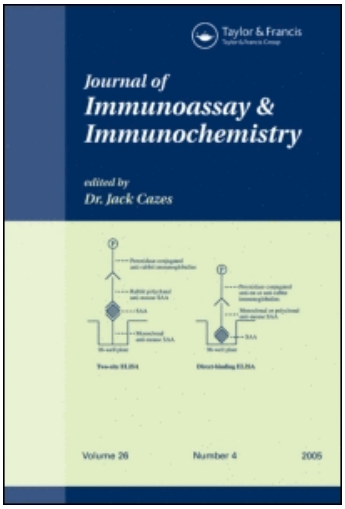

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ANA Detected by ELISA using Nucleus of Egg Cell as Antigen

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Abstract: Antinuclear antibodies, ANA, were usually detected with antigen of somatic cell nucleus. It has not been reported to detect ANA with egg cell nucleus as antigen. Enzyme linked immuosorbent assay, ELISA, coated with yolk was developed to detect ANA in our laboratory. A quality control test, cross absorption test, and cross antibody-induced test with yolk were performed. Results showed a good agreement between our method and IFA through measurement of the same samples from patients suspected of having rheumatic connective tissue diseases (Kappa = 0.668, P = 0.000). The results were not influenced by the RF and different sources of egg. CVs of inter-assay, were less than 10%. The cross absorption test was negative, as well; the ANA to somatic cell nucleus could be induced with egg cell nucleus. It is implied that there were both cross as well as overlapped Egg-ANA and Somatic-ANA. As egg nucleus, its volume was large, its purification was simple, so the better method might be established.

Keywords: ANA, Egg-ANA, Autoimmunodisease

INTRODUCTION

A humoral autoimmune response is a common manifestation of the rheumatic connective tissue diseases and often includes heterogeneous antinuclear antibodies (ANA). Heterogeneous ANAs were usually detected with a somatic antigen, such as bovine thymus, tumor cell line, liver cell of white rat, white blood cell, etc.[1–3] Immunofluorescence assay, IFA, has been generally used[4–6] This technique, although relatively simple to perform,

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has technical limitations with respect to assay sensitivity, interlaboratory standardization, and subjectivity of results.

During the past several years, enzyme-linked immunosorbent assays (ELISA), performed with antigens isolated from natural tissue sources, increasingly have been used to detect antinuclear antibodies.[7–9] However, these methods are still not completely satisfactory in terms of stability because of the difficulty of antigens purification from the nucleus. Although some studies with autoantigens produced by recombinant technology in microwell ELISA procedures have shown excellent specificity and sensitivity,[10] those recombinant autoantigens cannot represent a whole of nuclear antigens. Therefore, IFA has been the standard method for ANA, IF-ANA has also not been abandoned as the golden standard in clinical routine.[11–13] Our group has developed a new method, an ELISA involving coating with egg cell nucleus, instead of the classical somatic nucleus, to detect ANA, Egg-ANA-ELISA. It could resolve these problems.

EXPERIMENTAL

Sample

Seventy-nine sera were collected randomly from suspected patients with rheumatic connective tissue diseases, who were medical outpatients in the hospital of Dalian Medical University in China.

Egg-ANA-ELISA

Egg nuclear antigen was made by sucking out yolk with an injector and diluted 1:5 with pH 7.4 PBS. The above antigen was further diluted 1:100 with pH 9.6 CBS, then coated to ELISA plates overnight. The liquid was discarded. The product was stored at 4°C. Another test procedure was performed according to the common indirect ELISA.[14] The main parameters were as follows: dilution of sample (1:100) and enzyme-labeled staphylococcal protein A (1:40), coloring with tetramethyl benzidine (TMB), determination of absorption (A) ($\lambda = 450$ nm) Cut-off value with >2.1 (sample value/negative value). The negative control samples were mixed sera from 20 healthy subjects.

Stability of the Method

Three samples, positive, borderline positive, and negative, were selected and detected in 5 different runs (inter-assay). The CV was calculated.

The plate was coated with 5 yolks from different hens. A sample, borderline positive, was checked in a single run. The CV was also calculated.

IFA for Detection of ANA

ANA of samples, as above, were detected with common IFA.[3] The result was compared with our method. Measurement of agreement was determined by symmetric measure (Kappa). The calculations were carried out using SPSS software for Windows.

Interference Test

Thirty sera with negative ANA-IFA, including 10 RF positive and 20 RF negative sera by latex agglutination, were selected from the above samples. A_{450} were determined by ELISA described above. Significant difference A_{450} between groups was determined by Student's t-test for independent data (alpha = 0.05, two-tailed test) using SPSS software for Windows.

Cross Absorption Test

Ten samples, ANA positive with IFA, were mixed completely with yolk, respectively, then placed at 37°C for 30 minutes. The levels of ANA were determined by IFA. Significant differences between before and after absorption were determined by Wilcoxon Signed Ranks for two related samples (alpha = 0.05, two-tailed test) using SPSS software for Windows.

Cross Antibody-Induced Test

Forty mice (KM strain, 18–22 g) were used for this study. All mice were offered the same commercial diet, and tap water ad libitum, in a temperature-controlled (22°C) holding room. Half of them were injected intraperitoneally with 0.2 mL yolk (concentration of 3 g/L) per mouse, as primary immunization. One week later, the secondary immunization was performed; the immunization method and antigen quantity were identical with the primary immunization. The other half of the mice received no antigen stimulus (injected with normal saline); they served as the control. On the fourteenth day, blood samples were obtained by decapitation and sera were prepared by routine methods.

The ANA ELISA coated with the somatic nucleus kit with enzyme-labeled staphylococcal protein A instead of enzyme-labeled anti-human-IgG, purchased from Biotecx, USA, was used to detect the mice samples.

The chi-square test was performed to compare the two groups with SPSS software for Windows.

RESULTS

Stability of the Method

CVs of the inter-assay were 4.8%, 8.3%, and 8.9% for positive, borderline positive, and negative samples, respectively.

The CV of Egg-ANA-ELISA coated with yolks from different sources was 8.27% ($A_{450} = 0.145 \pm 0.012$) for a borderline positive sample.

Comparison with IFA

The result is presented in Table 1. The concordance rate was 83.5% (66/79). There was a good agreement between two methods (Kappa = 0.668, $P = 0.000$).

Interference Test

The raw data, both the RF positive and the negative group, are listed in Table 2. There was no significant difference between the two groups ($P > 0.05$).

Cross Absorption Test and Cross Antibody-Induced Test

The titres of ANA-IFA did not decrease after absorption with yolk. There was not a significant difference between before and after absorption ($P > 0.05$).

The ANA was induced with yolk in 90% of the mice. A significant difference was obtained, compared with control group ($P > 0.05$). The result is shown in Table 3.

Table 1. ANA detected with Egg-ANA-ELISA and IFA

	IFA	
Egg-ANA-ELISA	+	−
+	29	8
−	5	37

Table 2. Result of interference test with RF

Groups	n	Mean	S	p
Positive RF	10	0.1140	0.022	0.538
Negative RF	20	0.1095	0.010	—

Table 3. Result of ANA-induced with yolk in mice

Groups	n	Positive case	Positive rate (%)	p
ANA-induced	20	18	90	0.000
Control	20	0	0	—

DISCUSSION

ANA are autoantibodies which bind to cellular nuclear antigens. The ANA assay was widely used to screen autoantibodies associated with autoimmune diseases. Then, the problem of standardization impacted its diagnosis. Many kinds of somatic cell nuclei were used to detect ANA; what is more, it was more complex to purify somatic cell nuclei and, therefore, the problem of standardization was not easy to resolve.

Because ANA were able to react with many kinds of somatic cell nuclei, it was possible that ANA could react with egg nucleus. We used egg nucleus to coat the ELISA plate. The result showed that there was a good correlation between our method and IFA data. The results appeared not to be influenced by the RF and different sources of egg; CVs of inter-assay were less than 10%. These results suggested that ANA was able to react with egg nucleus and that Egg-ANA-ELISA was a better reliable method for detection of ANA.

The cross absorption test and cross antibody-induced test with yolk were performed to further observe the properties of Egg-ANA-ELISA. The result of the cross absorption was negative; also, the ANA to somatic cell nucleus could be induced with egg cell nucleus. It is implied that there were both cross and overlapping between Egg-ANA and Somatic-ANA.

The egg nucleus volume was large, its purification was simple, so a better method might be established. We believe that studies with the Egg-ANA will lead to development of a reliable method for detection of ANA.

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