QnAs with Michael Reth

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Michael Reth has made many landmark contributions to immunology during his career, including the discovery of the two signaling subunits (CD79a/b) of the B cell antigen receptor (BCR) and the immunoreceptor tyrosine-based activation motif. More recently, he has worked on elucidating the nanoscale organization of the plasma membrane of B cells and studying the B cell-specific membrane protein CD20, which is a target of therapeutic antibodies against B cell tumors and B cell-associated autoimmune diseases. Now a professor of molecular immunology at the University of Freiburg in Germany, Reth was elected to the National Academy of Sciences in 2018 as an International Member. In his Inaugural Article, Reth describes his findings on the role of CD20 as a gatekeeper of a receptor nanocluster on resting mature human B cells (1).

PNAS: How did you first start studying B cell membranes?

Reth: I'm a molecular immunologist by training and was originally working on the developmental program of pre-B cells before becoming interested in the structure and signaling function of the B cell antigen receptor on mature B cells. We recently started to map the location of the BCR and other receptors on the B cell plasma membrane at nanometer distances using electron and super-resolution microscopy and the proximity ligation assay.

Many people still think that receptors are moving and diffusing freely in the membrane in splendid isolation from each other, but this is an illusion of diffraction-limited light microscopy. If one studies the location and organization of receptors at nanometer distances, one comes to another conclusion. What we discovered in the 10- to 30-nanometer range on the B cell membrane is amazing. We found that the BCRs of the immunoglobulin M [IgM] and immunoglobulin D [IgD] classes are clustered separately from each other and are organized together with other proteins in different lipid environments. So, thousandfold below the resolution of a light microscope, there exists an enormous spatial and functional organization of receptors, and this is the exciting story of this new paper

Michael Reth. Image credit: Patrick Seeger/University of Freiburg.

(1). When people start to accept that the nanoworld of the plasma membrane is hardly explored, I think there will be more fantastic discoveries coming out of this.

PNAS: How did you become interested in studying CD20?

Reth: Our interest in the tetraspanning membrane protein CD20 came from the nanoscale explorer program of our Center for Biological Signaling Studies. We found that on resting B cells, CD19 and CD20 are part of the IgD-class nanocluster. Upon B cell activation, CD20 moves together with CD19 from the IgD-BCR to the IgM-BCR, where it promotes PI-3 kinase signaling. Interestingly, during this receptor rearrangement the CD20 molecule changes its functional identity from being an inhibitor, keeping CD19 under control, to an activator. Previously, the B cell-specific CD20 molecule was mostly studied as the target of the first successful therapeutic antibody, Rituximab. At present, several anti-CD20 antibodies are used in the clinic to eliminate B cells in patients with either a B cell tumor or a B cell autoimmune disease, such as rheumatoid arthritis. But, up to now, nobody knew what CD20 was doing. A while ago, I decided to shift my research from the mouse system to that of human cell lines. Living in the time of -omics—including proteomics, phosphoproteomics, surfaceomics, and metabolomics—we know now so much more about

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gene expression and function, and with the CRISPR/Cas9 method we can rapidly delete or change the genes of these cells. We also showed that we can do multiple gene knockouts. In this way, we removed all four components of the BCR from the human Burkitt B cell lymphoma line Ramos. We said, okay, let's generate CD20-deficient Ramos B cells, since this protein is a prominent pharmaceutical target and nobody knows what it's doing.

PNAS: What did you find about the role of CD20 in B cells?

Reth: The loss of CD20 resulted in an immediate rearrangement of the IgD-class nanoclusters on Ramos B cells and allowed CD19 to move to the IgM-BCR, where it starts signaling. So you don't need a cognate antigen or an antireceptor antibody to activate a human B cell; you also can achieve this by removing CD20. Luckily, we were patient enough to follow the CD20-deficient Ramos B cells over time, and we saw that they completely changed their identity and became plasma cells, which was pretty amazing. So CD20 is not only a gatekeeper of the IgD-class nanocluster but also a guardian of the mature B cell stage. We then had a closer look at Ramos B cells and normal human B cells exposed to Rituximab and found that an anti-CD20 antibody treatment induces similar changes on the B cell surface as the loss of CD20. I think our paper (1) will shed new light on the therapeutic effects of anti-CD20 antibodies, and maybe improve them, because they do not work as well in all cases.

PNAS: What follow-up experiments are you planning?

Reth: There are two directions. One is to learn more about how CD20 functions as a gatekeeper for the IgD nanocluster, and the other concerns the molecular requirements for the in vitro plasma cell development. CD20 has a short N terminus and a long C terminus, both pointing toward the cytosol, and very little is known about their functions. There must be connections to cytoskeletal elements, for example, and

maybe CD20 binds to certain lipids. Previous CD20 research was hampered by the lack of a functional assay, but now we can express CD20 mutants and test whether they compromise the gatekeeper function. We hope to identify new interaction partners of CD20 in the membrane and in the cytosol.

The other project is to identify molecules that support or prevent the in vitro B to plasma cell development. From a double-knockout analysis we already know that without CD19 or BCR, the CD20-deficient Ramos B cells do not become plasma cells. Thus BCR/CD19 signaling is required for in vitro plasma cell development. We now have the complete transcriptome and proteome of these cells and can also test for the functions of other human genes in this context.

PNAS: What are the other potential applications of this work?

Reth: A topic that we didn't foresee when we started our work with human B cell lines is the COVID-19 health crisis. For more than 20 years, I've been giving a yearly lecture on the 1918 Spanish flu and how it changed society and the scientific effort, but I never imagined experiencing a worldwide pandemic in my lifetime. When the coronavirus pandemic started, we joined the scientific mobilization against the virus. We are developing Ramos cells into a tool for a better understanding of SARS-CoV-2 biology and immunity. We expressed the spike proteins of different coronaviruses on BCR-null Ramos B cells and can now determine the anti-SARS antibody titer of an infected or vaccinated person by a spike flow assay. We are also generating Ramos B cells carrying a SARS-CoV-2 spike-specific BCR to understand how the virus is activating cognate B cells and why the humoral immune response against SARS-CoV-2 is rather short-lived. Is it possible that SARS-CoV-2 inhibits or kills cognate B cells? I think that, in the future, a more profound understanding of how viruses are interacting with the BCR and receptor nanoclusters on the B cell surface can translate to better vaccines. In closing, I want to thank my PhD and post-doc advisers Klaus Rajewsky and Frederick Alt for their continuous support of and interest in my scientific work.

1 K. Klasener et al., CD20 as a gatekeeper of the resting state of human B cells. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2021342118 (2021).