Immunoassays

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MILESTONES

Farr, RS: **A quantitative immunochemical measure of the primary interaction between ^{131}I-labelled BSA and antibody.** *Journal of Infectious Disease 1958,* 103:239-262

If you examine a list of publications having unusually high impact in a scientific field, close to the top will often be a breakthrough article on methodology. The ground-breaking report by Richard Farr[1] is an excellent example of this principle. At the time Farr's paper appeared, assays in use did not quantitate the amount of specific antibody present in a serum sample. They measured some activity that was a consequence of antibody binding to antigen. The level of that activity in a tested sample was expressed as its titer, usually the dilution beyond which the activity could no longer be detected.

Some of the consequences of antibody binding to antigen were measured *in vivo*, such as by Arthus reactions, anaphylaxis, or neutralization of a toxin or pathogen; others were measured *in vitro* with readouts such as complement fixation, precipitation, hemolysis or agglutination reactions. The specific hapten-binding capacity of an immune serum could be measured by equilibrium dialysis but that assay did not quantitate antibody.

Farr's classic paper appeared a decade before the start of the explosive growth of knowledge about antibody structure and function. It remained to be discovered that the various activities being titered were mediated by different classes and subclasses of antibodies. Immunochemists knew that some antigens scoring as weak inducers of antibody responses in one assay sometimes scored as strong inducers in a different assay and that the assays were not interchangeable. They recognized the curious properties of non-precipitating and blocking antibodies, and appreciated that most assays detected only some of the antibodies present in the sample tested. The lack of an assay that quantitated the primary interaction between antibody and antigen formed a serious gap in the tools available to immunochemists.

The Farr Assay became the first laboratory test to fill that void. The beauty of the Farr Assay resided in its ability to measure the primary interaction between antigen and antibody. It did not require the additional step of producing some functional activity such as precipitation or complement fixation.

Farr made the important discovery that at low concentrations, immune complexes could be separated from unbound antigen by differential solubility in concentrated solutions of ammonium sulfate. He observed that when bovine serum albumin (BSA) – trace labeled with radioactive iodine – was mixed at excess with diluted samples of anti-BSA antibody, the BSA:anti-BSA immune complexes – but not the unbound BSA – precipitated when the mixture was 50% saturated with ammonium sulfate. The assay provided a method to detect all the antibodies in the sample that combined with BSA to form immune complexes.

Farr's breakthrough paper reported more than a new assay. It was a tour-de-force of experiments and controls directed at understanding the interaction between antigen and antibody. Farr and his colleagues later adapted the assay to quantitate antibodies specific for ragweed antigen, Streptococcal M protein and endotoxin. Other antigens followed.

The principle of the Farr Assay – separating bound from unbound antigen – revolutionized the measurement of antibody responses. The assay detected immune complexes, the first step in antigen:antibody interactions. Farr's discovery launched the field of immunoassays, which as it grew led to numerous applications in clinical and research laboratories. Today, hundreds of immunoassays are routinely used for diagnosing diseases and monitoring responses to therapy. As the field of immunochemistry progressed, many investigators found clever new ways to detect antigen:antibody complexes. Some assays detected immune complexes using labeled antigen, some used labeled antibody, and others employed indirect methods.

Over the years, concerns about handling and disposing of radioactive materials led to the development of novel, non-radioactive readouts. Enzymatic production of color by immune complex:enzyme conjugates began to

replace the measurement of radioactivity present in antigen:antibody complexes.

The configuration of the Farr Assay made it a simple matter to measure competitive binding of labeled antigen by unlabeled antigen in a sample. Reference to standard curves allowed precise quantitation of the amount of antigen in a tested sample. The development of competitive radioimmunoassays rapidly led to their widespread use in the analysis of physiological fluids. It became possible to rapidly quantitate serum levels of drugs such as digoxin, and biologic mediators such as peptide hormones. While many derivative assays have replaced the original Farr Assay in routine laboratory testing, when comparisons are made, the Farr Assay consistently proves to be more sensitive. In a recent study, 173/178 serum samples from patients with Chagas disease were positive for Chagas-specific antibody by ELISA. By Farr Assay, 177 of the 178 samples were positive[2]. These findings have implications for the safety of blood transfusions. Studies of autoantibodies specific for double-stranded DNA and autoantibodies directed to laminin-5 confirm the superior sensitivity of the Farr Assay[3]. In laboratory testing for compounds present at extremely low concentrations, the Farr Assay still plays an important role.

Richard Farr made his breakthrough discovery fairly early in his research career. He subsequently went on to a long, productive and distinguished career as a physician-scientist at the National Jewish Hospital in Denver.

References

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