Facundo Batista: watching B cells spread and grab antigens

Batista combines high resolution imaging techniques with mathematical modeling to identify the molecules and mechanisms that B cells use to recognize antigens.

Facundo Batista

Early in an immune response, B cells latch on to antigens embedded in the surface membranes of follicular dendritic cells and macrophages. Engagement with antigen spurs a flurry of activity at the point of contact on the B cell surface—a phenomenon that Facundo Batista is documenting in molecular detail.

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During his post-doctoral research, Batista discovered that B cell receptors (BCRs) must exceed an affinity threshold to trigger B cell activation (1). He also found that B cells form an organized molecular structure, known as an immunological synapse, when they encounter membrane-tethered antigen. BCRs accumulate antigens at the synapse, then extract and internalize them for subsequent presentation to T cells (2).

After Batista set up his own laboratory at the London Research Institute, he began to follow these events in real time. His studies revealed that antigen-bound B cells reorganize their actin cytoskeleton to first spread across the surface of the antigen presenting cell and then to contract their membranes, concentrating the antigen at the synapse within a ring of adhesion molecules (3). High affinity antigens induce more spreading than low affinity antigens, leading to increased antigen capture.

Using a mathematical model based on this data, Batista's group hypothesized that deactivating the spreading mechanism would prevent cells from distinguishing between high and low affinity antigens—a prediction confirmed by their later experiments with spreading-defective B cells. The group has since identified the signaling molecules and pathways that enhance B cell adhesion at the synapse and contribute to activation (4–6). They have now begun to

use multi-photon microscopy techniques to image B cell behavior in real time in living tissue.

A NOBEL INSPIRATION

How did your journey into science begin?
Growing up in Argentina, I was really into things that involved nature and biology, and wanted to make documentaries about the Antarctic and things like that. But my interests changed when I started undergraduate studies at the University of Buenos Aires. I heard two professors, Daniel Goldstein and Alberto Kornblihtt, lecture on their research in molecular biology and I was hooked.

How did you end up earning a PhD in immunology from a program in Italy?
Ever since the military started to persecute university professors and other free thinkers in the 1960s, there was always a wave of scientists leaving the country. I came of age much later of course, but I also wanted to go abroad to study, as many of my friends were doing. So I took the necessary exams and was accepted at the International Center for Genetic Engineering and Biotechnology in Trieste, Italy.

At the time, I became interested in immunology because of César Milstein—an Argentinean scientist who won the Nobel Prize for his research on monoclonal antibodies. Inspired by his work, I joined Oscar Burrone's laboratory to work on the assembly of immunoglobulin E isoforms on the B cell surface.

ATTRACTED BY AFFINITY

Why did you move to England for post-doctoral work?
After I finished my PhD, I wanted to work on B cell activation, a field in which Michael Neuberger was doing brilliant work. I got a European Molecular Biology Laboratory (EMBL) fellowship to work in his laboratory in Cambridge for five years. Michael re-

ally shaped my attitude toward research by showing me how to think about science and ask questions that were wider in scope.

What problem did you work on in Michael's laboratory?
I was trying to understand how the affinity of interaction between the BCR and antigen impacted B cell activation. We first studied this issue using soluble antigens. We found that there was a minimum affinity threshold to activate the BCR and that the cells are very good at discriminating between affinities that are in the lower range. But the capacity of B cells to discriminate between higher range affinities is not so good, which suggests there is a "ceiling" during affinity maturation.

BECOMING DYNAMIC

How did you end up working on the immunological synapse?
During my post-doc, I came across the work of Mike Dustin and others who were working on immunological synapses in T cells. Their papers made me think that B cells in lymph nodes might mainly recognize antigens on surfaces of other cells, as this could reflect a more physiological means of antigen-induced B cell activation. Michael and I showed that B cells also form synapses after antigen encounter.

What was your next step?
The studies I did with Michael were very interesting, but they were limited by their static nature. So when I set up
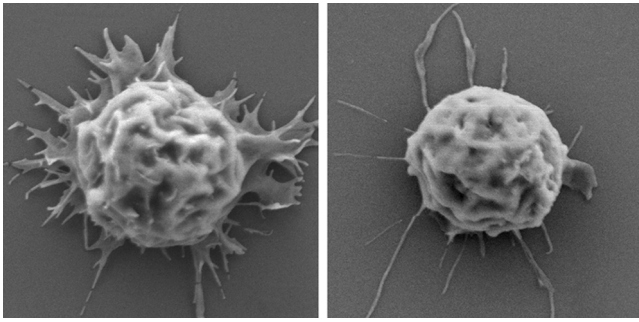
B cells that can spread (left) collect more antigens than B cells that can't (right).

my laboratory, I wanted to carry out dynamic studies of B cell antigen recognition. I called Mike Dustin, whom I'd never met, as he had developed a technique involving artificial bilayers to analyze T cell interactions. Mike kindly allowed me to learn these techniques in his laboratory. We have since modified and developed this method specifically for analyzing B cell interactions.

What's the advantage of this technique?
We can put specific ligands at any concentration onto lipid bi-layers on glass cover-slips. This gives us a unique focal plane for microscopy and allows us to be very quantitative about the information we get when cells bind to these ligands. The system allows us to study isolated receptors or the cooperation between two or more receptors or their interaction with other surface molecules. We used this system to show that B cells are better at recognizing low affinity antigens thanks to the activity of adhesion molecules such as LFA-1 on the B cell surface, which aid cell attachment and allow sampling of a greater area of the antigen-presenting surface.

How did you discover membrane spreading and contraction?
We realized that after initial antigen contact, it took ~10 min for the synapse to form. So we started to look more closely at what was happening at the surface during these early moments. My student Sebastian Fleire wanted to look at the cells interacting with antigens using electron microscopy. I thought the idea was rubbish because I didn't think

he would see anything interesting that way. But he came back with these amazing pictures; I never thought the spreading process would be so dramatic. And what was more interesting was that this process was driven by the cell's cytoskeleton. The B cell cytoskeleton has very much been ignored over the years, but not anymore.

MICROCLUSTERS AND MODELING

How does the cytoskeleton drive signaling?
The powerful combination of artificial lipid bilayers and total internal reflection microscopy showed us that cells polymerize actin and form surface microclusters of BCR and antigen as they spread. BCR engagement triggers the activation of a small GTPase called Rac2, which in turn activates the adhesion molecule LFA-1—so this is a form of inside-out signaling that increases B cell adhesion. Antigen binding also initiates the recruitment of other positive regulators such as CD19, which is critical for B cell activation in vivo. The microclusters thus form a platform for the assembly of all these molecules into what we've named "microsignalosomes".

Some molecules, such as CD19, are dynamically recruited to the BCR. Other bulkier molecules such as CD45 are excluded. This size-dependent exclusion fits the "kinetic-segregation" mechanism postulated by Anton van der Merwe and colleagues.

Are the surface changes that occur after activation different between naive cells and memory cells? And how do BCR signals compare with signals originating from cytokine receptors such as those that bind BAFF?
Those are very good questions that we plan to address in the future. It's possible that immune memory translates into cytoskeletal memory; memory cells might

recruit a different subset of signaling molecules. As for cytokine signals, we have yet to integrate them into the picture.

Are you also looking at these phenomena in vivo?
Yes. Yolanda Carrasco, one of my very talented post-docs, recently used two-photon microscopy to track antigen in vivo, and identified the precise area in the lymph nodes where B cells acquire particulate antigen. We're now trying to improve the resolution at which we can study this interaction. I've realized that it's one thing to buy a fancy microscope, but the key is to develop techniques to use it in the most effective way and push the limits of what we can use it for. I've started to do this by bringing people who are experts in microscopy and physics into my group.

How does mathematical modeling come into play in your work?
Our collaboration with Dennis Bray enabled us to appreciate the importance of mathematical modeling. The beauty of modeling is that we can answer questions about things we've yet to test biologically. We can include more parameters and get faster answers. This method pointed us toward the fact that a cell that will not be able to spread and contract will not be able to sense differences in ligand affinity.

Modeling is often criticized because people question the in vivo relevance of the data we get. For me, both approaches (theoretical and experimental) are essential and valid because we only learn more when a process that's been studied in vivo is revisited in a quantitative way, and vice versa.

"The beauty of modeling is that we can answer questions about things we've yet to test biologically."

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