
Preface

In 1884 Walther Flemming described histologically defined sites, within the follicles of secondary lymphoid organs, where large cells undergoing mitosis were present. Flemming called these sites germinal centers (GCs), in accordance with his proposal of these sites functioning as the places where cells originate in the body. Although the name of these sites remained, Flemming's hypothesis has since been disproven. Currently it is accepted that GCs are formed during immune responses and are the sites of B cell clonal expansion in T cell-dependent antibody responses to infection. In the GC, B cells undergo processes of DNA recombination, mutation, and differentiation by which long-lived high affinity antibody secreting cells (plasma cells) and memory B cells develop, assuring long-term protection against the infectious agents.

GCs are dynamic structures formed within B cell follicles and are composed of a diverse set of specialized cells. These include primarily highly proliferative GC B cells; follicular dendritic cells (FDCs), which provide migration and survival cues to GC B cells, and that retain antigen in their surfaces, important for affinity maturation under antigen-limiting conditions; GC T cells composed mainly of CD4^{pos} T follicular helper T cells (Tfh), key for the formation of GCs and for processes of GC B cell selection; and a smaller subset of CD4^{pos} Foxp3^{pos} regulatory T cells (Tfr), thought to limit the extent of the GC reaction. GC cellular populations also include tingible body macrophages (TBM) that phagocyte and eliminate dying B cells arising in the process of GC B cell selection, as well as other less well-characterized cellular subsets including CD8^{pos} T cells and bone marrow-derived dendritic cells for which a function remains to be determined.

Initial studies on GCs relied on tissue light microscopy, and despite the rudimentary technology available, key concepts on GC biology were established early on leading to highly active areas of research at the present time. An example of such is the observation of GC compartmentalization in two morphologically distinct areas: a dark zone (DZ) adjacent to the T cell zone and a light zone (LZ) contiguous to the splenic marginal zone or to the lymph node capsule. The lighter and darker appearances of the GC LZ and DZ resulted from the fact that B cells in the LZ are scattered among a network of FDCs and that these are largely absent from the DZ. GC T cells as well as FDCs localize in the LZ, where B cell selection takes place; TBM on the other hand are scattered among both areas.

The advent of cellular analysis by fluorescence-activated cell sorting, intravital microscopy, and gene expression profiling allows today the study GC biological processes to a high level of sophistication. Some of the initial concepts have since been solidified while others having to be reanalyzed. Initial studies using pulse labeling with radioactive nucleotides demonstrated the migration of cells in the DZ to the LZ, suggesting a precursor-progeny relationship between the two GC areas. Recently, studies using intravital microscopy showed a more complex migratory pattern of GC B cells, where bidirectional migration takes place, in accordance with the model of cyclic re-entry in which stepwise antibody affinity maturation takes place through multiple rounds of selection.

In vitro culture systems can mimic some of the features of GC B cell biology, including high proliferative index and class-switch recombination. Still studies in living organisms, and particularly in the mouse, have been crucial for an in-depth understanding of GCs. In addition to the use of wild-type animals, the field has been prolific in the generation

of genetically modified mice that allow the analysis of processes such as GC B cell selection, and affinity maturation. GC B cells arise from antigen-activated naïve B cells in a T cell-dependent manner through the course of an immune response. Therefore animals displaying impaired early development of these populations are improper for the study of GC biology. The generation of tools allowing targeted genetic mutational studies specifically to GC B cells, using primarily the Cre-Loxp system, has provided unique experimental approaches to tackle the relevance of genes, noncoding RNAs, and pathways for this stage of B cell development.

Animal model systems have also been used to address the concept of oncogenicity of the GC reaction. In the GC microenvironment B cells undergo processes that involve DNA recombination and mutation while rapidly dividing, namely class-switching and somatic hypermutation. These are essential for the development of high affinity antibodies; however infidelity in them may lead to oncogenic DNA lesions and be at the origin of cancer. In fact, and although human cancers may arise from B cells at several stages of development, cancers derived from GC B cells or from B cells that have passed the GC reaction are the most common hematological malignancy in adults overall.

GCs are key for antibody immune responses, vaccination, and are strongly implicated in cancer. GC biological processes involve cell-to-cell interaction; cellular activation, division, death, migration, selection, and differentiation; as well as DNA damage and repair. It is thus not surprising that GCs are a highly active research field and that its community includes scientist from the most diverse backgrounds. This MiMB volume provides key methods and protocols from those laboratories with the expectation of stimulating further research and to aid scientists in the study of GC biology and pathology.

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