Diversifying therapeutic antibodies: From one, come many with potential different uses

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A new method for producing antibodies against disease could result in a wider variety of drugs for infectious diseases, immune disease, and even cancer.

The immune system naturally produces enormous varieties of antibodies to fight diseases. Therapeutic antibodies—antibodies created against specific therapeutic targets—have been used for decades to either rev up the immune system or quiet it down, depending on the disease. But, some do not achieve maximum potential in terms of affinity—strength of its attachment to its target—and specificity—its ability to react only with its intended target.

"In this study we found a way to harness the power of the immune system to modify therapeutic antibodies," says Frederick Alt, Ph.D., director of the Boston Children's Hospital Program in Cellular and Molecular Medicine (PCMM), whose team developed the technique described in a paper in *PNAS*.

His team has developed a "high throughput" antibody producing mouse model approach to generate antibody variants with higher affinities or modified specificities. They used the method successfully to create diversified antibodies against human PD1, which inhibits T-cells in immune responses, such as those against tumor cells.

"Relative to in vitro antibody development platforms, our in vivo approach could yield some antibodies that are more suitable for clinical applications," Alt adds.

How antibodies are made

B cells produce antibodies by bringing together three types of gene segments in a process known as V(D)J recombination. Like a roll of the dice, these variable (V), diversity (D), and joining (J) genes rearrange to create large numbers of combinations. Once assembled, these gene segments ultimately lead to the creation of a large catalog, or repertoire, of antibodies that recognize antigens from infectious pathogens and even cancer cells.

"But most of the diversity of antibodies does not come from the different Vs, Ds, and Js themselves," says Alt. Instead, it comes from enzymatic activity that changes the V(D)J junctions where the gene segments come together. "That creates millions and millions, maybe billions, of fold greater antibody diversity than the combinations of the Vs, Ds, and Js" he adds.

Diversifying antibodies

Based on these principles, the team developed a humanized mouse model approach to diversify an

anti-PD1 therapeutic antibody—a version closely related to nivolumab, the FDA-approved antibody used in cancer immunotherapy—and produce variants with new properties. First, they took apart the anti-PD1 antibody into its various V, D, and J gene segments and introduced more junctional diversity near the most variable portion of the antibody—the area of the antibody that binds to its target, or antigen.

Living mice were then immunized with the antigen to that modified antibody. The mice then made a whole repertoire of different versions of the antibody. "Out of this whole set of antibodies, many mutations arose that really changed the original sequence tremendously," says Alt. The team then analyzed the diversified antibody collection, testing them for different properties, including affinity and specificity.

More antibodies, different functions

Not only did the method produce more diverse therapeutic antibodies from the original anti-PD1 antibody, but some of these new antibodies had different properties. "We found that many of the new antibodies can do the same thing as our original antibody, which we expected," says co-author Ming Tian, Ph.D., of the Alt laboratory, "But the most interesting thing is that we found antibodies that do the exact opposite; instead of inhibiting PD1 activity, it enhanced it. That means you can put this new type of anti-PD1 antibody to some other use." For example, it could be theoretically be used to suppress unwanted T-cell activity, such as in autoimmune diseases.

"This paper serves just as the beginning because we would like to use this method for creating many different therapeutic antibodies," Alt explains. For example, his team is already using the technique to create antibodies against SARS-CoV-2 proteins to treat COVID-19.

More information: Ming Tian et al. An in vivo method for diversifying the functions of therapeutic antibodies, *Proceedings of the National Academy of Sciences* (2021). DOI: 10.1073/pnas.2025596118

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