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Fishing for Biomarkers with Antigen Mimics

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Current efforts to identify antibodies that are biomarkers of disease rely on knowing the antigens they target. In many diseases, however, the relevant antigens are unknown. Reddy et al. (2010) now present an approach for discovering antibody biomarkers that avoids the need for antigen identification.

Biomarkers, objective indicators of a specific biological state, have the potential to illuminate the pathogenesis of disease and to transform its management. Biomarkers may aid in diagnosing disease, predicting disease onset, and selecting appropriate therapy. However, with a few exceptions, this promise has yet to be fulfilled (Rifai et al., 2006)—partly because, for many diseases, molecular biomarkers have yet to be identified. Antibodies are one type of molecular biomarker. Because antibodies function by binding specific antigens, attempts to identify antibody biomarkers have so far involved using antigens to capture antibodies that are overproduced in disease. The problem with this approach is that in many diseases, particularly autoimmune diseases, the antigen that triggers the immune response is unknown. In this issue of *Cell*, Reddy et al. (2010) report a new approach for the discovery of antibody biomarkers, one that requires no knowledge of the specificity of the immune response. Instead of putative antigens, the authors use an array of random synthetic molecules to pinpoint disease-associated antibodies (see Figure 1).

Identifying relevant antigens is at the heart of current approaches for discovering antibody biomarkers. Array-based approaches depend on exposing serum samples from patients to an ordered array of putative antigens, capturing those antibodies that bind antigens on the arrays, and measuring their levels (Robinson et al., 2002). Antibodies that are present at significantly higher levels in the serum of patients with the disease of interest (compared to control serum from either healthy patients or patients with an unrelated disease) are candidate biomarkers. One major drawback of these antigen arrays is that they are biased, given that antigens are selected based on the likelihood that they play a role in the disease. Antigen arrays are thus ill-suited to de novo discovery. Less biased are the high-density antigen arrays, which comprise clones of a human cDNA library expressed in bacterial or insect cells (Auger et al., 2009; Horn et al., 2006). However, because they comprise either recombinant proteins or biomolecules isolated from tissues irrelevant to the disease, neither high-density nor conventional antigen arrays recapitulate the spectrum of posttranslational modifications that can occur in humans. This omission is another major drawback because many of the antigens that play a role in autoimmune disease are molecules with posttranslational modifications that elicit disease only in their modified form (Doyle and Mamula, 2001). Unlike array-based approaches, mass-spectrometric approaches start with the extraction of biomolecules directly from diseased tissues and thus do take into account relevant posttranslational modifications. Immunoblotting with antibodies from patients' sera can then pinpoint specific disease antigens, which can be identified using mass spectrometry (Wu and Mohan, 2009). Nonetheless, mass-spectrometric approaches suffer from their own set of drawbacks; for instance, the selection of the diseased tissue to be analyzed, as well as imperfections in antigen isolation and sample preparation, introduce some bias.

Reddy et al. tackle the problem from a new angle, devising an unbiased, high-throughput approach that is predicated on posttranslational modification. They keep the array format and readout the same as that of current antigen arrays but change the content of the arrays—the antibody bait. They reason that the primary antigens (those that trigger the initial immune response) are most likely to be biomolecules that are not only modified but modified in an abnormal way, owing to a pathological process characteristic of the disease. This concept resonates with current thinking, for instance about rheumatoid arthritis, an autoimmune disease affecting the joints. A key target of the aberrant antibody response in rheumatoid arthritis is a protein that has undergone citrullination (Whiting et al., 2010), a posttranslational modification that occurs during inflammation and cell death. So rather than use unmodified biomolecules, the authors use a combinatorial library of unnatural, synthetic molecules that might by chance mimic the antibody-binding site of the primary antigen. The premise is that these synthetic molecules, termed peptoids, can form shapes that cannot be formed by unmodified biomolecules. Through mimicry, then, peptoids might be able to pinpoint antibodies that are important to the disease process and thus aid in the discovery of biomarkers.

To test their hypothesis, Reddy et al. initially use arrays of 4608 different peptoids to fish for antibodies associated

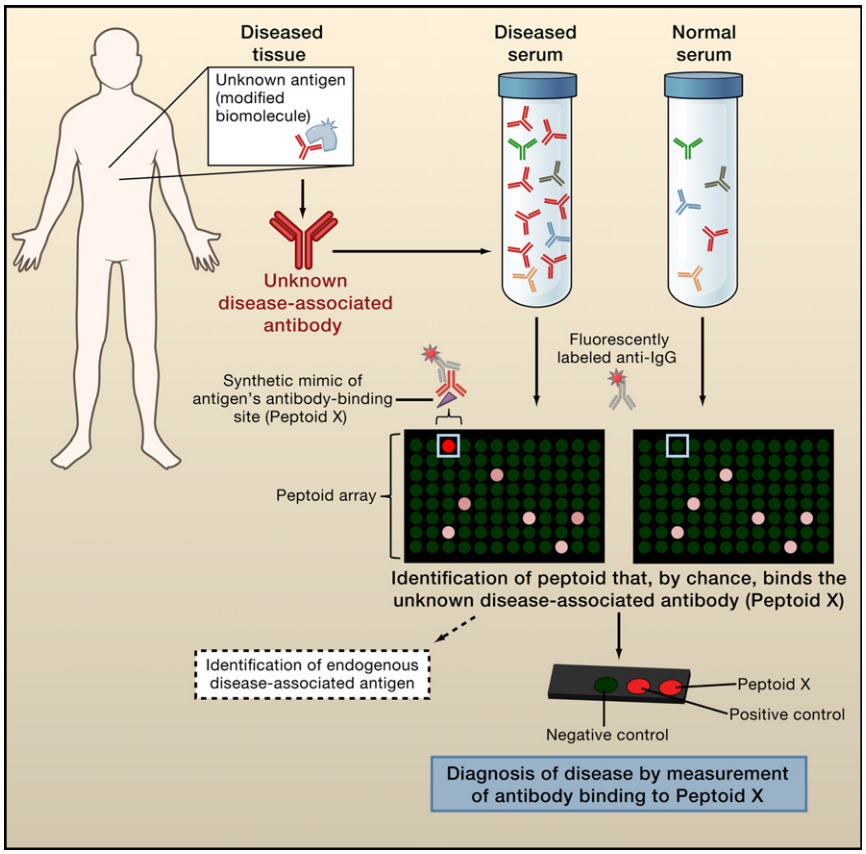

Figure 1. Using Peptoids to Discover Antibody Biomarkers of Disease

Reddy et al. (2010) describe a new approach for identifying antibody biomarkers of disease. They use arrays of synthetic molecules termed peptoids to capture antibodies from patients' serum. To measure the levels of the IgG antibodies they capture, the authors use a fluorescently labeled anti-IgG antibody. A peptoid (Peptoid X) that by chance mimics the antibody-binding site of a key disease antigen will retain much more antibody from patients' serum than from normal (nondiseased) serum. Antibody binding to Peptoid X could then serve as a marker of disease. In separate experiments, it may be possible to identify the endogenous target of the captured antibody by using the antibody to fish its antigen out of serum.

with experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (an autoimmune disease targeting myelin sheaths). They identify three peptoids (named AMogP1–3, after the Mog peptide used to induce EAE in mice) that bind much more antibody in serum from mice with EAE than in serum from healthy mice, control mice immunized with ovalbumin, or mice with systemic lupus erythematosus, another autoimmune disease. The authors go on to show that antibody binding to AMogP1–3 can differentiate between healthy mice and mice with EAE. These results define antibody binding to AMogP1–3 as a biomarker of EAE.

Although one particular antigen (the primary antigen) triggers the initial antibody response in autoimmune disease, additional antigens (secondary antigens) form as the disease progresses, leading to the production of additional antibodies. Compared to antibodies against secondary antigens, antibodies against the primary antigen are more likely to be specific to the disease and to therefore serve as biomarkers. If the antibodies that bind AMogP1–3 recognize the primary antigen in EAE, then Mog should be their endogenous target. The authors garner two pieces of evidence showing that this is the case. First, they show that antibody reactivity to AMogP1–3 arises at the same time as the reactivity to Mog itself. Second, and more importantly, serum from EAE mice (which have been immunized with Mog) no longer reacts with AMogP1–3 once it has been depleted of anti-Mog antibodies. These findings provide proof of concept that an unnatural molecule can uncover the antibody that recognizes a disease-triggering antigen.

But can such a peptoid-based approach be applied to human disease, which is much more varied than the carefully controlled disease induced in genetically identical laboratory mice? To address this question, the authors turn to Alzheimer's disease. Although not classically considered an autoimmune disease, Alzheimer's disease involves aberrations in levels of antibodies against β-amyloid (Britschgi et al., 2009) and ATP synthase (Vacirca et al., 2010). By screening serum samples against 15,000 peptoids, Reddy et al. identify three peptoids that can distinguish patients with Alzheimer's disease from age-matched healthy individuals. If further studies validate antibody binding to these peptoids as a biomarker of Alzheimer's (through evaluation in independent patient cohorts and in a larger number of samples), this new assay could greatly improve the management of Alzheimer's disease, a disease for which there is currently no objective diagnostic. Given that antibodies play not only pathogenic but also protective roles in Alzheimer's disease (Britschgi et al., 2009; Vacirca et al., 2010), searching for antibodies whose levels are abnormally low in Alzheimer's disease patients may lead to the discovery of additional biomarkers.

Although the authors' approach is compelling, key questions remain about its clinical usefulness and its ability to uncover antibodies that are meaningful in terms of pathogenesis. Are the Alzheimer's antibodies identified in this study true biomarkers of Alzheimer's disease? Do the findings in the mouse model of multiple sclerosis translate to the human disease? Do the antibodies detected in Alzheimer's disease target primary antigens, or do they target secondary antigens that might be unrelated to the pathogenesis of the disease? Knowing the identity of the relevant antigen in each disease is crucial for understanding disease pathogenesis and developing targeted therapies. Although not its primary objective, the peptoid assay could lead to the identification of endogenous targets of antibodies, if the antibodies captured in the peptoid assay are used to fish the complementary antigen out of serum.

But as Reddy et al. argue, for an antibody to be a useful biomarker, knowledge of its antigen is not necessary. In fact, antibodies of unknown specificity are already used in clinical diagnosis. For example, the cyclic citrullinated peptide test (CCP), which diagnoses rheumatoid arthritis, measures antibody binding to a collection of synthetic citrullinated peptides (Whiting et al., 2010). A big difference between the CCP test and a potential peptoid-based test, however, is this: Whereas the CCP test is the culmination of decades of research identifying citrullination as a key immunogenic process in rheumatoid arthritis, a peptoid-based test, requiring no prior knowledge of the disease at hand, could be developed in a fraction of that time. Thus, this new approach could prove to be of tremendous value in clinically managing the many immune-mediated diseases whose pathogenesis is unclear.

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