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Assay of Antinuclear Antibodies by ELISA Using Nuclei as Antigen

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Summary: An enzyme-linked immunosorbent assay to detect antinuclear antibodies in the sera of patients with autoimmune diseases is described. Goat liver nuclei were immobilized on polystyrene plates and antinuclear antibodies were used to standardize the assay. The effects of variables, such as the nuclei concentration, conditions of nuclei storage, and the length of the incubation period were investigated on the assay. Prototype sera with known antibody specificity were used to evaluate the assay. The method described is highly sensitive, autoantibodies being detectable at serum dilutions of 1 : 1000 or higher. According to the intra- and inter-assay coefficient of variation, the results were highly reproducible.

Introduction

Enzyme immunoassays have been successfully employed for the quantitation of human autoantibodies of defined specificities using purified antigen adsorbed on a solid matrix (reviewed in l. c. (1)). Some variations of the procedure include the use of nylon beads as the solid support (2–4) instead of a conventional polystyrene surface, and the use of a fluorogenic substrate (5) for the evaluation of enzyme activity.

In the present work, an enzyme-linked immunosorbent assay (ELISA) was evaluated as an alternative to the fluorescent antinuclear antibody (FANA) assay. Purified nuclei from goat liver were immobilized on polystyrene plates to detect antinuclear antibodies (ANA)[1]) in patients with systemic lupus erythematosus. The effects of the nuclei concentration, storage of nuclei below 0 °C, and the length of the incubation period was investigated on the assay. Sera from patients with systemic lupus erythematosus gave positive tests. In addition to the sera containing antinuclear antibodies, prototype sera with antibody specificity[1]) to nDNA, U1snRNP, Sm, nucleosomes, centromere, ANA (RNP, SS-B/La, SS-A/Ro) and Scl-70 autoantigens were used in this study. Positive results were obtained with these samples. Sera from normal individuals were used as controls.

[1]) List of antigens

nDNA	=	native DNA
U1snRNP	=	... small nuclear ribonucleoprotein
Sm	=	an antigen, named from the initials of the patient who showed corresponding antibodies; a nuclear glycoprotein
ANA	=	antinuclear antibodies
RNP	=	Ribonucleoprotein
SS-B/La	=	an antigen B, characterized by sera of certain patients with Sjögren syndrome (SS)
SS-A/Ro	=	an antigen A, characterized by sera of certain patients with Sjögren syndrome (SS)
Scl-70	=	an antigen of M_r = 70.000, identified by sera of certain patients suffering from scleroderma (Scl)

Materials and Methods

Materials

Polystyrene plates from Dynatech, USA, were used as the solid support for antigen binding. Anti-human IgG alkaline phosphatase conjugate, poly *D*-lysine, poly *D*-glutamate, calf thymus DNA and bovine serum albumin were from the Sigma Chemical Company, USA. *p*-Nitrophenyl phosphate was obtained from the C. S. I. R. Centre for Biochemicals, New Delhi. Nonidet P-40 was from BDH Chemicals, England. A Dynatech ELISA microplate reader MR 600 was used for absorption measurements.