

B Cell Diversification and Differentiation in the Periphery

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Humoral responses to thymus-dependent antigens begin in the T cell zones of secondary lymphoid tissues (1–5) where antigen-activated T and B lymphocytes make physical contact (6). Though both swift and brief (4, 7), this encounter initiates a complex program of B cell differentiation leading to specialization for early antibody production or immune memory. In the spleen, B cells initially proliferate in the T cell-rich periarteriolar lymphoid sheath (PALS) and then either develop locally into foci of antibody-secreting cells or migrate to the nearby lymphoid follicle to initiate germinal center (GC) formation (3–5).

In the follicle, these antigen-specific B cell immigrants interact with follicular dendritic cells (FDC) that are specialized for the retention and presentation of unprocessed antigen complexed with antibody (8, 9). This interaction results in further rounds of B cell proliferation within the FDC reticulum and leads to the generation of a GC (3, 4). GCs are finely structured in some species, containing a dark zone of rapidly proliferating centroblasts proximal to the PALS and more distal basal and apical light zones containing nondividing centrocytes (4). The light zones also contain the densest region of the FDC network and the few (5–10% of GC cells) T lymphocytes found in GCs (10) (Fig. 1). Interestingly, recent work (11, 12) has suggested that these T cells are antigen-specific and selectively recruited into the GC.

GCs are necessary for the generation of the B cell memory compartment (13) and are the site of Ig V-region hypermutation required for the affinity maturation of serum antibody (14–17). Typically, V-region mutations appear at about day 7 of the primary response, coincident with the end of the initial proliferative phase of the GC reaction and at the formation of the light and dark zones. Mutations are introduced in a stepwise manner for at least 14 d and the kinds and distribution of Ig mutations suggest that phenotypic selection also occurs within the GC microenvironment (14, 16–18). This selection generally favors higher affinity mutants and is believed to be driven by competition for antigen displayed on the FDC surface. GC B cells that survive repeated rounds of mutation and selection enter the memory cell compartment and dominate later responses (18).

The signals that mediate these processes of proliferation, migration, mutation, and differentiation are not known in detail; however, several recent studies have provided a glimpse of the molecular basis for these events. Genetic defects (19) or the early administration of antibodies (6) or fusion proteins (20) that prevent cognate (CD40:CD40L) or costimula-

tory (B7:CD28/CTLA-4) interactions between B and T lymphocytes block the GC reaction. It remains unclear if these agents inhibit the initial T-B interaction in the PALS or if they act after the GC has been formed.

In this issue of *The Journal of Experimental Medicine*, Virginia Pascual and her colleagues in Dardilly and Dallas demonstrate that multiparameter flow cytometry may be used to identify five developmental compartments within populations of human tonsillar lymphocytes (21). This tissue is rich in GCs and provides the basis for much of our knowledge about this remarkable microenvironment. Unfortunately, GCs are constitutively present in the tonsil, making it difficult to discern the temporal order of developmental events (22). Pascual et al. have neatly resolved this issue by convincingly defining a developmental series that includes, naive, GC, and memory B lymphocytes. Naive B cells are defined as small dense lymphocytes expressing high levels of surface IgD and

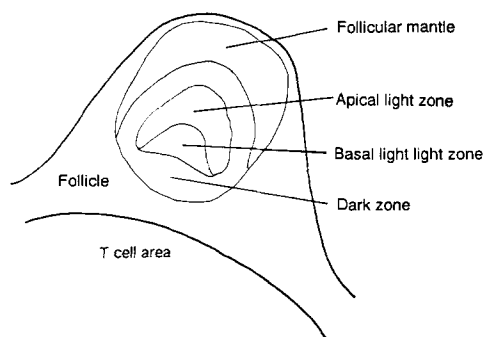


Figure 1. A generalized GC (after reference 10) illustrating the cellular compartments isolated by Pascual et al. (21). After activation through contact-dependent mechanisms in the T cell areas, e.g., the PALS of spleen, antigen-specific T and B lymphocytes migrate into the lymphoid follicle. There, B cells begin to proliferate within the FDC reticulum forming the nascent GC. After a period of intense proliferation, the dark and light zones are formed and Ig hypermutation commences. sIgD⁺ B lymphocytes (Bm1 and Bm2 cell fractions) enter the GC and there become sIgD⁻ and acquire CD38. CD38⁺ cells may be identified as dark zone centroblasts (CD77⁺; Bm3) or light zone centrocytes (CD77⁻; Bm4). It is believed that V-region mutations are acquired by the proliferating centroblasts whereas mutant centrocytes are selected for their ability to bind antigen displayed by the FDC within the apical light zone; GC B cells express very low levels of the antiapoptotic protein Bcl-2 and die in the absence of rescuing signals mediated by slg and the CD40 molecule. Recirculating, memory B lymphocytes (Bm5) lose CD38 expression and regain cytoplasmic Bcl-2. These cells are found in the follicular mantle.

IgM. These cells contain abundant Bcl-2 protein (23) in their cytoplasm, and are CD44⁺ and CD77⁻. This compartment is divided by the expression of CD23 (defined by the authors as the Bm2 fraction of cells) or its absence (Bm1 cell fraction). The CD38⁺ GC B cells lose surface IgD and cytoplasmic Bcl-2 but gain expression of the Ki67 antigen, a marker of cellular proliferation. CD38⁺ cells may be identified as centroblasts (CD77⁺; Bm3) or centrocytes (CD77⁻; Bm4). Memory B cells (Bm5) lack IgD, CD38, and the Ki67 marker but regain CD44 expression and cytoplasmic Bcl-2. Molec-

ular genetic analysis of the Bm1–Bm5 fractions indicate that mutation is absent before the Bm3 compartment, consistent with studies of murine responses, and is maintained as selected substitutions in the Bm5 memory cells. Thus, it should now be possible to isolate from tonsil the important cell compartments along the pathway to B cell memory. This approach has proven very successful for studies of B cell development in the bone marrow (24) and will permit investigation of the signals, molecules, and genes that control lymphocyte development and selection within the GC microenvironment.

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