# The germinal center reaction

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**List of Design Committee Members:** Dominique Gatto, PhD, and Robert Brink, PhD

**Activity Objectives**

1. To understand the formation and structure of the germinal center (GC) reaction.
2. To identify the role of GC regulation in pathological disease states.

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**The germinal center (GC) reaction is the basis of T-dependent humoral immunity against foreign pathogens and the ultimate expression of the adaptive immune response. GCs represent a unique collaboration between proliferating antigen-specific B cells, T follicular helper cells, and the specialized follicular dendritic cells that constitutively occupy the central follicular zones of secondary lymphoid organs. The primary function of GCs is to produce the high-affinity antibody-secreting plasma cells and memory B cells that ensure sustained immune protection and rapid recall responses against previously encountered foreign antigens. However, the process of somatic mutation of antibody variable region genes that underpins GC function also carries significant risks in the form of unintended oncogenic mutations and generation of potentially pathogenic autoantibody specificities. Here we review the current knowledge on the recruitment, selection, and differentiation of B cells during GC responses and the implication of defects in GC physiology for autoimmune, inflammatory, and malignant diseases. Recent advances in documenting cellular movement within GCs and some of the key migratory signals responsible for GC formation are also discussed. (J Allergy Clin Immunol 2010;126:898-907.)**

***Key words:*** *Germinal center, antibody response, B-cell differentiation, somatic hypermutation, affinity maturation, B-cell migration*

The presence of areas with high mitotic activity in lymph nodes and other lymphoid tissues was first described in 1884 by Walther Flemming.[1-3] He named these structures with strong cell division ***germinal centers*** (GCs) under the assumption that they were the main origin of lymphocytes. Although this notion was subsequently disproved, GCs remain a key source of effector B-cell populations and are crucial for the generation of humoral immunity. In particular, GCs function to generate the high-affinity antibodies that form a key defense against infectious pathogens and are crucial to the efficacy of virtually all vaccines. However, although GC reactions provide a cellular milieu for the affinity maturation of antibody responses, they also bear the risk of generating autoreactive B-cell clones and B-cell lymphomas. This article presents an overview of the current understanding of the GC reaction based on the study of human tonsils and murine lymphoid tissues and discusses the association of GCs with disease.

## INITIAL STAGES OF B-CELL ACTIVATION AND DIFFERENTIATION

GCs develop in B-cell follicles of secondary lymphoid organs in response to antigen challenge. Mature B cells continuously recirculate through secondary lymphoid organs in search of signs of infection and, on reaching the follicles, move rapidly within

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Terms in boldface and italics are defined in the glossary on page 899.

*Abbreviations used*
AID: Activation-induced cytidine deaminase
BCL-6: B-cell lymphoma 6
CD40L: CD40 ligand
CSR: Class-switch recombination
EBI2: Epstein-Barr virus-induced gene 2
FDC: Follicular dendritic cell
GC: Germinal center
ICOS: Inducible costimulator
IgV: Immunoglobulin variable region
SHM: Somatic hypermutation
STAT: Signal transducer and activator of transcription
T-B: T zone–B zone
$T_{FH}$: Follicular T helper
UNG: Uracil DNA glycosylase

them to survey these areas for antigen. On antigen encounter, B cells initially congregate at the boundary between B-cell follicles and T-cell areas in search of T-cell help.[4] This movement is directed by the rapid upregulation of the ***chemokine*** receptor CCR7 consequent on antigen activation.[5] Cognate encounters with T cells at the T zone–B zone (T-B) boundary drive initial B-cell proliferation and are required for the induction of GC responses. Notably, the interaction of the TNF receptor family member CD40, which is constitutively expressed by B cells, and its ligand, CD40L (CD154), which is expressed by activated $T_H$ cells, is crucial for formation of GCs.[6,7] Thus GCs are believed to be heavily dependent on $T_H$ cells, although transient GC formation has been observed in the absence of T-cell help under some experimental conditions.[8]

In addition to expressing CD40L, activated $T_H$ cells also secrete cytokines that deliver signals through specific cell-surface receptors that serve to drive B-cell proliferation and differentiation. Cytokine signals play a central role in triggering the molecular events that lead to the onset of immunoglobulin class-switch recombination (CSR) and thus the production of IgG-, IgE- and IgA-expressing B cells. Typically, signals delivered through cytokine receptors lead to the preferential targeting of the intracellular enzyme activation-induced cytidine deaminase (AID) to one of the switch recombination sequences located at the 5′ end of each of the γ, ϵ, and α immunoglobulin heavy chain constant region genes. Demethylation by AID of deoxycytidine residues in the targeted switch recombination sequences and the proximal μ heavy chain switch recombination sequence is followed by excision of the resulting deoxyuracil bases by uracil DNA glycosylase (UNG). This ultimately triggers recombination between the switch recombination sequences such that the downstream heavy chain gene assumes the original location of the μ heavy chain gene immediately 3′ of the rearranged immunoglobulin heavy chain variable region gene and is expressed as IgG, IgE, or IgA.[9] The process of immunoglobulin ***isotype switching*** can be

## GLOSSARY

**AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME:** Autoimmune lymphoproliferative syndrome is a disorder of lymphocyte apoptosis characterized by an increased incidence of autoimmunity, nonmalignant lymphoproliferation, and susceptibility to malignancy. Patients might have increased numbers $CD3^+\alpha\beta^+CD4^-CD8^-$ (double-negative) T cells. Most cases are due to mutations in Fas.

**CHEMOKINE:** Chemokines are the largest family of cytokines. They act by binding to G protein–coupled receptors. Their function in the immune system is to coordinate leukocyte trafficking and activation.

**DENDRITIC CELLS:** Dendritic cells are hematopoietic cells that function as antigen-presenting cells for naive lymphocytes. Their name is derived from their multiple, thin membranous projections.

**GERMINAL CENTER:** The germinal center is a specialized structure within secondary lymphoid organs in which responding B cells undergo somatic hypermutation and selection for increased antigen affinity (affinity maturation).

**GERMLINE:** The germline is the cellular lineage from which eggs and sperm are derived. Germline mutations can be passed to the next generation.

**INDUCIBLE COSTIMULATOR (ICOS):** ICOS is a member of the CD28 family of costimulatory receptors on T cells. ICOS binds to ICOS ligand on antigen-presenting cells. Function-loss mutations in the *ICOS* gene have been reported in patients with common variable immunodeficiency.

**INTEGRIN:** Integrins are cell-surface proteins that mediate adhesion. Leukocyte adhesion deficiency I is caused by mutations in a subfamily of integrins containing the conserved $\beta_2$ chain (CD18).

**ISOTYPE SWITCHING:** Isotype switching is the process of changing the class (isotype) of antibody production. There are 5 different antibody isotypes (IgM, IgD, IgG, IgA, and IgE), which are determined by the type of heavy chain present. Isotype switching allows an antibody-producing cell to alter the biological effects of its secreted product without affecting its specificity.

**OPSONINS:** Opsonins are various proteins (eg, complement or antibodies) that bind to foreign particles and microorganisms, making them more susceptible to the action of phagocytes. Mannose-binding lectin is an example of an opsonin that initiates complement activation.

**PLASMABLAST:** A plasmablast is an immature plasma cell still capable of proliferating and presenting antigens to T cells. A plasmablast can differentiate into a plasma cell, which is a terminally differentiated antibody-secreting B lymphocyte. Development of plasma cells is dependent on the induction of the transcription factor BLIMP-1.

**SILENT MUTATION:** A silent mutation is a mutation having no detectable effect, such as DNA base changes that do not alter the amino acid sequence of the encoded protein.

**SOMATIC MUTATION:** A somatic mutation is a mutation relating to nonreproductive parts of the body that is therefore not inherited. In the immune response antibody variable regions undergo intense somatic mutation (hypermutation) within germinal centers.

**SPLENIC MARGINAL ZONE:** The splenic marginal zone is the interface between the red pulp and the white pulp in the spleen. Antigen from blood first encounters B cells in the marginal zone.

**SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 3 (STAT3):** STAT3 is necessary for the development of $T_H17$ cells, and mutations in the *STAT3* gene are associated with hyper-IgE syndrome.

**TINGIBLE BODY MACROPHAGES:** Tingible body macrophages take on a distinct "starry-sky" appearance after phagocytosing cellular debris.

**TWO-PHOTON MICROSCOPY:** Two-photon microscopy is a form of laser scanning fluorescence microscopy that allows images of living cells and other microscopic objects.

**ZINC FINGER:** Zing fingers are small protein domains that use zinc ion binding to help stabilize the protein's folds. Zinc fingers are present in a variety of proteins, including those that participate in replication, repair, transcription, signaling, proliferation, and apoptosis.

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**TABLE I.** Chemokine receptors and ligands controlling B-cell migration in secondary lymphoid organs

| Receptor | Receptor expression | Ligand | Site of ligand production |
|---|---|---|---|
| CXCR5 | Naive B cells, activated B cells, GC B cells | CXCL13 | B-cell follicles |
| CCR7 | Activated B cells | CCL19, CCL21 | T-cell zone |
| CXCR4 | GC B cells (centroblasts mainly), plasmablasts, plasma cells | CXCL12 | GC dark zone, splenic red pulp, lymph node medullary cords |
| EBI2 | Naive B cells, activated B cells (but not GC B cells), plasmablasts, plasma cells | ? | Perifollicular and interfollicular areas, marginal zone bridging channels |

initiated within days of initial B-cell activation.[10,11] Thus although CSR can occur in the GC, it is not limited to this phase of the B-cell response.

After initial activation, proliferating B-cell blasts proceed down one of 2 independent pathways of migration and differentiation.[12,13] On the one hand, responding B cells migrate from the T-B boundary to extrafollicular areas, where they are induced to rapidly expand and differentiate into ***plasmablasts*** and plasma cells.[14] These transient antibody-secreting cells provide the most immediate source of antigen-specific antibodies and provide rapid protection before the slower GC response is established.[14] Alternatively, antigen-engaged B cells remain localized in B-cell follicles, where they seed GCs. Typically only 1 to 6 clones were found to colonize B-cell follicles, and thus GCs are believed to be of oligoclonal nature in both mice and human subjects.[15,16]

## RECRUITMENT OF B CELLS INTO THE PLASMABLAST VERSUS GC PATHWAY

Migration of activated B cells to the distinct microenvironments that support their differentiation into plasma cells or GC B cells is mediated by their differential expression of chemotactic receptors. Pivotal roles in the control of B-cell migration are played by the chemokine receptors CXCR5, CCR7, and CXCR4 and their respective ligands CXCL13, CCL19/21, and CXCL12, which are selectively produced in distinct anatomic regions of secondary lymphoid organs, such as the spleen and lymph nodes (Table I and Fig 1). Naive recirculating B cells express high levels of the chemokine receptor CXCR5 and therefore predominantly respond to its ligand, CXCL13, which is produced by stromal cells in B-cell follicles.[17,18] As B cells differentiate into plasma cells, they downregulate the chemokine receptor CXCR5 and upregulate CXCR4,[10] events that are critical for their localization in the splenic red pulp and lymph node medullary cords.[19] In addition, the expression of the orphan G protein–coupled receptor Epstein-Barr virus-induced gene 2 (EBI2), which is high on naive and recently activated B cells, plays an important role for their migration to the periphery of B-cell follicles, ***splenic marginal zone*** bridging channels, and interfollicular regions.[20,21] The EBI2-mediated localization of activated B cells to these areas of secondary organs is essential for the generation of robust extrafollicular plasmablast responses.[20] B cells seeding GCs, on the other hand, retain expression of CXCR5 and downregulate EBI2 expression.[20-22] The low levels of EBI2 on GC B cells compared with the bulk of naive follicular B cells cause their accumulation in the center of B-cell follicles, where GCs originate.[20,21] Downregulation of EBI2 on B cells committed to the GC pathway was shown to enable them to access the center of follicles and be required for efficient GC formation.[20,21] Thus the regulated responsiveness of activated B cells to chemokines and EBI2 ligand, as mediated by changes in expression of CXCR5, CXCR4, and EBI2, directs the cells to the distinct microenvironments that sustain their differentiation into plasmablasts or GC B cells (Fig 1).

B-cell differentiation decisions are linked to the migration of activated B cells to distinct regions of secondary lymphoid organs providing environmental clues that sustain their differentiation, but the signals controlling this early bifurcation of B-cell differentiation to plasma cells or GC B cells are not completely understood. Factors that influence the fate of responding B cells include transcriptional control, the availability and quality of T-cell help, and the strength of the B cell receptor-antigen interaction. Indeed, several studies with different experimental systems have indicated that the production of short-lived plasma cells increases with initial B-cell affinity for antigen, whereas B cells carrying a broad range of antigen affinities undergo GC B-cell differentiation.[10,23,24]

## STRUCTURE OF GCS

### Centroblasts and centrocytes

A classical view of GC organization has emerged from histologic analysis of human tonsils and murine lymphoid tissues (Fig 2). In mature GCs 2 compartments are established, termed the dark zone and light zone on the basis of their histologic appearance, which are surrounded by naive follicular mantle B cells. The dark zone is localized proximal to T-cell areas and contains a high density of large, proliferating B cells with downregulated surface immunoglobulin expression known as centroblasts.[3,25] At the distal pole lies the light zone, where the density of B cells is lower due to the presence of a follicular ***dendritic cell*** (FDC) network. Light-zone B cells, so called centrocytes, are small, nonmitotic B cells expressing surface immunoglobulins.[3,25] Compartmentalization of GC B cells into dark and light zones is dependent on the differential abundance of the chemokines CXCL12 and CXCL13 in these zones and the regulated expression of CXCR4 on centrocytes and centroblasts.[22] Centroblasts express higher levels of CXCR4 than centrocytes, and the ligand of this chemokine receptor, CXCL12, is more abundant in GC dark zones than in light zones. Thus upregulation of CXCR4 on centroblasts drives their localization to GC dark zones. On the other hand, accumulation of $CXCR4^{low}$ centrocytes in GC light zones is mediated by CXCR5 and the high levels of its ligand, CXCL13, present in these areas of GCs.[22]

### FDCs

FDCs are radio-resistant stromal cells with long processes that form a network occupying much of the GC light zone.[17] Unlike the professional antigen-presenting dendritic cells, FDCs are not derived from hematopoietic precursors and do not express class II MHC molecules. FDCs do, however, trap and retain

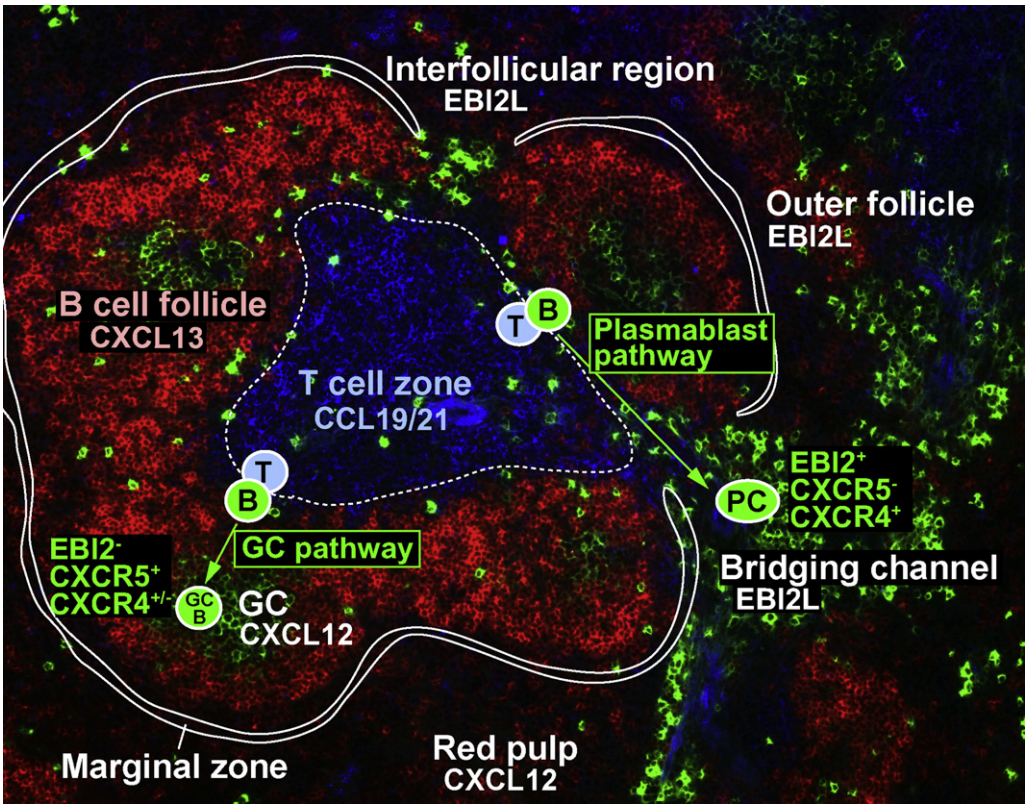


**FIG 1.** Early bifurcation in B-cell migration and differentiation. In the early stages of antibody responses, on interaction with T cells at the T-B boundary, activated B cells are recruited into the plasmablast or GC pathway of differentiation. The emerging spatially distinct populations of extrafollicular plasmablasts/plasma cells and GC B cells are guided in their localization by the regulated expression of the chemotactic receptors EBI2, CXCR5, and CXCR4. Plasmablasts and plasma cells downregulate CXCR5, retain EBI2, and upregulate CXCR4, which mediates their migration to the periphery of B-cell follicles and extrafollicular areas. Conversely, GC B cells downregulate EBI2 and retain CXCR5, which results in their accumulation in the center of B-cell follicles, where GCs are established. The histologic section of the spleen was stained with anti-IgD *(red)* and anti-CD3 *(blue)* to visualize B-cell follicles and T-cell areas, respectively. Hen egg lysozyme-specific B cells mounting a response were stained in *green* and can be observed to form GCs and plasma cell foci in bridging channels and red pulp. *EBI2L*, EBI2 ligand.

unprocessed antigen in the form of immune complexes through Fc receptors, such as FcγRIIb (CD32) and FcεRII (CD23), and complement receptors, such as CR1 (CD35), CR2 (CD21), and CR3 (CD11b/CD18).[17,26] Thus they serve as long-term antigen deposits and are generally believed to be important for selection of high-affinity GC B-cell clones, as well as the generation and maintenance of immunologic memory.[27] Interactions between FDCs and GC B cells are facilitated by the high expression of the ***integrin*** ligands vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 on FDCs.[28] In addition, FDCs are a major source of CXCL13 in GC light zones, where it accumulates on their processes.[17]

### Follicular T helper cells

A second population of accessory cells in light zones of GCs are follicular T helper ($T_{FH}$) cells. Access of this subset of $CD4^+$ T cells to B-cell follicles is mediated by their expression of CXCR5, which is induced on activation and mediates the chemotactic response of $T_{FH}$ cells to CXCL13 produced by follicular stromal cells.[29] $T_{FH}$ cells represent a minor population in GCs, only constituting 5% to 20% of GC cells.[29,30] However, it has recently become clear that this population is crucial for induction of GC responses because it provides important signals for GC B-cell survival.

## AFFINITY MATURATION AND SELECTION OF GC B CELLS

GCs are the primary site at which diversification and affinity maturation of antigen-experienced B cells is believed to occur. As B cells proliferate in GCs, they acquire high rates of mutations (in the order of $10^{-3}$ to $10^{-4}$ per base pair per generation) in their immunoglobulin variable region (IgV) genes through the process of somatic hypermutation (SHM).[31] This process is associated with DNA strand breaks and introduces mainly single nucleotide exchanges in the targeted IgV genes. Like CSR, SHM also requires the activity of the enzyme AID, which has been reported to be expressed primarily in the GC dark zone.[32] In this case AID targets deoxycytidine bases in both heavy and light chain IgV genes for demethylation, which are once again excised by UNG. Mutations are introduced by the "filling in" of the resulting gaps in the DNA sequence by an error-prone polymerase and result in random changes in B-cell receptor affinity and specificity.[33] GC B-cell

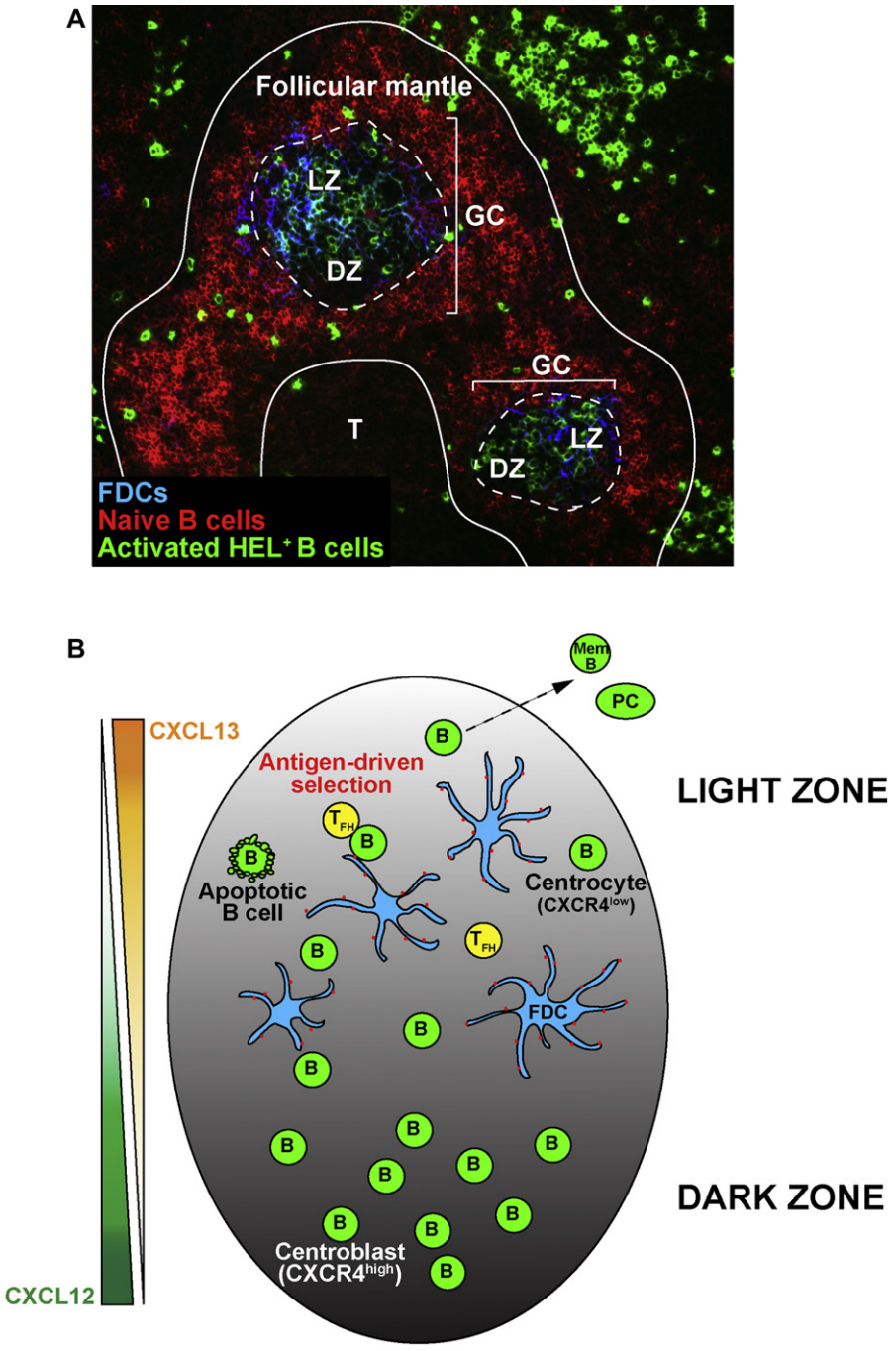


**FIG 2.** Organization of GCs. **A**, Histological staining of hen egg lysozyme *(HEL)*–specific B cells *(green)* organized into GCs in response to immunization with HEL coupled to sheep red blood cells. Naive follicular mantle B cells are stained with anti-IgD *(red)*, and FDC networks in GC light zones are detected with anti-CD21/35 *(blue)*. *DZ*, Dark zone; *LZ*, light zone; *T*, T-cell area. **B**, Schematic diagram of the structure of GCs. Centroblasts and centrocytes are organized in GC dark and light zones, and their positioning in the respective compartments is controlled by the chemokine-chemokine receptor pairs CXCL12-CXCR4 and CXCL13-CXCR5. CXCR4 is more abundant on centroblasts than centrocytes, whereas CXCR5 is expressed at similar levels on all GC B cells. FDCs, which display native antigen and express CXCL13, and $T_{FH}$ cells are localized in GC light zones. Interaction of GC B cells with FDCs and $T_{FH}$ cells drives affinity maturation and leads to differentiation of selected GC B cells to memory B cells and long-lived plasma cells.

clones expressing variants with increased binding to the immunizing antigen are selectively expanded, whereas changes that result in impaired antigen binding induce apoptosis of GC B cells. This is reflected in the enrichment of replacement mutations compared with ***silent mutations*** in the antigen-binding regions of immunoglobulins expressed by GC B cells and an increase in the average affinity of secreted antibodies as the response progresses.[34,35]

The selection process underlying affinity maturation has classically been viewed to involve sequential events in the spatially and functionally separated dark and light zones of GCs. According to this model, termed the cyclic re-entry model, centroblasts undergo cell division and SHM in the dark zone before exiting the cell cycle, re-expressing surface immunoglobulin, and migrating into the light zone to form centrocytes.[25] In light zones mutant GC clones with increased affinity for antigen are preferentially selected by their interaction with antigen retained on FDCs and survival signals provided by $T_{FH}$ cells. High affinity for antigen provides a competitive advantage for the limited amount of antigen, recruitment of T-cell help, and potentially positive signals derived from FDCs.[36] Selected centrocytes were proposed to subsequently return to the dark zone for further rounds of proliferation and selection, whereas apoptotic cells are taken up by a specialized subset of macrophages that reside within GCs known as the ***tingible body macrophages***.[25] These cells are named after the tingible (stainable) bodies within the cells that represent the degrading components of phagocytosed GC B cells.

The model in which GC B cells undergo alternating proliferation-dependent migration between the light and dark zones was recently tested in studies of GC dynamics by using real-time imaging with ***2-photon microscopy***. This analysis indicated that GC B cells in both the light and dark zones were highly motile and morphologically similar, exhibiting irregular, constantly changing shapes.[37-39] Contrary to previous belief, cell division and cell death were found to occur at a similar rate in both zones.[37,38] Thus the morphology and mitotic properties of dark- and light-zone GC B cells were more similar than previously thought and indicated by their division into centroblasts and centrocytes. The approach of intravital 2-photon microcopy also provided the first direct evidence for the transit of B cells between the dark-zone and light-zone compartment, but the extent of this exchange remains controversial.[37-39] In addition, an interesting finding reported from the visualization of GC dynamics was that some naive follicular B cells entered ongoing GC reactions, indicating that GCs are open structures that can be colonized by unrelated B-cell clones.[39] Consistent with this finding, high-affinity B cells and memory B cells have been shown to join established GC reactions.[39,40] The entry of follicular B cells into GCs has been proposed to be one of the mechanisms mediating antigen transport and deposition into GCs.[41]

## POST-GC B-CELL DIFFERENTIATION

One of the hallmarks of the GC reaction is the generation of humoral memory. B-cell clones positively selected within GCs differentiate into memory B cells or long-lived plasma cells and exit the GC reaction. Memory B cells have been shown to persist for long periods of time after antigen exposure and to recirculate through secondary lymphoid organs in addition to colonizing the splenic marginal zone.[42] As a result of their selection in GCs, memory B cells express immunoglobulins carrying ***somatic mutations*** and therefore are capable of rapidly responding to antigen re-encounter with secretion of high-affinity antibodies.[43] Long-term humoral memory is further maintained by a population of GC-derived long-lived plasma cells, which reside in the bone marrow.[44] Although both memory B cells and bone marrow plasma cells are enriched for improved affinity for antigen, the requirements for entry of GC B cells into these 2 post-GC compartments are distinct. Several studies have indicated that differentiation of GC B cells into plasma cells requires delivery of signals above a certain threshold that is determined by the B-cell receptor affinity for antigen, as well as costimulatory signals, through complement receptors and CD40.[45-47] In contrast to the selective accumulation of high-affinity clones in the bone marrow plasma cell population, entry into the memory B-cell compartment has been reported to be less stringently controlled by affinity for antigen.[45,46] Thus survival in the GC has been proposed to be sufficient to allow differentiation of GC B cells to memory B cells.[46] In addition, *in vitro* studies have suggested that cytokines produced by activated T cells might also play a role in the regulation of the commitment of GC B cells to the memory B-cell or plasma cell compartment.[48]

## TRANSCRIPTIONAL CONTROL OF GC FORMATION

The transcription factor B-cell lymphoma 6 (BCL-6) has emerged as the master transcriptional regulator of GC differentiation.[49,50] BCL-6–deficient mice do not generate GC responses, despite normal B-cell development and despite mounting normal extrafollicular plasmablast responses.[51,52] In the B-cell compartment BCL-6 protein is specifically expressed in GC B cells, and its expression is tightly regulated on B-cell activation.[53,54] BCL-6 is induced in antigen-experienced B cells as they proliferate in nascent GCs, but its expression remains silent in B cells committed to the extrafollicular plasma cell fate.[52] BCL-6 is a ***zinc finger***–containing nuclear transcription factor that acts as a transcriptional repressor. Among the target genes of BCL-6 are genes involved in apoptosis and cell-cycle control, such as p53, cyclin D2, and the cdk inhibitor p27kip1, which sustains the high proliferation rate of B cells in GCs.[50,55] A second major function of BCL-6 is to inhibit the expression of genes involved in lymphocyte activation, including *CD69*, *CD44*, and signal transducer and activator of transcription (STAT) 1, as well as terminal plasma cell differentiation.[50,55] Inhibition of the plasma cell differentiation program by BCL-6 is thought to be mediated by repression of *Prdm1*, the gene encoding B lymphocyte-induced maturation protein-1, a transcription factor that is crucial for plasma cell differentiation.[55] A further important component of the activity of BCL-6 is the repression of EBI2 expression.[50,55] As discussed above, downregulation of EBI2 in B cells committed to the GC pathway is both necessary and sufficient for activated B cells to localize to the center of B-cell follicles to establish GC reactions.

The critical requirement for BCL-6 for GC formation is further attributed to its important role in driving the programming of $T_{FH}$ cells.[56,57] BCL-6 is selectively expressed in the $T_{FH}$ cell population and promotes expression of $T_{FH}$-related genes, such as *CXCR5*, programmed death-1, and ***inducible costimulator (ICOS)***, while inhibiting those associated with development to other T cell lineages.[56,57] Thus T cells lacking BCL-6 have been shown to be unable to differentiate into $T_{FH}$ cells and to support GC responses.[56,57]

## REGULATION OF GC REACTIONS

GC formation and termination is regulated by a number of variables, and the kinetics of GC reactions are strongly dependent on the nature of the immunizing antigen. Studies using haptenated protein for immunization have suggested that GCs arise as early as 4 to 5 days after immunization and have a duration of 2 to 3 weeks.[12,13] On the other hand, viral particles have been

demonstrated to induce GC reactions persisting for many months after primary immunization/infection driven by long-term antigen deposits in the form of immune complexes or low-level chronic infection.[58,59] Thus antigen availability is believed to play an important role in maintaining GC responses. As antigen levels decrease through uptake, degradation, or sequestration by antigen-specific antibodies, GC B cells face increasing pressure for antigen-mediated survival signals. GC B cells are intrinsically prone to apoptosis because of their proapoptotic program of gene expression characterized by low levels of BCL-2 and BCL-$X_L$ and high levels of BIM and Fas (CD95).[60,61] This limits the lifespan of GC B cells that do not receive survival signals and leads to their apoptosis *in situ.*

## DEFECTIVE GC FORMATION IN PATIENTS WITH IMMUNE DEFICIENCIES

The interaction of GC B cells with T cells in the light zones of GCs plays an important role in the survival of GC B cells. Expression of surface molecules by $T_{FH}$ cells, such as CD40L, ICOS, and PD-1, have been shown to be required for GC development and maintenance in mice.[62-64] In addition, the population of $T_{FH}$ cells is unique in the expression of the cytokine IL-21, which, along with IL-4, provides proliferation, survival, and differentiation signals to GC B cells.[65,66] Mutations or deficiencies in the signaling of the costimulatory molecules CD40, CD40L, and ICOS and defects in secretion of IL-21 have been shown to abolish GC formation and underlie immunodeficiencies in human subjects.[67-69] Genetic defects in CD40L and CD40 are common in patients with hyper-IgM syndrome, which is characterized by markedly decreased serum IgG and IgA levels and normal or increased levels of IgM. In patients with hyper-IgM syndrome, lymph nodes are devoid of GCs, and the proportion of memory B cells and the frequency of SMH are reduced, which underscores the importance of the CD40L-CD40 interaction for the maturation of antibody responses in human subjects.[68,69] In a subgroup of patients with hyper-IgM syndrome, the failure to generate isotype-switched antibodies and memory B cells is caused by mutations in AID and UNG.[69] Defects in molecules expressed highly by $T_{FH}$ have also been associated with immunodeficiency. Mutations that lead to homozygous loss of ICOS expression have been characterized in some patients with common variable immunodeficiency and result in disruption of GC formation and absence of memory B cells.[68,69] The study of patients with autosomal-dominant hyper-IgE syndrome has suggested that defects in ***STAT3***–dependent IL-21 signaling play a role the antibody deficit observed in these patients.[67]

## DYSREGULATED GC REACTIONS IN AUTOIMMUNITY

Dysregulation of the mechanisms controlling GC development and maintenance can lead to exaggerated or chronic GC reactions. GCs have been reported to be numerous in autoimmune-prone murine strains and to arise spontaneously in the absence of immunization or infection.[70] Spontaneous GC formation is associated with the production of pathogenic antibodies, which show evidence of SHM and antigen selection in both human subjects and mice.[71-73] Several mechanisms have been proposed to lead to dysregulated GC formation and secretion of autoantibodies. Defects in Fas-mediated apoptosis of GC B cells in mice, as a result of genetic deletion, have been shown to disrupt B-cell homeostasis and lead to autoimmunity.[74] Similarly, in human subjects mutations in Fas are associated with ***autoimmune lymphoproliferative syndrome*** and autoimmunity.[75,76] There is also evidence to support the involvement of $T_{FH}$ cells in the abnormal GC formation that accompanies autoimmune diseases. Increased numbers of $T_{FH}$ cells are observed in many murine strains prone to systemic lupus erythematosus–like disease, and their accumulation is thought to sustain spontaneous GC formation.[77,78] Overexpression of molecules involved in GC formation on $T_{FH}$ cells, such as ICOS, has been shown to cause exaggerated GC responses and autoimmunity in mice,[78] and increased ICOS expression by T cells has been reported in human subjects with systemic lupus erythematosus or rheumatoid arthritis.[79,80]

GCs are sites of marked apoptosis, and the clearance of apoptotic debris by the specialized tingible body macrophages is crucial for preventing autoimmunity. Defects in molecules that facilitate recognition, uptake, and digestion of apoptotic cells by macrophages, such as ***opsonins*** and their receptors, make mice and human subjects susceptible to autoimmune diseases.[70] It is believed that the exposure of intracellular autoantigens, such as DNA, RNA, and nuclear proteins, leads to the recruitment and activation of autoreactive B cells that would normally remain quiescent and be excluded from GC reactions. Breakage of B-cell tolerance during GC reactions can potentially occur as a result of diversification of IgV genes through SHM. Accumulation of somatic mutations in IgV regions can lead to the generation and expression of antibodies with self-reactivity.[70] Under normal circumstances, autoreactive GC B-cell clones are thought to be deleted by the interaction with self-antigen.[81,82] Although failure to recruit survival signals provided by T cells has been shown to play a role in the negative selection of autoreactive GC B cells,[81,82] the mechanism of tolerance induction in GCs is still incompletely understood.

## GC-DERIVED B-CELL LYMPHOMAS

Not only can the process of somatic diversification of GC B cells lead to the emergence of autoreactive clones, but also the genetic instability arising from the rapid cellular proliferation and somatic mutation of GC B cells is conducive to their malignant transformation. The majority of B-cell lymphomas originate from GC B cells, as indicated from their expression of somatically mutated IgV genes.[83,84] The frequency of SHM in GCs is about one million times higher than the mutation rate of housekeeping genes,[32] and if not tightly regulated and targeted to immunoglobulin genes, it can result in genetic alterations that lead to malignant transformation. Aberrant actions of the SHM machinery on nonimmunoglobulin loci is thought to be responsible for the introduction of mutations in the 5′ regulatory region of multiple genes, including the *MYC* proto-oncogene and *BCL6.*[85,86] These changes can lead to the constitutive expression of proto-oncogenes in GC B cells, which disrupts their apoptosis or differentiation and leads to lymphoma development.[87] In addition to the aberrantly targeted introduction of mutations, malfunctions in immunoglobulin gene remodeling during CSR and SHM can cause chromosomal translocations. Chromosomal translocations frequently involve the immunoglobulin locus with *MYC*, which is characteristic of Burkitt lymphoma, or with *BCL6*, which leads to diffuse large B-cell lymphoma.[87,88] Constitutive expression of BCL-2 as a result of a reciprocal chromosomal translocation is

found in follicular lymphoma and is thought to override the proapoptotic program of GC B cells, thereby promoting malignant transformation.[87,88]

## ECTOPIC GC FORMATION

Although GCs normally form in secondary lymphoid organs in response to foreign antigen, they can form in nonlymphoid tissues in patients with inflammatory and autoimmune conditions. Such ectopic GCs have been observed in exocrine glands, the liver, the synovium, the thymus, and brain meninges of patients with autoimmune and chronic inflammatory diseases, including rheumatoid arthritis, Hashimoto thyroiditis, Sjogren syndrome, multiple sclerosis, and chronic hepatitis C infection.[89] Interestingly, ectopic GCs are also found in chronically rejected grafts.[90]

GC reactions are usually transient and turned off when the causative antigen has been eliminated. However, when the antigen persists, as a result of pathogens escaping immunosurveillance or constant replenishment of self-antigen by the tissue, chronic inflammatory responses are generated, and ectopic lymphoid structures containing GCs form at the site of tissue pathology. Ectopic GCs contain the same main cellular components and often display a similar polarized structure as GCs of secondary lymphoid organs.[91,92] Proliferating B cells are intermeshed with FDCs, which develop *de novo* as a result of the differentiation of local fibroblasts or from stromal cell precursors located in the inflamed tissue or recruited from the blood.[93] Aggregates of T cells surround the GC structures and sustain the formation and progression of the GC response. The action of a panel of cytokines and chemokines, such as lymphotoxin α, CCL21, and CXCL13, is required for the initiation of ectopic GC formation, but it is unclear whether naive or memory B cells give rise to ectopic GCs.[89] In addition to the microanatomic similarities to conventional GCs, ectopic GCs are functional and support local clonal expansions, SHM, immunoglobulin class switching, and plasma cell differentiation.[94-96] In patients with autoimmune diseases, ectopic GCs fulfill many of the criteria for having a pathological role in disease progression, and indeed, production of tissue-specific, disease-relevant autoantibodies by ectopic GC-derived plasma cells has been shown in patients with myasthenia gravis, Sjogren syndrome, and Hashimoto thyroiditis.[91,92,97]

## GC REACTIONS IN ALLERGIC INFLAMMATION

Although ectopic GC reactions are well documented in target organs of patients with autoimmune diseases, there is evidence that GC-like reactions also occur at sites of allergic inflammation. In a murine model of allergic pulmonary inflammation, formation of GCs was shown to occur within the parenchyma of the inflamed lungs.[98] FDC networks were present in these GCs, and antigen-specific plasma cells secreting IgE were closely associated.[98] Local formation of GC-like structures in patients with allergic disease has been supported by the presence of IgE variable region sequences of oligoclonal origin containing somatic mutations in nasal biopsy specimens from patients with allergic rhinitis.[99] Consistent with the high number of mutations, AID expression was detectable in the nasal mucosa of these allergic patients.[99] These studies also demonstrated that CSR to IgE can occur in the periphery in addition to the local clonal expansion and SHM.

Although the observation of an accumulation of $IgE^+$ plasma cells in the respiratory tract during allergic inflammation might suggest local class switching, the exact location of IgE switching remains unclear. GCs are generally believed to be sites at which switching to downstream isotypes occurs, in particular at later stages after immunization/infection or in response to prolonged antigen exposure. However, the GC microenvironment is poorly conducive to IgE class switching. As discussed above, $T_{FH}$ cells secrete IL-21, which has been shown *in vitro* to suppress IL-4–induced switching to IgE.[100] Consistently, mice deficient in IL-21 or IL-21 receptor show increased serum IgE levels,[101,102] and patients with hyper-IgE syndrome are deficient in STAT3–activating cytokines, such as IL-21.[67] In addition, BCL-6 inhibits $C_\epsilon$ germline transcripts by repressing the $C_\epsilon$ promoter,[103] thus making GC B cells unlikely to undergo IgE class switching because of their high BCL-6 expression. In a murine model of IgE responses, $IgE^+$ B cells were largely found outside GCs, despite showing signs of SHM and affinity maturation.[104] Thus it was proposed that high-affinity IgE antibodies are generated through sequential switching of $IgG1^+$ B cells exiting the GC reaction and differentiating into plasma cells.[104]

## CONCLUDING REMARKS

The GC microenvironment provides a dynamic niche in which activated B cells come together with concentrated deposits of their antigen retained on FDCs and $T_{FH}$ cells, which provides the basis for the generation of long-term humoral immune responses. However, although the output of GC reactions is crucial for immunity, autoreactive B cells and B-cell lymphomas can be detrimental byproducts of dysregulated GC B-cell differentiation. In the more than 120 years of research since their discovery, the cellular events that occur during GC responses and the factors regulating GC B-cell selection, survival, and differentiation have been elucidated. The challenge of future research will be to find strategies to modulate recruitment and proliferation of B cells in the GC and thus be able to limit the propagation of GC reactions in autoimmune, inflammatory, and malignant diseases or enhance their formation for vaccination purposes.

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