Immunity
Previews

Germinal-Center B Cells in the Zone

Robert Brink[1,*]
[1] Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst NSW 2010, Australia
*Correspondence: r.brink@garvan.org.au
DOI 10.1016/j.immuni.2007.05.002

Visualization of lymphocyte dynamics in vivo has only recently become feasible. In this issue of *Immunity*, Hauser et al. (2007) gaze into the germinal-center reaction and find B cells making some unexpected moves.

Germinal centers (GCs) were initially named by Walther Flemming in 1885 according to his theory that these structures were the sites of de novo lymphopoiesis. It was eventually determined that the intense areas of cellular proliferation observed by Flemming were in fact responses or reactions of lymphocytes to the entry of foreign antigen into the body (reviewed by Nieuwenhuis and Opstelten [1984]). We now know GCs to be the major site at which antigen-specific B cells undergo somatic hypermutation (SHM) of their immunoglobulin genes and selection for mutant clones that have acquired increased affinity for antigen (affinity maturation). These high-affinity cells form the progenitors of both the long-lived antibody-secreting cells and the memory B cells that sustain serological immunity after infection.

Observations made with a range of analytical techniques—including electron microscopy, immunohistology, and flow cytometry—have led to the synthesis of a model of GC function that has widespread acceptance (MacLennan, 1994). GCs form as early as 5 days after initial exposure of the immune system to T dependent antigen via the migration of activated antigen-specific B cells and T follicular helper (T_{FH}) cells into the B cell-rich follicles of peripheral lymphoid tissues such as lymph nodes, tonsils, and spleen. Antigen trapped in the form of immune complexes is localized on the follicular dendritic cells (FDCs) resident within the follicle (Figure 1A) and, together with T_{FH} cells, drives rapid B cell proliferation.

As the GC matures, it resolves into two distinct areas termed the light zone (LZ) and dark zone (DZ). The LZ is located distal to the T cell-rich area adjacent to the primary follicle and is where FDCs and the majority of T_{FH} cells are ultimately located (Figure 1A). The B cells of the LZ are termed centrocytes and are thought to be nonmitotic. The DZ contains a high density of large proliferating B cells, known as centroblasts, that undergo immunoglobulin gene SHM (Figure 1A). The prevailing view is that this arrangement of the GC serves to compartmentalize the alternating steps required for affinity maturation of the B cell response. Thus centroblasts undergo cell-division-dependent SHM in the DZ and then migrate to the LZ to become centrocytes and "test" their new surface-immunoglobulin antigen receptors against the antigen displayed on FDCs. Centrocytes with improved antigen affinity preferentially interact with antigen and are therefore selected via the provision of T_{FH}-cell-derived help to migrate back to the DZ and undergo further proliferation and SHM (Figure 1A) (MacLennan, 1994). This dynamic model of GC function based on alternating cell-division-dependent migration between the LZ and DZ has been termed the "cyclic-re-entry" model.

The recent development of intravital multiphoton microscopy has for the first time allowed visualization of immune cells as they move through living lymphoid tissues (Cahalan et al., 2002). In this issue, Hauser et al. (2007) employ this powerful technique to directly test whether the movements of B cells in active GCs conform to those predicted by the cyclic-re-entry model. The approach taken by Hauser et al. (2007) allowed simultaneous visualization of antigen-specific GC B cells, naive resting B cells, and FDCs in lymph nodes from immunized mice. In this way, the GC itself was defined by the paucity of resting B cells, and the LZ and DZ areas of the GC by the presence and absence of FDCs, respectively. Through a combination of genetic and physical labeling techniques, responding antigen-specific B cells were visualized via green fluorescence, naive resting B cells by blue, and FDCs by red (Figure 1A).

A striking initial finding was the high motility and irregular, constantly changing shape of antigen-specific B cells within the GC. Motile GC B cells continually projected and retracted cellular extensions as they moved, often assuming very polarized shapes. Importantly, neither the morphology nor the motility of GC B cells varied between the LZ and the DZ. This picture of GC B cell morphology contrasts with the rounded images of these cells in tissue sections, a striking endorsement of the ability of intravital-microscopic analysis to minimize artifacts arising from tissue processing.

How then did the movement of cells correlate with the predictions of the cyclic-re-entry model? Surprisingly, the majority of GC B cell movement was found to be within the LZ or the DZ rather than migrations between the two. Thus in one hour of recording, which at this point is the technological limit of this approach, only 5% of the tracked GC B cells crossed from the LZ to the DZ or vice versa. According to a detailed mathematical model provided by the authors, this degree of movement is not sufficient to sustain a cyclic-re-entry model in which centroblasts divide once in the DZ and return to the LZ. In short, Hauser et al. (2007) found GC B cells to have both a morphology and an

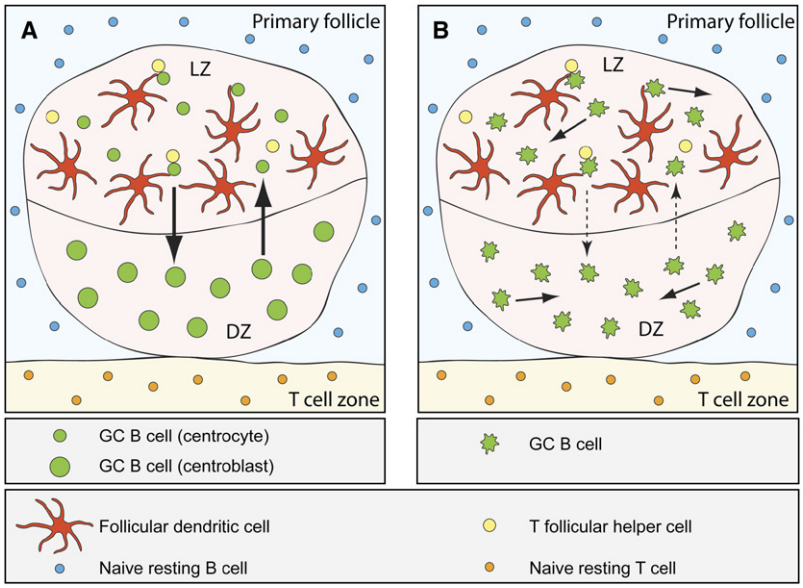

Figure 1. Conventional and revised views of germinal center B cell morphology and migration

Germinal centers (GCs) form within the primary B cell follicle and consist of a light zone (LZ) and a dark zone (DZ), which are distal and proximal to the T cell zone, respectively. Follicular dendritic cells (FDCs) and T follicular helper cells (T_{FH}) localize within the LZ.

(A) The conventional view of the GC is that LZ B cells (centrocytes) are nonproliferating and relatively small, whereas DZ B cells (centroblasts) are large, dividing cells. According to the cyclic-re-entry model, there is frequent passage of B cells between the LZ and DZ.

(B) The results of Hauser et al. (2007) and others indicate a modified picture of the GC in which both LZ and DZ B cells have an irregular and constantly changing morphology, migrate extensively within either the LZ or DZ, and can divide within either zone. Migration of B cells between the LZ and DZ can occur at relatively low frequencies, but can also be quite extensive (Schwickert et al., 2007).

interzonal-migration frequency that differ (Figure 1B) from the conventional GC model (Figure 1A).

Studies of GC B cell movement with similar intravital-microscopic strategies have recently been reported elsewhere (Allen et al., 2007; Schwickert et al., 2007). Comparison of the three independent studies reveals consistent findings that alter the conventional picture of GC B cell behavior. Irregular and changing morphology of GC B cells and a common morphology and motility for LZ and DZ B cells were reported in each case. These observations challenge prevailing views not only on GC B cell morphology, but also on the assignment of LZ and DZ GC B cells as centrocytes and centroblasts (Figure 1A). Thus, at least in mice, the phenotypes of GC B cells in these two areas may not be as different as was previously thought (Figure 1B). This is supported by the observations of both Hauser et al. (2007) and Allen et al. (2007) that GC B cell division does in fact occur in the LZ as well as the DZ.

A point of difference between the studies was the frequency with which GC B cells traversed between the LZ and DZ. The figure of 5% per hour obtained by Hauser et al. (2007) was similar to that obtained by Allen et al. (2007) (7% per hour) but substantially less than the 26% per hour observed by Schwickert et al. (2007). The reason for this difference is unclear but may reflect any one of a number of different response parameters. For instance, Schwickert et al. (2007) utilized carrier-primed T_{FH} cells that may supply a quantitatively or qualitatively distinct form of T cell help. Indeed, the motility of naive B cells in this model and their entrance into the GC were far greater than in the other two cases, suggesting that the immunization strategy utilized may produce particularly high gradients of the chemokines that drive GC B cell migration (Allen et al., 2004).

It appears, then, that the mechanics of the GC reaction may well vary between responses and potentially over the course of an individual response. Where does this leave the cyclic-re-entry model? Although migrations between the LZ and DZ can take place at a frequency consistent with the cyclic-re-entry model (Schwickert et al., 2007), this does not seem to apply to the response observed by Hauser et al. (2007). A simple explanation is that cyclic re-entry is not necessarily "monocyclic"—that is, cells may divide multiple times before traversing from the DZ to the LZ to encounter FDC-associated antigen. An alternative model favored by Hauser et al. (2007) is one in which migration of DZ B cells to the LZ is not required for affinity-based selection. In this case, antigen must be available in the DZ in a form capable of mediating selection. There is indirect evidence that this may be possible (Haberman and Shlomchik, 2003), but the mechanism in this case is currently unknown. To help clarify the roles of the LZ and DZ in affinity maturation, it will be of great importance to understand why the rate of migration between the LZ and DZ varies in different immune responses and how this relates to rates of proliferation, SHM, and selection.

Several other questions are raised by these studies. What is the significance of the cell division in the LZ, particularly because GC B cells undergoing DNA synthesis in this area rapidly disappear from the LZ (Allen et al., 2007; Hauser et al., 2007)? Are these cells migrating to the DZ or perhaps leaving the GC to become memory or plasma cells? What also are the actual differences between LZ and DZ GC B cells? Finally, what is the significance of the tendency of GC B cells to migrate toward the outer edge of the GC (Hauser et al., 2007)? Could this reflect a need for interactions with T cells or antigen-bearing cells bordering the GC, particularly in the DZ? Wider and increasingly sophisticated application of the intravital analysis employed by Hauser et al. (2007) appears to hold the greatest promise for providing the answers.

REFERENCES

Allen, C.D., Ansel, K.M., Low, C., Lesley, R., Tamamura, H., Fujii, N., and Cyster, J.G. (2004). Nat. Immunol. *5*, 943–952.

Allen, C.D., Okada, T., Tang, H.L., and Cyster, J.G. (2007). Science *315*, 528–531.

Cahalan, M.D., Parker, I., Wei, S.H., and Miller, M.J. (2002). Nat. Rev. Immunol. *2*, 872–880.

Haberman, A.M., and Shlomchik, M.J. (2003). Nat. Rev. Immunol. *3*, 757–764.

Hauser, A.E., Junt, T., Mempel, T.R., Sneddon, M.W., Kleinstein, S.H., Henrickson, S.E., von Andrian, U.H., Shlomchik, M.J., and Haberman, A.M. (2007). Immunity *26*, this issue, 655–667.

MacLennan, I.C. (1994). Annu. Rev. Immunol. *12*, 117–139.

Nieuwenhuis, P., and Opstelten, D. (1984). Am. J. Anat. *170*, 421–435.

Schwickert, T.A., Lindquist, R.L., Shakhar, G., Livshits, G., Skokos, D., Kosco-Vilbois, M.H., Dustin, M.L., and Nussenzweig, M.C. (2007). Nature *446*, 83–87.