Unveiling B cell receptor structure

A roadmap for understanding and controlling B cell activation

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B cells took center stage in the COVID-19 pandemic as the world waited anxiously for a vaccine. Nearly all vaccines in use today depend on activating B cells by binding to the surface-expressed B cell receptors (BCRs) that recognize antigens and stimulate antibody production. However, even recent vaccines were developed without a detailed understanding of how BCRs function and many fail to provide optimal long-lived immunity. On pages XXX and YYY of this issue , Ma *et al.* (*1*) and Su *et al*. (*2*), respectively, reveal the structures of the BCR complex, composed of membrane forms of antibodies [immunoglobulin M (IgM), IgG etc] bound to two transmembrane proteins, Igα (CD79A) and Igβ (CD79B). These structures are likely to inform the development of highly effective vaccines, therapies that eliminate cancers, and treatments that control and prevent autoimmune diseases.

Earlier studies showed that the membrane antibody of the BCR has the familiar Y shape with two Fab arms connected through hinges to one Fc leg, which in this case is linked to a transmembrane domain. Igα and Igβ chaperone the membrane antibody to the B cell surface and, crucially, initiate biochemical signaling inside the B cell when the membrane antibody binds antigen (*3*, *4*). The critical interactions holding the membrane antibody to Igα and Igβ are buried within the cell membrane (5) making the BCR resistant to structural studies by crystallography.

The BCR is a multifunctional machine responsible for most functions of a B cell. Under the appropriate conditions, antigen binding triggers B cell proliferation and differentiation into antibody-secreting cells. However, this activation requires second signals, without which BCR signaling leads to cell death. Many second signals are provided by $CD4^+$ helper T cells. To solicit T cell help, the BCR captures the antigen and delivers it to intracellular vesicles for proteolytic processing and loading onto major histocompatibility complex class II (MHC II) molecules, which are recognized by antigen-specific helper T cells. T cells also stimulate class-switching from the default IgM and IgD antibodies to IgG, IgA or IgE. All of these antibody classes form distinct BCRs on cell surfaces and fine-tune the responses to antigens (*6*). For example, IgG BCRs stimulate antibody secretion better than IgM BCRs.

Another important feature of the BCR is sensing the affinity of the antigen binding. Rather than being an on-off switch, the BCR grades the responses to advantage B cells that are better at recognizing the antigen at the expense of B cells that bind it poorly. This is the basis for antibody affinity maturation, which is mediated by the selection of B cells that have acquired somatic mutations in the antigen-binding site of their antibodies (*7*). The BCR is not even idle in the absence of antigen but provides survival signals to resting B cells (*8*). Clearly, understanding all the nuances of B cell activation is not possible without a detailed structure of the BCR.

The physical signal through which antigen binding is transmitted through the BCR to the interior of the cell has itself been an enigma. Originally, antigens were thought to simply aggregate BCRs on the B cell surface because antigens are overwhelmingly multivalent

(e.g. viral particles). BCR aggregation was an attractive mechanism because it obviated the need for conformational changes induced by antigen binding to be propagated to the cytoplasm through the highly flexible hinge region of the BCR antibody. Instead, the model proposed that aggregation of the Igα-Igβ intracellular domains leads to cross-phosphorylation of their immunoreceptor tyrosine-based activation motifs (ITAMs) by associated tyrosine kinases (*9*). However, phosphorylation was observed to spread to BCRs that were not engaged by antigen, suggesting that the process can also work the opposite way: resting BCR oligomers can be separated by antigen binding, exposing their ITAM phosphorylation sites (*10*). Furthermore, given that the BCR often needs to wrestle antigens from other cell surfaces, a mechanical tug on the BCR could induce distant conformation changes or clustering (*11*), likely in conjunction with regulation from the submembrane cytoskeleton, which helps to keep the BCR in the resting state (*12*). The new structures presented by Ma *et al.* and Su *et al.* are the first steps toward testing these models in B cell activation or pathology.

To provide a high-resolution view of the BCR, both groups purified the BCR from cells and imaged them using cryogenic electron microscopy (cryo-EM). It took more than six million single BCR particles to refine each of the final structures up to atomistic detail (both papers present structures of the IgM BCR, and Ma *et al*. also report that of the IgG1 BCR). The structures agree with each other and confirm in vivid detail what was previously only depicted in schematic cartoons. The membrane antibody retains its Y shape in which the Fab arms remain free, whereas the Fc portion binds the Igα-Igβ heterodimer with 1:1 stoichiometry (*10*). Linker peptides introduce the extracellular portions into the cell membrane, where the transmembrane helices form a tight bundle, glued by numerous polar residues interacting inside the hydrophobic environment. These intramembrane interactions are highly conserved and explain why this region is so critical for BCR assembly (5). By contrast, the cytoplasmic tails of the BCR are invisible in the reconstruction, indicating that they are highly flexible.

The structures also reveal unexpected features. They demonstrate the central importance of the linker peptides. The tightly knitted linkers are stabilized by a network of interactions, suggesting that they are essential in keeping the BCR together and in the correct orientation. One of the antibody linkers threads in between Igα and Igβ. The BCR complex thus must assemble from the individual chains while they are still folding and will be difficult to dismantle once fully formed.

Although the interactions within the membrane are conserved between the IgM and the IgG BCRs, the two BCRs differ in their extracellular domains. The IgM membrane antibody sits closer to the membrane and interacts with Igα-Igβ on its side. In contrast, the IgG is spaced further out and sits on top of the Igα-Igβ. This structural disparity may underlie some of the signaling differences between IgM and IgG BCRs. It also predicts that BCRs of other classes adopt distinct structures, which may be important for class-specific functions and therapeutic targeting. Additionally, the Fab arms of the IgM BCR show only limited flexibility compared to soluble IgM (*13*). Apparently, association with Igα alters the position of one of the Cμ3 domains in the IgM Fc which in turn stabilizes the hinge movement of the Cμ2 domains. This suggests that in the IgM BCR there is a communication between the Fab movement and the Igα-Igβ heterodimer. It will be interesting to see if such communication is involved in signal transmission after antigen binding, and how this may contrast with the IgG BCR, in which the Fab arms move freely.

Where might these pioneering BCR structures lead us? One hope is that cryo-EM can solve the structures of the antigen-engaged BCR complexes. Activated BCR structures could address the mechanisms by which antigens stimulate B cells and guide the engineering of vaccines for optimal B cell activation (*14*). Another area of interest is to prevent unwanted activation of B cells. Rational approaches to inhibit the BCR may help to quell B cells recognizing self-antigens in autoimmunity. Similarly, targeting the BCR can be beneficial in chronic lymphocytic leukemia and some types of B-cell lymphomas, where BCR signaling

drives malignant proliferation (*15*). Inhibiting intracellular signaling or marking the diseased cells for elimination will extend the range of therapeutic options in these diseases.

References and notes

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