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REVIEW ARTICLE

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Transcriptional regulation of memory B cell development

Abstract Antigen-reactive B cells in the spleen of mice immunized with T cell-dependent antigens generate antibody-producing foci in periarteriolar lymphoid sheaths (PALS) or migrate into follicles to form germinal centers. Germinal center B cells clonally expand, have somatic hypermutation in IgV-region genes, are selected by apoptosis on the basis of antigen-specific signals, and differentiate to memory B cells. Two transcription factors (Bcl6 and c-Fos) in B cells play a critical role in the development of germinal centers. (1) Bcl6 is highly expressed in germinal center B cells, and defects in B cells perturb the formation of germinal centers but not that of PALS-associated foci, indicating the essential role of Bcl6 in the differentiation. (2) Overexpression of c-Fos in germinal center B cells induces apoptosis and perturbs the formation of memory B cells. Overexpression of Bcl-2 cannot rescue c-Fos-induced apoptosis in germinal center B cells. Since c-Fos is induced in mature B cells which have reacted with antigens, and clonal deletion of self-reactive B cells is insensitive to overexpression of Bcl-2, c-Fos may play a causal role in the clonal deletion of germinal center B cells. Thus, these factors provide a unique opportunity to investigate the molecular mechanisms of memory B cell development.

Key words Bcl6 · c-Fos · Germinal center · Memory B cells

Introduction

Immunological memory results in a secondary antibody response which gives a higher affinity to non-IgM isotypes of immunoglobulin (Ig).[1] The feature of the secondary antibody response is important in protecting the host against repeated infection. The induction of immunological memory depends upon the antigen reaching and being available in the lymphoid organs in a dose- and time-dependent manner. Thus, antigen-presenting cells are of critical importance in transporting antigen from the periphery to local organized lymphoid tissue. Furthermore, the formation of germinal centers in lymphoid organs is essential for the development of immunological memory.[2] However, the molecular mechanisms of memory development through germinal centers are poorly understood.

When mice are immunized with T cell-dependent (TD) antigens in the peritoneum, antigen-reactive B cells in the spleen are activated by interaction with $CD4^+$ helper T cells in the periarteriolar lymphoid sheaths (PALS) (Fig. 1).[2,3] These activated B cells generate antibody-producing foci in the PALS (PALS-associated foci) or migrate into follicles to form germinal centers that are identified by their binding capacity to peanut agglutinin (PNA). The great majority of antibodies (IgM, IgG, and IgE) produced in the first 12 days after immunization (the primary antibody production) are derived from PALS-associated foci.[4] During this primary antibody response, germinal centers develop in follicles in the first 3 weeks after immunization.[5] Germinal center B cells mutate the genes that encode their Ig variable regions.[6,7] This can increase the affinity of the antibody, but can also induce autoreactive B cells. Selection mechanisms allow the cells with high affinity for the provoking antigen to persist, while other B cells recruited into the response die.[8] Furthermore, germinal center B cells have an Ig-class switch to non-IgM isotypes and differentiate to memory B cells. We have recently worked on the transcriptional regulation of memory B cell development in germinal centers by Bcl6 and c-Fos using knockout mice[9] and transgenic mice,[10] respectively. Here we review our work on a role of Bcl6 and c-Fos in memory B cell development.

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Development of memory B cells in germinal centers

Antigen-reactive B cells are activated in PALS and migrate into follicles to form germinal centers. The germinal centers

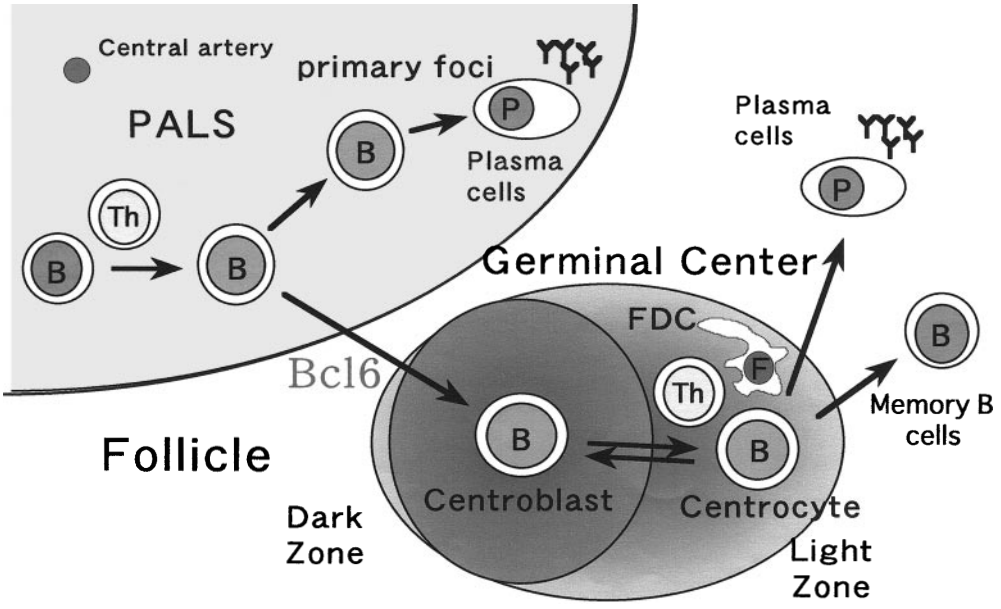

Fig. 1. Differentiation of antigen-reactive B cells in the spleen of mice immunized with T cell-dependent (TD) antigens. Antigen-reactive B cells in the spleen are activated by interaction with $CD4^+$ helper T cells in the periarteriolar lymphoid cells (*PALS*), and further differentiate to PALS-associated foci to produce primary antibody production, or to germinal center B cells in the follicles

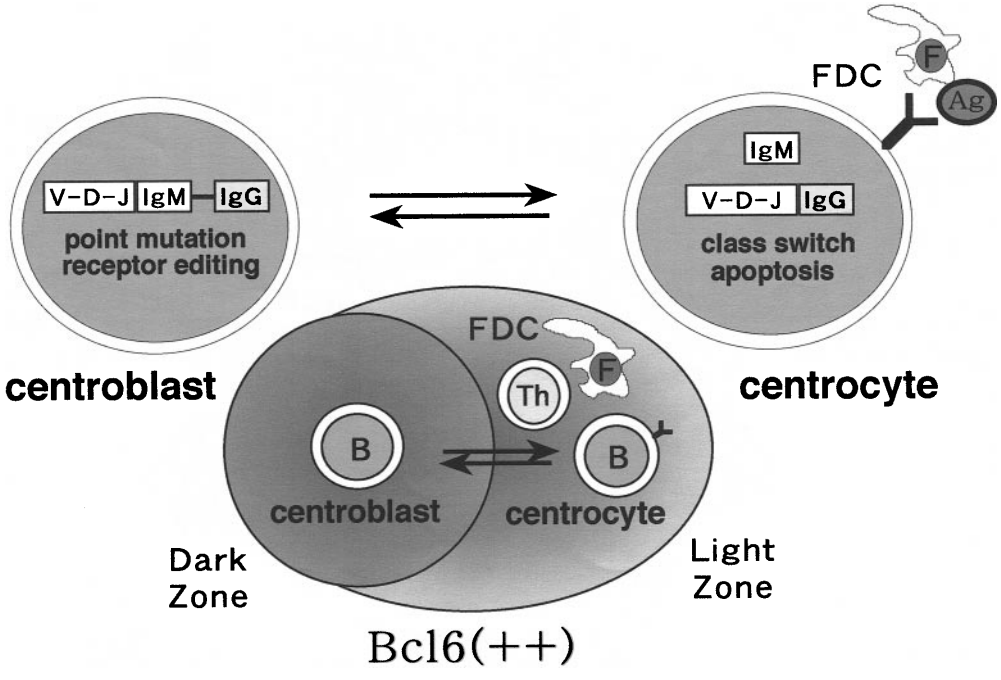

Fig. 2. Memory B cells are developed in germinal centers. Germinal center B cells undergo massive clonal expansion to form a dark zone occupied by centroblasts with somatic hypermutations of IgV genes. These B cells differentiate into sIg-positive centrocytes in a light zone. The light zone contains a rich network of follicular dendritic cells (*FDC*) with antigen on their surface. Centrocytes with a higher affinity to the antigen further differentiate into antibody-producing cells or memory B cells

formed are oligoclonal; on average three B blasts colonize each follicle.[2] Mature germinal centers are divided into dark and light zones (Fig. 2). Nascent germinal center B cells undergo massive clonal expansion to form a dark zone occupied by surface Ig (sIg) negative centroblasts. Somatic hypermutations of the Ig gene occur in centroblasts.[6,7] These cells differentiate into sIg-positive centrocytes at a light zone in the germinal centers. The light zone contains a rich network of follicular dendritic cells (FDCs) that have the capacity to take up antigen and hold this on their surface for periods of more than a year.[11] Centrocytes appear to be selected by their ability to interact with antigen held on FDCs. Centrocytes with a higher affinity to self-antigens or with a lower affinity to immunized antigen undergo selective apoptosis,[12,13] and those with a higher affinity to the antigen further differentiate into antibody-producing cells or memory B cells.[14] In the differentiation process, an Ig class-switch occurs in germinal center B cells, mainly in centrocytes.[13] Germinal centers persist for about 3 weeks following immunization, but after this, memory B blasts continue to proliferate in follicles throughout the months of T cell-dependent antibody responses.

There are two types of memory B cells, recirculating memory B cells, and nonrecirculating memory B cells at the

marginal zones of the spleen and equivalent areas of other secondary lymphoid organs.[15,16] The recirculating memory pool is not sustained for more than a few weeks in the absence of antigen. Marginal zone memory B cells do not appear to move spontaneously to follicles for periodic renewal. They will only leave the marginal zone if a fresh supply of antigen reaches them at that site. Recirculating B cells are able to respond to antigen already held on FDCs. Those memory B cells differentiate to antibody-producing cells to result in secondary antibody production.

Bcl6 in B cells is essential for germinal center formation

Chromosomal translocations involving band 3q27 are often present in B cell lymphomas. The *Bcl6* gene was identified from the breakpoint of these translocations. Bcl6 is a 92- to 98-kDa nuclear phosphoprotein that contains six Krüppel-type zinc finger motifs in the C-terminal region and the POZ/BTB domain[17,18] in the N-terminal region (Fig. 3). These zinc finger motifs have been shown to bind to specific DNA sequences in vitro. We have isolated a murine homologue of human Bcl6, and the predicted amino acid sequence of murine Bcl6 was 95% identical to that of human Bcl6, indicating that the *Bcl6* gene is well conserved between humans and mice.[19] Two noncontiguous regions of Bcl6 (the BTB/POZ domain and the middle portion) express transrepressor activity. Since the BTB/POZ domain of Bcl6 interacts with the silencing mediator of retinoid and thyroid receptor protein (SMRT),[20] and SMRT forms a repressive complex with histone deacetylase 1 (HDAC 1),[21,22] Bcl6 may repress the transcription of target genes by locally inducing a repressive (hypoacetylated) chromatin structure through mechanisms involving SMRT recruitment and histone deacetylation.

Expression of the *Bcl6* gene is ubiquitously detected in adult mouse tissue, including lymphatic organs, and is induced in activated lymphocytes as an immediate early gene.[19] Furthermore, the expression is predominant in germinal center B cells[23,24] (see Fig. 2). However, Bcl6 expression was not detected in PALS-associated foci[9] and antibody-producing cells.[7,8] There are two distinct types of B cell in germinal centers; centroblasts and centrocytes. Several unique molecular events, such as somatic hypermutation of the Ig gene, Ig isotype class-switching, selective cell death, and differentiation into memory B cells, occur separately in each type of germinal center B cell. Bcl6 expression is continuously upregulated in both types of germinal center B cell, suggesting that Bcl6 is required not for each specific molecular event in germinal center B cells, but for more fundamental events throughout germinal center reactions.

In order to examine the functions of Bcl6 in germinal center cells, Bcl6-deficient ($Bcl6^{-/-}$) mice were developed.[9,25,26] $Bcl6^{-/-}$ mice display growth retardation and an inflammatory response in multiple organs that was characterized by the infiltration of eosinophils.[27] Since the increased expression of Th2-type cytokines, including IL-5, is induced in T cells from $Bcl6^{-/-}$ mice by anti-CD3 activation, the mechanisms of this eosinophilic inflammation could be partly explained by the functional dominance of Th2 in $Bcl6^{-/-}$ mice.[25,26] The functions of Bcl6 in germinal center cells were investigated in RAG1-deficient ($RAG1^{-/-}$) mice reconstituted with bone marrow (BM) cells from $Bcl6^{-/-}$ mice ($Bcl6^{-/-}$RM).[9] Flow cytometric analysis of BM cells, thymocytes, and splenocytes from $Bcl6^{-/-}$RM mice 3 months after transplantation revealed no abnormality in early lymphocyte development, indicating that Bcl6 is not essential for lymphogenesis in primary lymphoid tissues. When $Bcl6^{-/-}$RM mice were immunized with TD antigens, the $Bcl6^{-/-}$RM mice produced the primary IgM and IgG antibodies at levels comparable to those of $Bcl6^{+/+}$RM mice. However, none of $Bcl6^{-/-}$RM spleens showed PNA-binding B cells in follicles, although primary B cell follicles, marginal zones, PALS, and PALS-associated foci were histologically identified. Furthermore, germinal center formation was detected in the spleen from mice reconstituted with B cells from $Bcl6^{+/+}$RM mice and T cells from $Bcl6^{-/-}$RM mice. In contrast, co-transfer of B cells from $Bcl6^{-/-}$RM

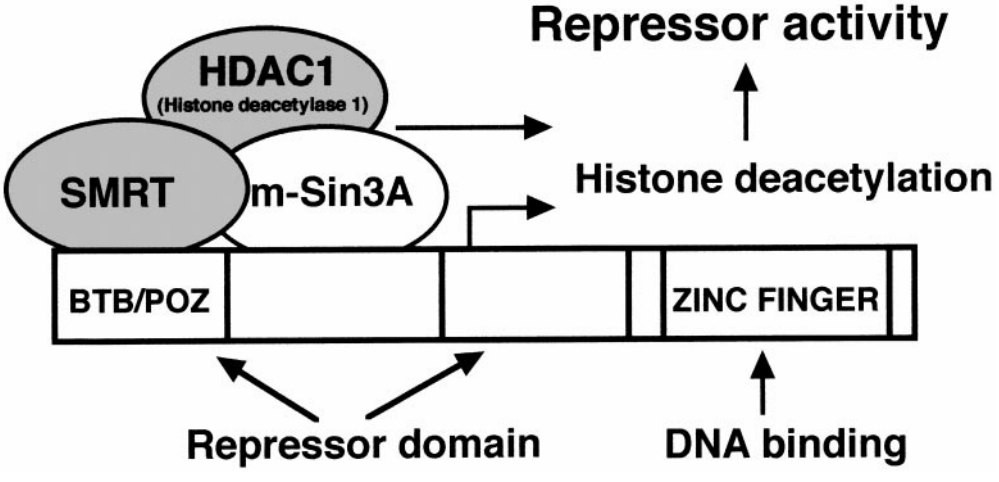

Fig. 3. Structure of Bcl6 protein. The BTB/POZ domain and the middle portion recruit a SMRT/mSin3A/HDAC complex to express transrepressor activity by histone deacetylation

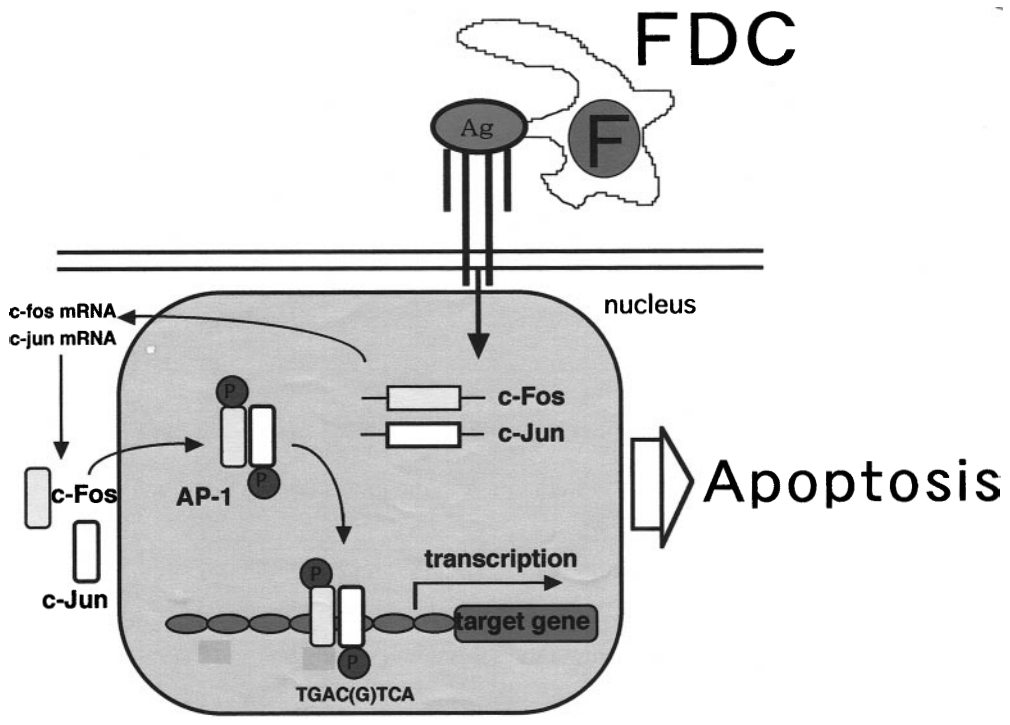

Fig. 4. A possible mechanism of self-tolerance in germinal center B cells. After c-Fos is strongly induced in germinal center B cells reacting with self-antigens, apoptosis is induced in the germinal center B cells

mice and T cells from Bcl6$^{+/+}$RM mice did not restore germinal center formation, indicating that Bcl6 expression in B cells but not in T cells is essential for germinal center formation (see Fig. 1). Additional work is required to elucidate specific target genes for Bcl6 to develop germinal center B cells.

Overexpression of c-Fos in B cells perturbs germinal center formation

c-Fos in a complex with products of another proto-oncogene c-jun (AP-1) regulates the expression of AP-1 binding genes at the transcriptional level. c-Fos is transiently induced in numerous cell types by many agents and conditions (Fig. 4).[28] Thus, a function of c-Fos may be implicated in the transduction of signals induced by growth and differentiation factors. When c-Fos transgenic (H2-c-fos) mice were generated[29] and immunized with TD antigens, the mice could produce primary IgM but not IgG antibodies, and failed to generate memory B cells.[30] The perturbation of IgG production in H2-c-fos mice was due to apoptosis in Ig class-switching B cells.[29] Although primary B cell follicles, marginal zones, and PALS were histologically identified in the spleen of H2-c-fos mice, the development of germinal center B cells was very poor in spleen from H2-c-fos mice immunized with TD antigens.[10]

Apoptosis is a physiological type of cell death, and some apoptosis in B cells can be rescued by overexpression of Bcl-2.[31] H2-c-fos mice were mated with Ig-bcl-2 transgenic mice.[32] F1 progeny were immunized with TD antigens, and germinal center formation was histologically analyzed in the spleen. PNA-binding germinal center B cells were detected in the spleen of Ig-bcl-2 mice, and the number of PNA-binding B cells in each germinal center was larger than that from control mice. Although germinal center formation was detected slightly in the spleen of H2-c-fos mice, PNA-binding germinal center B cells were clearly identified in the spleen of doubly transgenic mice, indicating that the exogenous Bcl-2 can rescue the perturbation of germinal center formation in H2-c-fos mice. All F1 progeny produced the primary IgM antibody at a comparable level. However, the amounts of IgG antibody in doubly transgenic mice were still very low. These results suggest that the exogenous Bcl-2 can delay the time of cell death in B cells during differentiation in germinal centers, but cannot rescue perturbation of mature B cell differentiation into IgG producing cells in H2-c-fos mice.

In B cell development at the germinal center stage, deletion by apoptosis occurs at the transition from centroblasts to centrocytes. Centrocytes with a higher affinity to self-antigens undergo selective apoptosis.[12,13] This apoptosis in germinal center B cells is insensitive to Bcl-2.[33] c-Fos-induced apoptosis in Ig class-switching B cells may also be insensitive to Bcl-2, since overexpression of Bcl-2 cannot rescue the production of IgG antibodies in doubly transgenic mice at control levels. Therefore, the deregulation of c-Fos may augment signal transduction to induce apoptosis in germinal center B cells, and the c-Fos-induced apoptosis may mimic the deletion of self-reactive B cells in germinal centers (see Fig. 4). This notion is supported by the evidence that this selective apoptosis in germinal center B cells requires prolonged Ig cross-linking.[34] Ig cross-linking induces transient expression of c-Fos in mature B cells,[35] and the prolonged Ig cross-linking may induce prolonged

overexpression of c-Fos. In that case, c-Fos may play a causal role in the deletion of germinal center B cells with Ig receptors for self-antigens.

Conclusion

Mature B cells activated with antigens and helper T cells in PALS of the spleen migrate into follicles to form germinal centers, and further differentiate to memory B cells. Two transcriptional factors (Bcl6 and c-Fos) play a critical role in germinal center formation. Bcl6 in B cells is essential for germinal center formation (see Fig. 1). c-Fos may play a causal role in the clonal deletion of germinal center B cells (see Fig. 4). Thus, the products of the genes regulated by those factors are important for memory B cell development.

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