protein PRC1 is one of the key molecules required for the integrity of this structure. Endogenous PRC1 can be interacted with KIF14. KIF14 targets the central spindle via its interaction with PRC1 and has an essential function in cytokinesis [88].

3.3.5. Cluster C5

Cluster C5 was the fifth group to be inducted transcendently and it has 99 genes. *MCM10* and *PCNA* genes were the most differentially expressed in the cluster.

MCM10 encodes one of the highly conserved mini-chromosome maintenance proteins (MCM) that are involved in the initiation of eukaryotic genome replication. Human MCM10 regulates the catalytic subunit of DNA polymerase-α and prevents DNA damage during replication [89]. MCM10 interacts with RECQ4 (RecQ helicases 4) and is important for efficient replication [90, 91]. PCNA encodes a cofactor of DNA polymerase delta in nucleus. The protein acts as a homotrimer and helps increase the processivity of leading strand synthesis during DNA replication. PCNA was well studied in plants and had the ability to stimulate the activity of DNA polymerase δ and the ability to interact with p21, a regulator of the cell cycle [92].

3.3.6. Cluster C6

Cluster C6 was the sixth group to be inducted transcendently and it contains 128 genes. *CCR4* and *HIST1H1C* genes were the most differentially expressed in the whole process during naïve B cell activation with IL4 and anti-CD40 signal stimulation.

CCR4 is the receptor of CCL17 and CCL22. It is later inducted, which means that the three may work in late stage of CSR. CCR4 was previously detected in nongerminal center cells. The possible functional roles in CSR were discussed in Section 3.1.1. Histone H1 has previously been shown to influence mast cell–mediated type-I hyperreactivity in mice [93].

3.4. Cluster D group

Cluster D group has three profiling clusters to show transient downregulation during CSR according to the time of downregulation.

3.4.1. Cluster D1

Cluster D1 was the first group to be inducted transcendently and it contains 99 genes. *GPR18* and *TP53INP1* genes were the most differentially expressed in the cluster.

GPR18 encodes G protein–coupled receptor 18. The activity of this receptor is mediated by G proteins, which inhibit adenylyl cyclase [94], and it contributes to regulation of the immune system. GPR18 also mediates NAGly-induced process of reorganization of actin filaments and induction of acrosomal exocytosis. Stimulation of human spermatozoa with the GPR18 ligand N-arachidonoylglycine induced the phosphorylation of 12 protein kinases. N-arachidonoylglycine affects the cytoskeleton by changing levels of F-actin and inducing the acrosome reaction in human spermatozoa in a concentration-dependent manner. GPR18 might be involved in physiological processes of human spermatozoa [95]. Tumor protein 53-induced nuclear protein 1 (TP53INP1) is a tumor suppressor. It was described as a p53 target gene involved in cell death, cell-cycle arrest and cellular migration [96]. TP53INP1 is also able to interact with ATG8-family proteins to induce autophagy-dependent cell death by caspase-dependent autophagy [97].

3.4.2. Cluster D2

Cluster D2 was the second group to be inducted transcendently during CSR in B cells. It contains 69 genes. *RAB6B* and *PM20D1* genes were the most differentially expressed.

RAB6B (RAB6B, member RAS oncogene family) has the ability for GTP binding and myosin V binding. Members of the RAB subfamily of small GTPases play an important role in the regulation of intracellular transport routes [98]. RAB6B is predominantly expressed in brain and the neuroblastoma cell line SK-N-SH. In brain, RAB6B was found to be specifically expressed in microglia, pericytes and Purkinje cells. Endogenous RAB6B localizes to the Golgi apparatus and to ERGIC-53–positive vesicles. RAB6B displayed lower GTP-binding activities, and in overexpression studies, the protein is distributed over Golgi and ER membranes [99]. A secreted enzyme, peptidase M20 domain containing 1 (PM20D1), is enriched in UCP1(+) versus UCP1(−) adipocytes. These data identify an enzymatic node and a family of metabolites that regulate energy homeostasis [99].

3.4.3. Cluster D3

Cluster D3 was the third group to be inducted transcendently in CSR of human B cells and the cluster has 113 genes. *IKZF2* and *ADCY1* genes were the most differentially expressed.

IKZF2 encodes a member of the Ikaros family of zinc-finger proteins. Three members of this protein family (Ikaros, Aiolos and Helios) are hematopoietic-specific transcription factors involved in the regulation of lymphocyte development. This protein forms homo- or heterodimers with other Ikaros family members and has a function predominantly in early hematopoietic development. Helios is preferentially expressed at the mRNA level by regulatory T cells (Treg cells) and is potentially a specific marker of thymic-derived Treg cells. It raises the possibility that a significant percentage of Foxp3+ Treg cells are generated extrathymically [100]. *ADCY1* gene encodes a member of the adenylate cyclase family that is primarily expressed in the brain. This protein is regulated by calcium/calmodulin concentration. Cyclic AMP (cAMP) production, which is important for mechanotransduction within the inner ear, is catalyzed by adenylate cyclases (AC). ADCY1 has an evolutionarily conserved role in hearing, and cAMP signaling is important to hair cell function within the inner ear [101].

The most significantly differential genes in each cluster and major pathways in each cluster are listed in **Table 1**. The most interesting clusters are Cluster A1 and Cluster B1 and the full gene names of these two clusters are listed in **Tables 2** and **3**.

Cluster	Numbers	Activation during CSR	Most significant genes	Pathways involved	References
A1	153	Expression on	*CCL22*; *CCL17*; *TRAF*; *BCL2L1*; *MYB*; *VIM*; *TRIP10*; *FAS*; *PTGIR*; *EBI3*; *AHR*; *NCF2*	ERK signaling, TRAF pathway; insulin pathway; NF-kB pathway	[105–108]
A2	83	Expression off	*MARCH1*; *FOSB*; *DUSP1*; *FOS*; *CR1*; *CR2*; *RGS2*; *PLD4*; *CCR6*; *RASGRP2*; *MOP-1*; *FCRLA*	Toll-like receptor signaling pathways, MAPK signaling pathway; innate immune system pathway	[109–111]

Cluster	Numbers	Activation during CSR	Most significant genes	Pathways involved	References
B1	126	Sustained induction	*BHLHE40*; *IL17RB*; *NFIL3*; *HOMER2*; *AICDA*; *BATF3*; *ARID5A*; *DUSP4*; *CD80*; *TNFAIP2*; *XBP1*; *MTHFD2*	Circadian rhythm pathway; IL-17 family signaling pathways; IL4-mediated signaling pathway	[57, 112, 113]
B2	112	Sustained induction	*EPHB1*; *TNFSF4*; *DPYSL2*; *RPS6KA2*; *SLC41A1*; *AMICA1*; *MIIP*; *RGS9*; *CISH*; *LRRC32*; *AUH*; *SLC37A3*	EPH-Ephrin signaling; TNF superfamily pathway; transport of glucose pathway	[114–116]
C1	79	Transient induction	*TFEC*; *RRP12*; *SLC29A1*; *GPATCH4*; *SSRP1*; *BCL2A1*; *CYB561*; *NME1*; *TTLL12*; *FASN*; *NETO2*; *SLC27A4*	C-MYB transcription factor network; apoptosis modulation and signaling	[117, 118]
C2	112	Transient induction	*LMNB2*; *B4GALT5*; *SLC43A3*; *ESPL1*; *EZH2*; *PSMC3*; *SUV39H2*; *MREG*; *FSCN1*; *SRC*; *PHOSPHO2-KLHL23*	Apoptosis pathway; glycosaminoglycan metabolism pathway	[119, 120]
C3	105	Transient induction	*UHRF1*; *CHEK1*; *FANCI*; *CHAF1B*; *DTL*; *CDC6*; *EXO1*; *MCM6*; *CHAF1A*; *CDC45*; *TCF19*	Chromatin regulation/ acetylation pathway; DNA double-strand break repair pathway	[121, 122]
C4	151	Transient induction	*KIF14*; *PRC1*; *NDC80*; *NUF2*; *HMMR*; *DEPDC1*; *AURKA*; *ARHGAP11B*; *BRCA1*; *FAM72B*; *HIST1H4L*; *DLGAP5*; *HIST1H1B*	Signaling by Rho GTPases; cell cycle pathway; DNA double-strand break repair pathway	[85, 123, 124]
C5	99	Transient induction	*MCM10*; *PCNA*; *TCFL5*; *HELLS*; *ZC3HAV1L*; *PHF19*; *CARM1*; *VDR*; *LIMA1*; *MYH10*; *SEMA4A*; *TMOD1*	Telomere C-strand synthesis pathway; apoptosis modulation and signaling; chromatin regulation/ acetylation	[125–127]
C6	128	Transient induction	*CCR4*; *HIST1H1C*; *CHRNA6*; *HIST1H3I*; *CCL1*; *GPR55*; *SYT11*; *PSTPIP2*; *KIAA1549L*; *HIST1H1D*; *PSAT1*; *TFDP2*	Signaling by GPCR; apoptosis induced DNA fragmentation; nicotine pathway	[128–130]
D1	69	Transient downregulation	*GPR18*; *TP53INP1*; *IFIT2*; *RNASET2*; *LBH*; *DOK3*; *FGD3*; *CD69*; *OAS1*; *ABCG1*; *PNOC*; *PARP15*	Signaling by GPCR; p53 pathway, innate immune system; B cell development pathway	[96, 131–133]
D2	69	Transient downregulation	*RAB6B; PM20D1; CYP2C19; CPNE4; TNFSF8; HIST1H2BD; METTL7A; ADHFE1; TMEM140; JMJD7; KLHL24; POU2AF1*	Vesicle-mediated transport; drug metabolism; ERK signaling	[134–136]
D3	113	Transient downregulation	*IKZF2*; *ADCY1*; *APOBEC3H*; *VAMP5*; *PDCD1LG2*; *CYP2C18*; *ILDR1*; *ADRB1*; *TM6SF1*; *GCSAM*; *CHAC1*; *ENPP3*	mRNA editing—C to U conversion; NF-kB signaling; cytochrome P450 pathway	[137–139]

Table 1. The most significantly differential genes and pathways in each cluster during CSR.

Cluster A1: 153 genes									
CCL22	*CCL17*	*TRAF1*	*BCL2L1*	*MYB*	*VIM*	*TRIP10*	*FAS*	*PTGIR*	*EBI3*
AHR	*NCF2*	*BCAT1*	*MGLL*	*SEMA4C*	*SIRPA*	*TFPI2*	*ARNTL2*	*STAT5A*	*HYOU1*
ADA	*ICAM1*	*CFLAR*	*MYO1C*	*IGSF3*	*CCR7*	*SEPT11*	*WSB2*	*IL13RA1*	*CDK2AP1*
CLIP2	*NFKB2*	*PGD*	*HIPK2*	*CD58*	*MARCKS*	*SNX8*	*TNFAIP3*	*TNIP1*	*SLAMF7*
SLC43A2	*FADS1*	*FAM129A*	*RAP2A*	*YWHAG*	*CYFIP1*	*LOC101929479*	*GNG8*	*IRF5*	*TMEM120A*
FCER2	*IPO7*	*CBX6*	*CDK6*	*BLVRA*	*AP1S3*	*XPO5*	*PIK3R5*	*LSP1*	*FNBP1*
TAGLN2	*RBBP9*	*SPINT2*	*FYTTD1*	*GPR137B*	*PEF1*	*PPA1*	*RANGAP1*	*LSM11*	*RHOF*
WDR91	*ATP1A1*	*MIR17HG*	*SUPT16H*	*USP14*	*CPNE8*	*MMP7*	*EEF2K*	*PPME1*	*ANXA7*
EHD4	*CDR2*	*SRPK1*	*BLMH*	*UBE2Z*	*KDM2B*	*PPP1R7*	*CAPRIN1*	*SF3B6*	*TALDO1*
LMNA	*MCOLN2*	*SCD*	*PCGF5*	*HERPUD1*	*NFKB1*	*CHURC1-FNTB*	*ERH*	*TJP2*	*BSG*
VOPP1	*KEAP1*	*CEP85*	*NAE1*	*UBTD2*	*TKT*	*GOT2*	*SZRD1*	*APOL1*	*NSUN4*
CHMP4B	*TRIM28*	*KCNN4*	*SLC16A1*	*SLC9A7*	*SLC20A1*	*MAP4*	*ZNF48*	*ECE1*	*ADCY3*
STX11	*LARP4*	*OTUD7B*	*GPR183*	*PSMD8*	*ZAK*	*SLC35F2*	*COA7*	*ANXA5*	*IDE*
SLC39A1	*MDFIC*	*GSTP1*	*OGFOD1*	*SEC23B*	*IMPDH2*	*HDLBP*	*HTT*	*KIF13B*	*GTF3C4*
ACSL4	*MPC1*	*PITPNB*	*TNIP2*	*EXOC3*	*MDH1*	*PLEKHA7*	*UBE2D4*	*JADE3*	*POR*
PEA15	*PIK3CD*	*TP53BP2*							

Table 2. The lists of Cluster A1.

Cluster B1: 126 genes									
BHLHE40	*IL17RB*	*NFIL3*	*HOMER2*	*AICDA*	*BATF3*	*ARID5A*	*DUSP4*	*CD80*	*TNFAIP2*
XBP1	*MTHFD2*	*CD86*	*CD59*	*CAMK4*	*MFHAS1*	*SLC1A5*	*SRGN*	*USP46*	*CHDH*
HDGFR3	*PIGX*	*FLT1*	*RNF19B*	*LTA*	*NOD2*	*ZNF788*	*AARS*	*ATXN1*	*RFC5*
WARS	*PXDC1*	*PPP1R14A*	*DENND5A*	*QSOX1*	*STK38L*	*PRR5L*	*RGS10*	*SLC7A5*	*SCCPDH*
RRAGD	*LY75-CD302*	*ADAMDEC1*	*YARS*	*GPHN*	*TRIM16L*	*IRF4*	*NINJ1*	*SLC7A1*	*SOCS1*
CD274	*ECHDC3*	*NECAP2*	*TSPAN33*	*SEC11C*	*LOXL3*	*AHRR*	*RALB*	*ARID3A*	*RDX*
CSF1	*THG1L*	*SLC39A8*	*SAMSN1*	*TXNL1*	*STK35*	*DARS*	*TARS*	*CLDND1*	*C12orf5*
SEL1L	*CARS*	*FAM162A*	*VCL*	*SEPHS1*	*XPOT*	*ACSL1*	*GOT1*	*PFKM*	*NSMCE1*
RBM47	*CEP19*	*ATXN2L*	*DHRS3*	*RAB39B*	*DCTN2*	*PABPC4*	*HIVEP1*	*CCDC126*	*ACADVL*
MTX2	*AEN*	*TFG*	*RBPJ*	*SLC25A20*	*ETFDH*	*COPA*	*NR4A3*	*GPX4*	*ITFG3*
DUSP22	*CTNS*	*IL2RA*	*RAP1A*	*TNFAIP1*	*PAM*	*SLC37A1*	*DCTN6*	*AKAP2*	*RIPK2*
RAB21	*RPS23*	*KIAA1279*	*MARS*	*ZNF267*	*CLCN5*	*NFKBID*	*PRPSAP1*	*NEDD1*	*ZNF382*
CDKN1A	*PRRT3*	*LYSMD1*	*NCK2*	*AZIN1*	*KIF5B*				

Table 3. The lists of Cluster B1.

4. The future research on CSR in human B cells

A total of 1399 genes were shown to have differential expression during CSR in human B cells, and the novel genes have the roles in immune system process, cellular amine and DNA process and cell cycle phase or ribonucleoprotein biogenesis. Understanding the precisely functional roles of these novel genes in CSR in human B cells will bring new insights into the mechanisms of CSR and find potential therapeutic targets for human immune disorders such as allergic asthma and autoimmune diseases.

The next stage of research will also focus on determining how the naïve B cells turn into the specific IgE-, IgA- or IgG-releasing cells after T cell cytokines signal stimulation. The different stages of CSR in human B cell may contain unique transcription regulators for the destiny for each single cell. The development of single-cell sequencing provides a unique opportunity to explore the subsets of the human B cells to generate IgE, IgA and IgG. Obtaining high-quality single-cell sequencing data from B cells depends on efficient isolation of individual cells and amplifications of the genome or transcriptome of single cell to acquire sufficient materials for downstream analysis, identifying true variations from technological biases [102]. One of the major challenges of analyzing single-cell genomics data is to develop tools that differentiate technical artifacts and noise introduced during single-cell isolation, whole genome amplification, whole transcriptome amplification and sequencing from true biological variation. There are many factors that can influence the single-cell analysis. During single-cell isolation, the population of cells can be biased through the selection of cells based on size, viability or propensity to enter the cell cycle. Cells from cell lines as control may be problematic as they

may not be diploid, and they can be highly aneuploid or even polyploidy. These will affect experimental performance [102]. Our understanding of human B cell function in CSR will derive from comparisons between healthy individuals and those with particular immunological diseases, and among groups of patients having the same disease with different clinical outcomes. For example, human SLE is clinically heterogeneous [103], making treatment decision challenging. It is important to know which B cell subsets are responsible for which functions in immune diseases, in addition to identifying how a "signature" profile for an individual subject's collection of subsets may correlate with disease outcome that could eventually allow greater optimization of targeted therapies [104].

Abbreviation

AC	adenylate cyclases
AID	activation-induced cytidine deaminase
AP-1	activating protein-1
APC	antigen-presenting cell
APE	apurinic/apyrimidic endonuclease
BCRs	B cell receptors
Be-1	B effector 1
Be-2	B effector 2
CB	cord blood
CLPs	common lymphoid progenitors
CSR	class switch recombination
DCs	dendritic cells
DSBs	double-strand DNA breaks
Eph	erythropoietin-producing hepatocellular
FIP	FOS-interacting protein
FO	follicular
GCs	germinal centers
HDM	house dust mite
hnRNPs	heterogeneous nuclear ribonucleoproteins
IgH	Ig heavy-chain
MAPs	microtubule-associated proteins

MAREs	Maf-recognition elements
MCM	mini-chromosome maintenance proteins
MZ	marginal zone
NF-kB	NF-kappa B
NHEJ	nonhomologous end-joining
NO	nitrogen oxide
PKC-β	protein kinase C-β
PRC1	protein regulator of cytokinesis 1
RAG	recombination activating gene
RTKs	receptor tyrosine kinases
SLE	systemic lupus erythematosus
TD	T lymphocyte dependent
TFEC	transcription factor EC
TI	T lymphocyte independent
TNF	tumor necrosis factor
TRAF	TNF receptor associated factor
TRAP	tartrate-resistant acid phosphatase
UNG	uracil DNA glycosylase

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