Researchers develop new technique to tap full potential of antibody libraries

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In hopes of more fully tapping the libraries' potential, a group of Scripps Research Institute scientists, led by Scripps Research President Richard A. Lerner, M.D., has for the first time developed a new screening technique that enables antibody screening against equally massive libraries of targets. This technique makes it possible to accelerate searches for new treatments against cancer and other diseases.

The work is being reported in this week's Early Edition of the journal *Proceedings of the National Academies of Science (PNAS).*

The immune system produces antibodies to immobilize invaders, such as bacteria and viruses, by attaching to proteins referred to as antigens on those invaders. For many years, researchers have been producing huge collections of synthetic antibodies that collectively dwarf the number of antibodies humans produce naturally. These resources are a synthetic immune system with almost limitless potential, but existing techniques have only enabled screening the millions upon millions of available antibodies against handfuls of antigens.

"Many scientists have long recognized that efficient and sufficient access to the libraries demands an effective technique for also screening target antigens by the millions," said Lerner. "This work now makes that possible."

Traditional antibody research has involved developing systems in which the antibodies to be tested are incorporated into yeast cells, bacterial

viruses known as phages, or some other form of "display" for testing against a target antigen protein. Past attempts to instead screen antibody libraries against antigen libraries have been stymied by a variety of technical challenges.

A key aspect to the success of the Lerner group's technique is using yeast cells to display the antibodies for screening, while using phages for the antigens, with each display labeled by a different colored fluorescent protein.

Screen results are determined using flow cytometry, a technique that allows the researchers to examine images of the yeast cells and phage particles and manipulate them. Using the differing displays means that antibody-antigen pairs that bind can be easily identified, because they show both fluorescent dye tag colors. Bound pairs are then filtered out of the mix for identification of the antibody and antigen involved, which requires genetic sequencing.

"It took us a while to get to the right conditions," says Diana Bowley, Ph.D., a Scripps Research staff scientist and the paper's first author with Teresa Jones, a Scripps Research scientific associate, "but now that we have, it's quite easy to visualize and isolate the antibody-antigen pairs."

To prove the concept, the group focused its initial experiments on a known interaction between a specific antibody and a fragment of a protein found on the outside of HIV particles. The group worked with some 10 million antibodies, but the library was weighted to include a known antibody. The antigen library was of similar size and comparably weighted to include the known HIV antigen. The weighting guaranteed the existence of an antibody-antigen pair, which in turn allowed the group to tweak its initial concept until it could identify pairings at the expected rate.

The group was able to successfully identify the expected pairings, proving the new technique's potential to enable screening of large antibody and antigen libraries. "We're still deciding where to take it next," says Bowley. One likely direction would be to work with a broad group of cancer proteins, which should identify antibodies with potential as new cancer treatments.

Source: Scripps Research Institute