LEADING ARTICLE

dsDNA -- MEASUREMENT BY ENZYME LINKED IMMUNOSORBENT ASSAY AND CLINICAL USEFULNESS

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Antibodies reactive with double-stranded DNA(dsDNA) are a characteristic finding in patients with systemic lupus erythematosus (SLE). These antibodies are important for the diagnosis of SLE and closely correlate with clinical activity (1). They are thought to play a key role in the pathogenesis of the disease, especially when deposited as immune-complexes(2). However, the exact mechanism of tissue injury by DNA-anti-DNA complexes remains uncertain and controversial(3).

The demonstration of precipitating antibodies in SLE reactive with DNA by agar gel diffusion(4) led to a plethora of investigations to define optimal serologic assays to detect, characterize and quantitate these antibodies. Serologic evaluation of anti-DNA antibodies is complicated by the intrinsic properties of the antigen DNA and anti-dsDNA antibodies. DNA has a double helical configuration and the molecule is susceptible to formation of single stranded regions. It manifests non-immunologic interactions with serum proteins such as Clq, fibronectin and factor B which may interfere in binding tests. Antibodies against DNA are known to be heterogeneous with respect to class, complement fixing properties, specificity and avidity(5,6).

Numerous methods have been reported for the detection of anti-DNA antibodies, indicating that none is perfect and that each method has various drawbacks. The methods include counter-immunoelectrophoresis (CIE), complement fixation, indirect haemagglutination, immunofluorescence using the nuclei of Crithidia luciliae as the antigen substrate, radioimmunoassay(RIA) and enzyme immunoassays. These methods differ in the sensitivity and specificity and the ease of performance. Of these, indirect immunofluorescence and RIA are the most commonly used.

The enzyme linked immunosorbent assay (ELISA) is a simple, rapid and versatile assay used to detect antibodies directed against a variety of biological substances. These assays have the advantage of providing measurements with sensitivity in many instances comparable to a radioimmunoassay without the requirements and hazards of radioactive material. ELISA assays are specific for immunoglobulin binding and provide direct measurement of antibody capable of reacting with a specific antigen without the possible interference from non-immunoglobulin binding material.

Briefly, the ELISA assay for anti-DNA antibodies is a procedure in which dsDNA is applied to the wells of polystyrene plates that are pre-coated with poly-L-lysine or other charged molecules(7). The amount of anti-DNA antibody bound to the solid phase is measured colorimetrically after addition of an alkaline phosphatase substrate (p-nitrophenyl P04).

The advantages of the ELISA methodology are considerable. It provides a sensitive and quantitative measurement of anti-DNA antibodies from patients with SLE and can be used for diagnostic screening of patients with syndromes suggestive of SLE. Less than 5% of patients with drug induced lupus and rheumatoid arthritis were positive for anti-dsDNA(8).

The Farr assay which uses 50% saturated ammonium sulphate to precipitate DNA-anti-DNA immune complexes requires a 10 fold quantity of antibody for a three fold increase in percentage binding(9). In contrast, the ELISA assay when expressed as OD (optical density) units may have a linear relationship to the amount of antibody present.

The ELISA system may be utilised to assay a variety of properties of the antibody such as immunoglobulin class, complement fixing properties and relative avidity. Inhibition studies suggest that ELISA is a sensitive method for detecting low concentrations of serum DNA(10). The ELISA assay is capable of detecting lower avidity anti-dsDNA antibodies than RIA, haemagglutination or CIE. The antibody binding avidity is another factor which may account for differences between the ELISA test and RIA. The Farr assay tends to select for high-avidity antibodies which can retain DNA binding under high salt conditions. There is experimental evidence suggesting that a large proportion of antibodies to dsDNA in SLE sera are high-avidity, since DNA-anti-DNA immune complexes formed in vitro dissociate slowly in the presence of high salt or excess DNA(11).

Although the above advantages of the ELISA assay for anti-dsDNA have been long recognised, it has not been widely used. One important limiting factor is the difficulty in adhering dsDNA preparations to solid-phase supports. Precoating of ELISA wells with a positively charged compound using protamine sulphate contributes to non-specific immunoglobulin binding leading to false positives(7). In addition, the presence of antibodies to single stranded DNA in sera of SLE patients may be responsible for false positive or spuriously high titers of anti-dsDNA unless the conditions of the assay maintain the double-stranded structure of DNA. The antigenic determinants present on DNA immobilised on the plastic could be influenced by conditions of the assay(12). Assays performed in salts such as citrate, phosphate or borate may prevent detection of certain antibody specificities leading to inaccurate determination of quantitative levels. The specificity of the antibodies present in the conjugate could also influence the ability to detect certain antibodies. It has been observed that there are differences in the quantitative measurements of certain anti-dsDNA antisera using goat as opposed to rabbit anti-immunoglobulin conjugate(13).

Although useful as a screening and research tool, the application of ELISA to anti-DNA detection has been plagued by high backgrounds, false positives and poor reproduci-

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bility(14). Studies have shown that ELISA results correlate well with values anti-DNA antibodies obtained by the Farr assay(10). However, ELISA method may not be as sensitive or reproducible as a solid phase RIA for characterisation of certain monoclonal antibodies(15).

Various commercial ELISA kits for anti-dsDNA have been marketed for the past several years. The specificity and reproducibility of these kits may vary considerably. Although these variations occur mainly with sera that contain low levels of anti-DNA antibodies, they nevertheless may be clinically important. Evaluation of intra-assay variability and comparison of kits produced by different manufacturers has not been reported. Therefore the manufacturer's claims of accuracy cannot be accepted at face value without assessing inter-lot and intra-lot variability. It is mandatory that technical sources of error be minimised and quality control ensured.

It must be emphasised that caution be exercised in the interpretation of results of anti-dsDNA antibodies detected by similar but not identical methods because none of the current assays available is ideal. The search for new anti-DNA assays continues.

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