

## Invited Review

# Memory B cells and CD27

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**Summary.** Following antigen activation in germinal centers, B cells develop into memory B cells or plasma cells. Triggering via B-cell immunoglobulin receptors by antigens, cytokines and direct cell-to-cell contact by B and T cells plays an important role in the B cell differentiation into memory or plasma cells. Adult human peripheral blood B cells are separated into three subtypes by the expression of IgD and CD27, which belong to the tumor necrosis factor receptor (TNFR) family: IgD<sup>+</sup> CD27<sup>-</sup> naive B cells, IgD<sup>+</sup> CD27<sup>+</sup> and IgD<sup>-</sup> CD27<sup>+</sup> B cells. CD27<sup>+</sup> B cells are larger cells with abundant cytoplasm carrying somatic hypermutation, and have an ability to produce immunoglobulin, indicating that CD27 is a memory marker of B cells. The ligation of CD27 yields crucial signals that positively control the entry of B cells into the pathway to plasma cells. We review observations on subpopulations and differentiation of mature B-cells by T/B cell interaction via CD27/CD70 as compared with CD40/CD154 interaction, and discuss about memory B cells.

**Key words:** Memory B cells, CD27, CD70, Plasma cells

### Introduction

Accumulated research and clinical data over the past several years have demonstrated convincingly that T-B cell interactions in germinal centers (GCs) or the periarteriolar lymphoid sheath (PALS) play key roles in somatic hypermutation, B cell activation, proliferation and differentiation into memory B cells or plasma cells (Kelso, 1995). The differentiation of precursors along the pathway of B-cell development has been well characterized in bone marrow: stem cells differentiate into pro-B cells, large pre-B cells, small pre-B cells and immature B cells (Ghia et al., 1998). The pre-B cells contain  $\mu$  in the cytoplasm and immature B cells express IgM receptor on the surface. When the IgD receptor is

expressed on the surface, immature B cells become mature B cells. The mature B cells become activated in the T cell zones of PALS and then migrate into B cell zones to form germinal centers.

To produce antibodies, the differentiation of B cells into specific antibody-secreting cells (plasma cells) is required. Triggering via B cell immunoglobulin receptors by antigens, cytokines such as IL-2, IL-6 and IL-10, and direct cell-to-cell contact between T and B cells plays an important role in the differentiation of mature B cells. However, the mechanism of differentiation toward memory B cells or plasma cells from mature B cells has been unclear until now. Recently, we have found that CD27 on B cells is memory marker of B cells and CD27<sup>+</sup> B cells differentiate toward plasma cells by contact with CD27 ligand (CD70) transfectants in cooperation with stimuli such as IL-10 (Agematsu et al., 1997, 1998a,b). Here, we discuss the function of CD27 molecule in B cells as a memory marker.

### TNFR/TNF family in B cell function

With regard to the T-cell help by direct T/B cell interaction, the members of a new superfamily, tumor necrosis factor receptor (TNFR) superfamily (Mallet and Barclay, 1991), such as TNFR-I, TNFR-II, nerve growth factor receptor (NGFR), CD27, CD30, CD40, CD95 (Fas), CD134 (OX-40), CD137 (4-1BB), play an important role in the activation, proliferation, differentiation and cell death of B cells.

As for the effects of NGF on immune systems, it has recently been demonstrated that NGF is constitutively produced by B cells and maintains viability of cells with the surface phenotype of memory B cells, indicating that NGF is an autocrine survival factor for memory B lymphocytes (Torcia et al., 1996).

CD40/CD40 ligand (CD154) interaction is important for the B cell proliferation and immunoglobulin production. However, recent reports demonstrated that the CD40-mediated signal induced B cell proliferation and differentiation into memory B cells, but suppressed their capacity to differentiate along the plasma cell pathway (Arpin et al., 1995; Silvy et al., 1996),

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### Memory B cell and CD27

**Table 1.** Characteristics of peripheral blood B cells separated by the expression of IgD and CD27.

	IgD+ CD27-	IgD+ CD27+	IgD- CD27+
Percentages in adult B cells	60%	10%	30%
Percentages in cord blood B cells	>95%	<5%	<5%
Morphology	small scant cytoplasm	large abundant cytoplasm	large abundant cytoplasm
Immunoglobulin production			
SAC+IL-2 stimulation			
IgA	-	-	+++
IgM	-	+++	+++
IgG	-	++	+++
IL-4+anti-CD40 stimulation			
IgE	++	ND	+++
Somatic hypermutation	-	+	+

indicating that CD40 signaling pathway is instrumental for the clonal expansion of memory B cell pool, but does not operate in the late of the response.

The OX40-OX40 ligand pair promotes B-cell proliferation and differentiation, and increases immunoglobulin secretion in mice (Stuber et al., 1995; Stuber and Strober, 1996). OX40 ligand cross-linking results in the down-regulation of the transcription factor B cell-specific activator protein (BSAP). They also showed that blocking of OX40-OX40 ligand interaction in vivo results in a profound decrease of the anti-hapten IgG response and inhibition of the development of the periarteriolar lymphoid sheath-associated B cell foci, indicating that OX40-OX40 ligand interaction in vivo is necessary for the differentiation of activated B cells into Ig-producing cells. However, in our experiments in human system, OX40-transfectants did not enhance IgA, IgM and IgG secretion in the presence of stimuli (unpublished data).

#### CD27 and immunoglobulin production

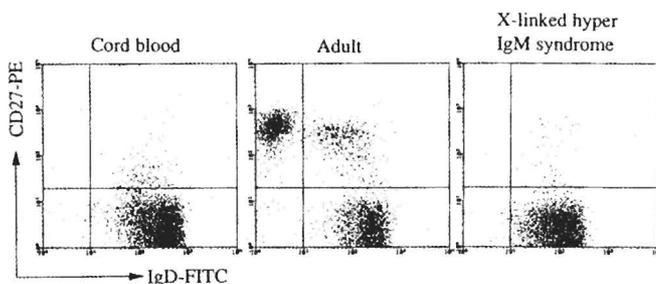
Recently, we have demonstrated that the interaction between CD27 and CD27 ligand (CD70), which is expressed not only on activated B cells but also on T cells, especially activated CD4+ CD45RO T cells (Agematsu et al., 1995a), can enhance immunoglobulin production by B cells (Agematsu et al., 1995b; Kobata et

al., 1995). CD27/CD70 interaction can induce the production of IgA, IgG, IgM, IgE and IgG subclass (Nagumo and Agematsu, 1998; Nagumo et al., 1998). Adult peripheral blood B cells are separated into at least two subtypes: IgD+ CD27- B cells and IgD- CD27+ B cells. CD27+ B cells produce large amounts of immunoglobulins in the presence of stimuli, but CD27- B cells do not (Maurer et al., 1992). The immunoglobulin synthesis from CD27+ B cells is greatly enhanced by the contact with its ligand, CD70 (Nagumo and Agematsu, 1998; Nagumo et al., 1998). CD27+ B cells are furthermore separated into IgD+ and IgD- cells (Fig. 1). IgD- CD27+ B cells produce IgG, IgM and IgA, whereas IgD+ CD27+ B cells predominantly produce IgM (Table 1) (Agematsu et al., 1997).

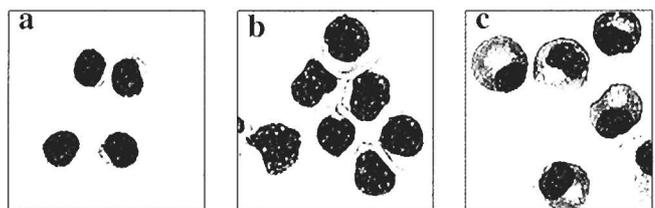
#### CD27 as marker of memory B cells.

CD27+ B cells are significantly larger than CD27- B cells and have abundant cytoplasm (Fig. 2). In addition, CD27+ B cells produce IgA, IgM and IgG by SAC+ IL-2, and IgE by IL-4 + CD40 signaling, whereas CD27- B cells do not produce IgA, IgM and IgG by SAC+ IL-2 (Table 1). Cord blood B cells do not express CD27 and CD27 expression on B cells increases with age (Fig. 1). On the basis of these findings we advanced that CD27+ B cells are memory ones and CD27- B cells are naive (Agematsu et al., 1997).

Antigen-specific differentiation of naive to memory



**Fig. 1.** B cell subpopulations separated by IgD and CD27. Three color analysis was carried out by gating CD20-PerCP positive B cells.



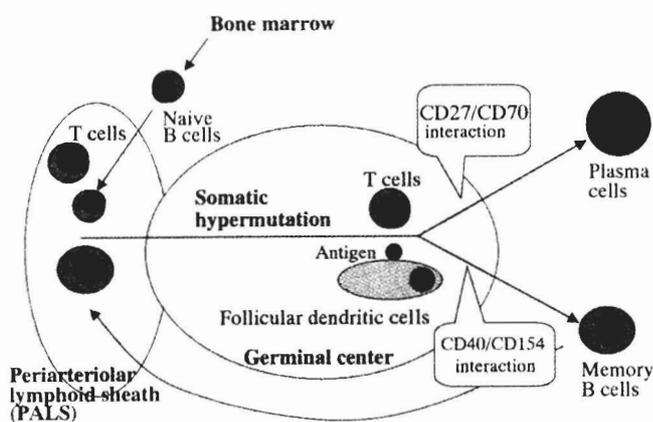
**Fig. 2.** B cell subpopulations and plasma cells. **a.** CD27- naive B cells. **b.** CD27+ memory B cells. **c.** plasma cells induced by CD70 transfectants in the presence of IL-10.

B cells is generally believed to occur within the germinal center, in which activated naive B cells undergo vigorous proliferation and somatic hypermutation of immunoglobulin variable (V) region genes. Therefore, naive B cells are expressing V region genes without somatic hypermutations, whereas memory B cells carry mutated V genes. Recently, it has been demonstrated that human B cells expressing CD27 in peripheral blood (Klein et al., 1998) and spleen (Tangye et al., 1998) carry somatically mutated V region genes. In lymphoid tissue, memory B cells are believed to reside in marginal zone. Immunohistology by using anti-CD27 mAb revealed that marginal zone B cells in human spleen (Tangye et al., 1998) and human tonsil (our unpublished data) were positive for CD27. These findings indicate that CD27 cell surface antigen represents a general marker of memory B cells.

X-linked hyper-IgM syndrome (XHIM) has been shown to result from mutations in the CD40 ligand (CD154) gene, resulting in impaired CD40/CD154 interactions and germinal center formation. We demonstrated that IgD- CD27+ B cell population was absent in patients with XHIM (Agematsu et al., 1998a). Since CD40/CD154 interactions may promote the differentiation into memory B cells in germinal centers (Arpin et al., 1995), these findings also support a view that CD27+ B cells are memory cells.

### Generation of plasma cells by CD27 signaling

Plasma cells are finally differentiated cells of the B-cell lineage, but the exact pathway and regulation of their differentiation have not been clarified in detail until now. The striking function of CD27 in B cells is its great promotion to plasma cells (Fig. 2). We demonstrated that the CD27 signal resulted in the terminal differentiation of peripheral blood memory B cells into plasma cells in



**Fig. 3.** B cell development in lymphoid tissue. B cells become activated in the T-cell zones (called periarteriolar lymphoid sheath) and migrate into B-cell zone to form germinal center. After germinal center B cells undergo somatic hypermutation and pick up antigen from follicular dendritic cells, the B cells are directed toward memory B cells by CD40 ligand (CD154) or toward plasma cells by CD27 ligand (CD70).

B cell activation systems using IL-10, augmented by the addition of IL-2. In contrast, the CD40 signal increased the number of B cells, but not of plasma cells (Jacquot et al., 1997; Agematsu et al., 1998b; Nagumo et al., 1998b). Thus, CD27 is crucial in controlling the differentiation of memory or activated B cells into plasma cells and the expression of CD27 on memory B cells is important for prompt differentiation into plasma cells.

### Concluding comments

In conclusion, by virtue of their morphology, increased expression with age, immunoglobulin production, localization within the marginal zone, the presence of mutations in Ig V region genes, and their enhanced ability to differentiate to plasma cells, CD27+ B cells are memory B cells.

In the immune response, activated helper T cells not only secrete cytokines but also express molecules on the surface, such as CD154 and CD70. The cell-to-cell interaction between T and B cells via two signalings, CD40/CD154 and CD27/CD70, strictly regulates the B cell activation, proliferation, differentiation and cell death. CD40/CD154 interaction acts on an early phase of B cell activation and induces the expansion of a memory B cell, and then the memory B cells differentiate into plasma cells via CD27/CD70 (Fig. 3).

We finally comment on the difference in B cell response to CD27/CD70 interaction between human and mice. Our findings presented here should be testified in the murine system including CD27<sup>-/-</sup> mutant mice (Gravestien et al., 1996) and blocking antibody injection, whereas effect of CD27/CD70 interaction on B cell immunoglobulin synthesis between the murine and human systems are somewhat different (J. Borst and T. Kobata, personal communication). In human, since memory B cells and naive B cells can be clearly separated by CD27 surface expression, CD27 will become a powerful tool for analyzing the immune system and diseases such as immunodeficiency, autoimmune diseases and allergy.

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