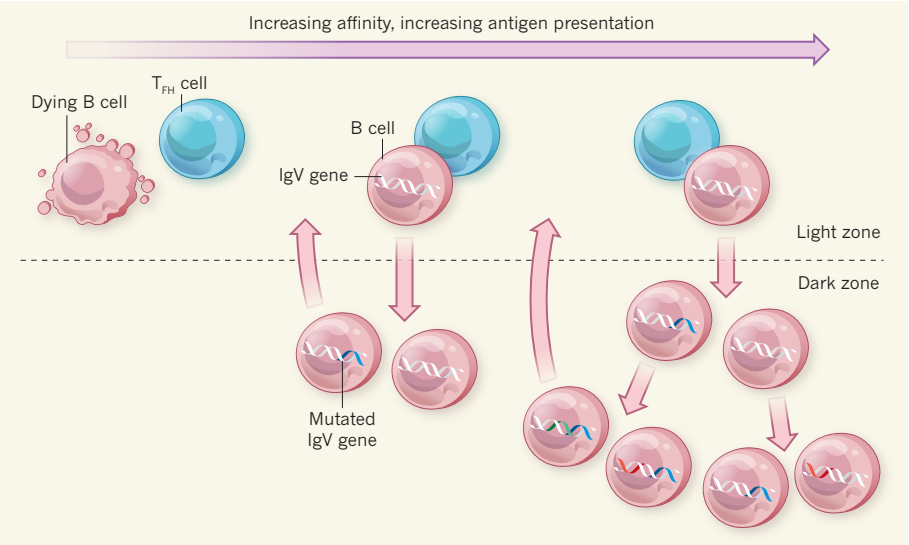

Figure 1 | Selection in the germinal centre. During an immune response to a specific antigen, B cells undergo antibody-affinity maturation in germinal centre (GC) regions of the lymphoid organs. This process involves the introduction of mutations in the B cells' IgV genes as the cells divide in the dark zone of the GC. The B cells then migrate to the GC light zone, where interactions with follicular helper T cells (T_{FH} cells) determine which cells re-enter the dark zone — B cells in which IgV-gene mutation leads to the generation of higher-affinity antibodies survive and migrate, whereas those with diminished affinity die. Gitlin *et al.*[1] show that the amount of antigen captured by a light-zone B cell and presented on its surface to T_{FH} cells determines the number of rounds of division and mutation that the cell subsequently undergoes in the dark zone, and the time taken for this, before re-migration. Because higher-affinity cells, which correlate with more mutations, capture more antigen, this results in a feed-forward mechanism, ensuring that these cells dominate the response.

from zone to zone, undergoing proliferation and mutation, and then selection, as they move between zones.

But what actually happens to a positively selected B cell in the light zone to promote its dominance? Is it subject to enhanced survival, increased movement between zones or enhanced proliferation that bypasses the cyclical routine? More pragmatically, how can affinity maturation be analysed, when it may occur at a low frequency with unpredictable timing, and when the high-affinity cells thus generated may be identifiable only retrospectively and as a population, making their behaviour around the time of selection difficult to follow?

Gitlin *et al.* address these very questions. First, they studied the effect, in mice, of delivering extra antigen to a small minority of B cells in established GCs. They observed increased proliferation of the boosted antigen-specific B cells, initially in the dark zone and to an extent proportional to the amount of antigen delivered. Although this clearly demonstrated that extra antigen enhances the proliferation of B cells in the dark zone, it did not distinguish between an acceleration of the normal cyclic migration of B cells between zones and an increased number of divisions per dark-zone B cell before migration to the light zone.

To address this issue, the authors isolated B cells from GCs and quantified the relative proportion in the 'actively dividing' or 'non-cycling' phases of the cell cycle. They found unboosted B cells in the actively dividing phases in both GC zones and observed that, once in the dark zone, the cells underwent, on average, two cell divisions. Boosting with antigen increased the proportion of actively dividing B cells in the dark zone from 60% to almost 90%, while halving the migration of cells from the dark zone to the light zone. Together, these findings show that B cells undergoing selection in the light zone can be programmed to divide in the dark zone a variable number of times on the basis of their previous interactions with T_{FH} cells; such interactions are, in turn, determined by the amount of antigen the B cell presents to the T_{FH} cells (Fig. 1). These *in vivo* observations are strikingly similar to the finding[7] that the number of divisions that B cells undergo *in vitro* is dictated by the strength of the signal passing through the CD40 pathway — one of the drivers of B-cell behaviour in GCs that is provided by T_{FH} cells.

To relate these observations to 'real-world' immune responses, Gitlin and colleagues applied their GC B-cell division tracking to cases in which antigen availability was not manipulated after the initial immunization. They found that the B cells undergoing the most proliferation had six times more affinity-enhancing mutations in their IgV genes than the least-proliferating cells. Cells undergoing the most proliferation also contained the highest number of somatic mutations. These findings confirmed the relationship between proliferation, mutation and affinity maturation.

The ultimate conclusion of this work is that affinity maturation works through a feed-forward mechanism, in which improved affinity begets a 'stronger' T_{FH}-cell signal that begets increased dark-zone B-cell proliferation and mutation, begetting further improvements in affinity, antigen acquisition and thus even more proliferation and mutation. Such a mechanism allows an ever more rapid expansion of high-affinity cells in the population, overwhelming both low-affinity and nonspecific B cells.

Is this the end of the road for GC biology? Thankfully, no. Although it provides extraordinary insight into the mechanics of B-cell dominance, Gitlin and colleagues' study leaves unexamined the means by which such favoured GC B cells extricate themselves from the GC and become circulating memory B cells and long-lived antibody-secreting cells in the bone marrow. There is evidence for stringent, affinity-based selection of GC B cells into the long-lived bone-marrow population[8], but how such selective interactions differ from those measured by the authors remains unclear.

Moreover, it has been shown[9] that interactions with T_{FH} cells mediate the differentiation of GC B cells into antibody-secreting cells, which cease affinity maturation and leave the GC to migrate to the bone marrow. So it is puzzling that when GC B cells are provided with almost unlimited amounts of T_{FH}-cell-derived signals, as in Gitlin and colleagues' study, they prefer to divide rather than differentiate. Perhaps this is an issue of kinetics, an incorrect hypothesis of what induces differentiation or an indication of nuanced types of signalling through T_{FH} cells or other, regulatory, T cells. Resolving such issues will further our understanding of how the orchestration of B-cell behaviour in GCs helps to optimize immediate and long-term immune protection. ■

David M. Tarlinton *is at the Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria 3052, Australia, and in the Department of Medical Biology, University of Melbourne, Parkville.*
e-mail: tarlinton@wehi.edu.au

1. Gitlin, A. D., Shulman, Z. & Nussenzweig, M. C. *Nature* **509,** 637–640 (2014).
2. MacLennan, I. C. *Annu. Rev. Immunol.* **12,** 117–139 (1994).
3. Muramatsu, M. *et al. Cell* **102,** 553–563 (2000).
4. Victora, G. D. & Nussenzweig, M. C. *Annu. Rev. Immunol.* **30,** 429–457 (2012).
5. Allen, C. D. C. *et al. Nature Immunol.* **5,** 943–952 (2004).
6. Crotty, S. *Annu. Rev. Immunol.* **29,** 621–663 (2011).
7. Turner, M. L., Hawkins, E. D. & Hodgkin, P. D. *J. Immunol.* **181,** 374–382 (2008).
8. Smith, K. G. C., Light, A., Nossal, G. J. V. & Tarlinton, D. M. *EMBO J.* **16,** 2996–3006 (1997).
9. Tarlinton, D. & Good-Jacobson, K. *Science* **341,** 1205–1211 (2013).

CORRECTION

In the News & Views article 'Sensory biology: Radio waves zap the biomagnetic compass' by Joseph L. Kirschvink (*Nature* **509,** 296–297; 2014), Figure 1a was wrongly credited. It should have been credited to Marianne Hanzlik.