SCIENCE & SCILIFELAB PRIZE

Stop, Go, and Evolve

Mouse genetics and intravital microscopy are combined to investigate how antibody evolution correlates with, and depends on, B lymphocytes.

Gabriel D. Victora

Pathogens evolve fast. RNA-based viruses, such as influenza and HIV, incorporate mutations in their genomes at the brisk rate of one change per 10^5 bases each time they replicate (the error rate in our own genome is one per ~10^{10} bases) (*1*). DNA-based viruses and bacteria have per–base pair rates closer to our own, but these rates still dwarf ours because of their vastly shorter generation times (*2*). How can our immune systems cope with this astounding rate of evolution? Part of the answer lies in a remarkable real-time Darwinian process that enables our defenses to adapt at breakneck speed.

Antibodies have the unique ability to bind—with high affinity and pinpoint precision—to a virtually limitless number of molecular structures. Much of this flexibility comes from the combinatorial assembly of antibody genes from hundreds of individual V, D, and J gene segments (*3*), which can potentially generate billions of different variants (*4*). Yet even this extreme variability may fail to deliver a perfect match, and antibodies produced immediately after contact with antigen are often of low potency. So how do high-affinity antibodies emerge? The answer is that, like the pathogens they fight, antibodies themselves are capable of rapid evolution.

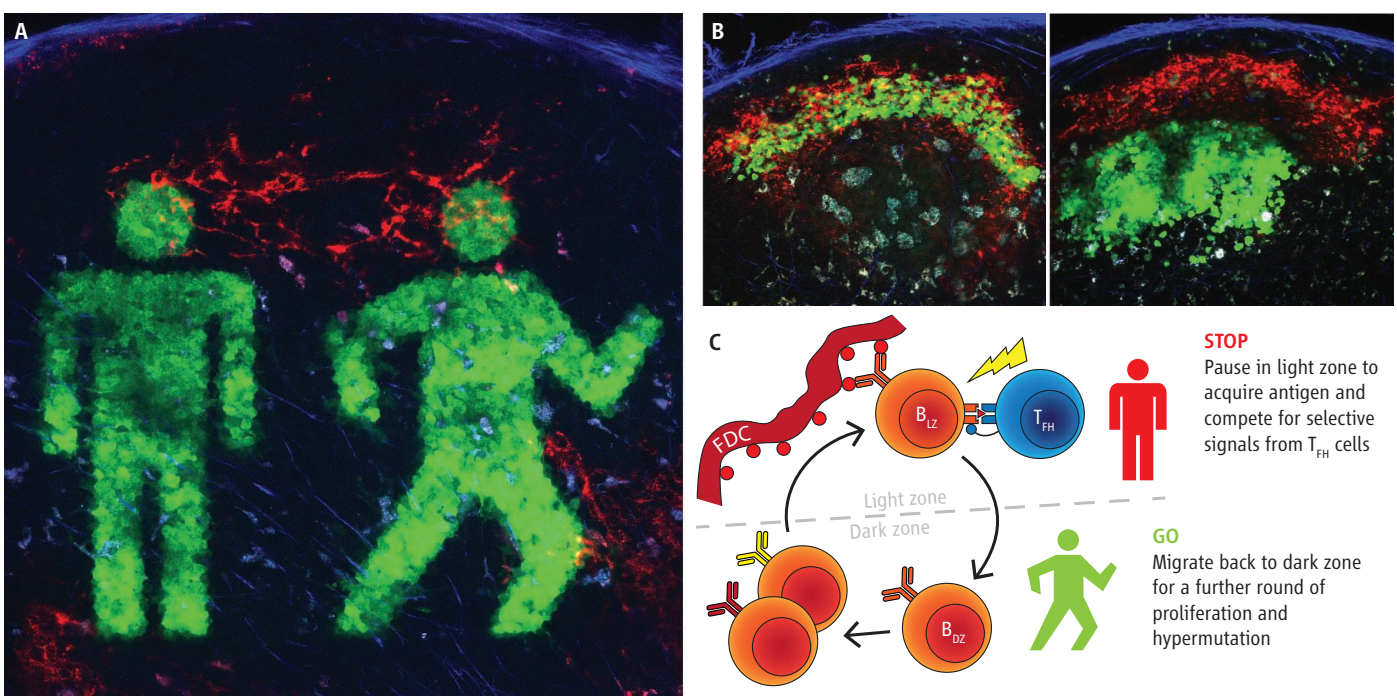
When they first meet an antigen, responsive B cells coalesce into foci of rapidly dividing cells known as germinal centers (GCs). While in these structures, B cells express the "hypermutator" enzyme, activation-induced cytidine deaminase, or AID (*5*). AID inserts small lesions into the DNA that encodes the antibody gene and thus accelerates the basal rate of DNA mutation at that locus one million-fold, to roughly 100 times that of HIV. This process is known as somatic hypermutation (SHM) (*6*, *7*).

Science and SciLifeLab are pleased to present the essay by Gabriel D. Victora, the 2013 first runner-up of the *Science* & SciLifeLab Prize for Young Scientists.

The product of SHM is a pool of B cells bearing slight differences in the antibodies they produce. As in Darwinian evolution, most mutations are either neutral or detrimental to the antibody. However, a small number of cells acquire mutations that increase their antibodies' antigen-binding abilities. The job of the GC is to act as a cellular cherry-picker, by eliminating cells with deleterious mutations while selecting the best binders for survival, proliferation, and differentiation into antibody-secreting plasma cells (*8*, *9*).

Associated with this process is a stereotypical pattern of B cell migration between the GC's two anatomical regions—the "dark zone," where B cells divide and mutate, and the "light zone," where mutant B cells encounter antigen and helper T cells, the effectors of selection. As a joint Ph.D. student in Michel Nussenzweig's laboratory at

Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, MA 02142, USA. E-mail: victora@wi.mit.edu



(**A**) Two-photon micrograph showing photoactivation of an explanted popliteal lymph node from a PA-GFP–transgenic mouse, illustrating the precision of in situ photoactivation. Green, photoactivated cells; red, follicular dendritic cells; blue, collagen. (**B**) As in (A), but showing labeling of germinal center light and dark zones (left and right, respectively). Adapted from (*10*). (**C**) Cartoon illustrating the interplay between migration and selection in the germinal center. LZ, light zone; DZ, dark zone; FDC, follicular dendritic cell; T_{FH}, T follicular helper.

2013 First Runner-Up

Gabriel D. Victora for his essay in the category of Molecular and Cellular Biology is the first runner-up. Dr. Victora is a Whitehead Fellow at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts, where he heads the Laboratory of Lymphocyte Dynamics. He received his Ph.D. from New York University School of Medicine for work done jointly at this institution and at the Rockefeller University. His work combines mouse genetics with intravital microscopy to study the development of high-affinity antibodies in the germinal center. He is a recipient of the 2011 Weintraub Award for Graduate Research, the 2012 March of Dimes Foundation Basil O'Connor Scholar Award, and the 2012 National Institutes of Health director's Early Independence Award.

For the full text of all winning essays and further information, see the *Science* site at http://scim.ag/SciLifeLab.

Rockefeller University and Mike Dustin's lab at New York University Medical School, I investigated how this choreography translates into antibody evolution (*10*).

I began by asking how light and dark zone B cells differed from each other. Flow cytometry was initially of little use, because it required the complete dispersal of lymph node cells, which obliterates essential anatomical information. To circumvent this, we generated transgenic mice expressing a photoactivatable variant of green fluorescent protein (PA-GFP) (*11*). Cells from these mice, when illuminated at a certain wavelength, irreversibly turn on green fluorescence. By illuminating intact lymph nodes under a two-photon microscope, we could photoactivate cells in anatomical areas corresponding to either light or dark zones (see the figure, A and B), permanently highlighting these cells for subsequent identification. This technique yielded the first gene expression profiles of anatomically defined light and dark zone B cells. On the basis of these profiles, we developed a method to distinguish between light and dark zone populations by surface markers alone, a strategy that would become critical to our subsequent investigation of the cellular and molecular mechanisms of GC selection.

Photoactivation can also be performed in live mice. By marking light and dark zones within surgically exposed lymph nodes, we tracked cell migration over a period of many hours, obtaining accurate estimates of the rates of B cell migration between the two GC compartments. The result of these experiments—namely, a large net vector of migration from dark to light zones—provided the first solid experimental evidence for the cyclic reentry model, which proposes that GC B cells evolve by iterative rounds of mutation followed by selection (*12*).

Finally, with flow cytometry markers in hand, we investigated the cellular mechanisms that control B cell migration between GC zones—and, by extension, GC selection. We developed a system to trick B cells into interacting with GC-resident T follicular helper cells on demand. Forcing such interactions triggered B cells to mimic the choreography of bona fide selection—a momentary arrest in the light zone (STOP) while gathering signals from helper T cells, followed by massive migration to the dark zone (GO), accompanied by proliferation and further hypermutation (EVOLVE) (see the figure, C). These experiments placed the T follicular helper cell at the very center of the antibody evolutionary mating game.

These findings spawned a series of subsequent studies. Our ability to force B cells to engage T follicular helper cells showed that B cell competition begins even before GCs coalesce (*13*). With our collaborators, we found that the transcriptional programs of light and dark zone B cells are conserved between mice and humans and are co-opted by different types of B cell lymphomas (*14*), and we determined the crucial role of the transcription factor c-Myc in triggering GC selection downstream of T cell help (*15*). More recently, we used photoactivation to provide a first glimpse into the long-term dynamics of T follicular helper cells themselves (*16*).

Despite these efforts, our understanding of the black box that lies between SHM and affinity maturation is incomplete, and our ability to control GC selection is still in its infancy. Among the key remaining questions: What drives a B cell to exit the GC as an effector rather than persist through further cycles of mutation? An answer to this would unlock the possibility of coaxing B cells to stay in GCs long enough to accumulate the ~100 mutations (within a 300–base pair variable region) required for broad HIV neutralization (*17*, *18*). With any luck, mapping the steps of this intricate dance will help us design better vaccines against rapidly changing pathogens.

References

1. J. W. Drake, B. Charlesworth, D. Charlesworth, J. F. Crow, *Genetics* **148**, 1667 (1998).
2. J. W. Drake, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7160 (1991).
3. N. Hozumi, S. Tonegawa, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3628 (1976).
4. C. A. Janeway Jr., P. Travers, M. Walport, M. J. Shlomchik, *Immunobiology: The Immune System in Health and Disease* (Garland Science, New York, ed. 5, 2001), chap. 4, part 1; www.ncbi.nlm.nih.gov/books/NBK27140/.
5. M. Muramatsu *et al.*, *Cell* **102**, 553 (2000).
6. M. G. Weigert, I. M. Cesari, S. J. Yonkovich, M. Cohn, *Nature* **228**, 1045 (1970).
7. C. Berek, C. Milstein, *Immunol. Rev.* **96**, 23 (1987).
8. I. C. MacLennan, *Annu. Rev. Immunol.* **12**, 117 (1994).
9. G. D. Victora, M. C. Nussenzweig, *Annu. Rev. Immunol.* **30**, 429 (2012).
10. G. D. Victora *et al.*, *Cell* **143**, 592 (2010).
11. G. H. Patterson, J. Lippincott-Schwartz, *Science* **297**, 1873 (2002).
12. T. B. Kepler, A. S. Perelson, *Immunol. Today* **14**, 412 (1993).
13. T. A. Schwickert *et al.*, *J. Exp. Med.* **208**, 1243 (2011).
14. G. D. Victora *et al.*, *Blood* **120**, 2240 (2012).
15. D. Dominguez-Sola *et al.*, *Nat. Immunol.* **13**, 1083 (2012).
16. Z. Shulman *et al.*, *Science* **341**, 673 (2013).
17. J. F. Scheid *et al.*, *Science* **333**, 1633 (2011).
18. F. Klein *et al.*, *Cell* **153**, 126 (2013).

10.1126/science.1247567

CREDIT: KATHLEEN DOOHER

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Science **342** (6163), 1186.
DOI: 10.1126/science.1247567