MedicalXpress

Newly identified B-cell selection process adds to understanding of antibody diversity

June 9 2014

As elite soldiers of the body's immune response, B cells serve as a vast standing army ready to recognize and destroy invading antigens, including infections and cancer cells. To do so, each new B cell comes equipped with its own highly specialized weapon, a unique antibody protein that selectively binds to specific parts of the antigen. The key to this specialization is the antigen-binding region that tailors each B cell to a particular antigen, determining whether B cells survive boot camp and are selected for maturation and survival, or wash out and die.

Now, using high-throughput sequencing technology and computational and systems biology, investigators from Beth Israel Deaconess Medical Center (BIDMC) have discovered that B cells can be selected for survival independent of their antigen binding regions. Described online this week in the journal *Proceedings of the National Academy of Sciences* (*PNAS*), the findings add a surprising new dimension to the understanding of antibody repertoires – each individual's complement of millions of B cells—and the potential for shaping these repertoires to better fight disease.

"B cells play essential roles in vaccination, infection, autoimmunity, aging and cancer," explains senior author Ramy Arnaout, MD, DPhil, an investigator in the Department of Pathology at BIDMC and Assistant Professor of Pathology at Harvard Medical School whose work focuses on the emerging field of high-throughput multimodality immunology, also known as immunomics. "We were surprised and excited to find that B cell survival could be influenced by a non-antigen-binding region of

the antibody, specifically the 'elbow' region that connects the antigen-binding regions to the signaling domain."

Each new B cell makes its own unique antibody by mixing and matching from a set of a few hundred genes, taking one each from subsets called V, D and J. The most diverse part of an antibody is the region where the three genes come together, a stretch called the third complementarity-determining region, or CDR3.

"CDR3 is thought to be the single most important determinant of antigen binding," explains Arnaout. As a result, in understanding how the body fights infections and in developing new vaccines, immunologists have primarily focused their attention on CDR3, while considering other parts of the antibody, including the elbow region, to play secondary roles.

In their new study, Arnaout and colleagues sequenced 2.8 million VDJ-recombined heavy-chain genes from immature and mature B-cell subsets in mice. "We initially wanted to ask how selection on CDR3 changed antibody repertoires during B-cell maturation," says Arnaout. But, unexpectedly, during the course of the investigation, they found they were instead focused on the antibody's 'elbow' region."

They found that B cells for which antibodies use V genes that encode 'looser' elbows were more likely to mature, regardless of their CDR3 sequence. This effect was both distinct from, and larger than previously described maturation-associated changes in CDR3 in the mice. Furthermore, it had a unique source: Differences in the V genes were hard-coded into the genome, as opposed to the mixed-and-matched combination of V, D and J genes that typically differs from B cell to B cell.

"This discovery was a little like going to watch a concert pianist perform and being mesmerized by her fingers only to realize that music was also

coming from her elbows," says Arnaout. "It was something of a shock."

One explanation for how this "loose elbow" promotes survival relates to the bending process of the antibody. "B-cell selection and maturation depend on signaling," he explains. "Antigen binding is the signal, but for it to get to the cell it has to go through the elbow. It, therefore, makes sense that previous experiments have found that disrupting the elbow abolishes signaling without affecting antigen binding. We think a loose elbow might affect how the cell perceives binding, which then determines whether the B-cell soldiers are able to divide and form an elite antigen-fighting platoon, or turn in their weapons and retreat."

Ultimately, the authors write, "This discovery adds a surprising new dimension to the understanding of antibody repertoires and might one day help us shape them ourselves."

More information: Antibody repertoire deep sequencing reveals antigen-independent selection in maturing B cells, *PNAS*: www.pnas.org/cgi/doi/10.1073/pnas.1403278111

Provided by Beth Israel Deaconess Medical Center