抗 2 本鎖 DNA 抗体に対する紫外線処理プレートを用いた 酵素免疫測定法の再評価

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Reevaluation of enzyme-linked immunosorbent assay with ultraviolet-treated polystyrene microtiter plates for measurement of antibodies to dsDNA

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【Summary】

We reevaluated the efficacy of enzyme-linked immunosorbent assay (ELISA) with ultraviolet (UV)-treated polystyrene microtiter plates (UV-ELISA) for the detection of human serum anti-dsDNA antibodies. The subjects consisted of 38 patients with systemic lupus erythematosus (SLE). The titers of IgG serum anti-dsDNA antibodies by UV-ELISA were significantly ($p<0.01$) higher in sera from 26 active SLE patients than in those from 12 inactive SLE patients. ELISA with poly-L-lysine coated microtiter plates (PLL-ELISA) revealed the similar results. However, the background ratio was only $1.0\pm0.9\%$ by UV-ELISA, which was significantly ($p<0.01$) lower than that by PLL-ELISA ($16.8\pm10.8\%$). These results demonstrated the efficacy of ELISA with UV-treated polystyrene microtiter plates for the measurement of human serum anti-dsDNA antibodies in patients with SLE.

Key words : anti-dsDNA antibodies,
enzyme-linked immunosorbent assay,
systemic lupus erythematosus

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【概 要】

ヒト血清中抗2本鎖(ds)DNA抗体を測定するために、紫外線処理ポリスチレンマイクロタイタープレートを用いた酵素免疫測定法(ELISA: 以下, UV-ELISA法)の有用性を再評価することを目的とした。SLE 38例を対象とし、その血清を試料とした。抗dsDNA抗体測定法の対照として、ポリ-L-リジン(poly-L-lysine)でdsDNAを固相化するELISA(以下, PLL-ELISA法)を行った。Background比は、(DNA非固相化ウエルの吸光度)÷(DNA固相化ウエルの吸光度)×100(%)で求めた。UV-ELISA法によるIgG抗dsDNA抗体価は、PLL-ELISA法における成績と同様に、非活動期SLE 12例に比べ、活動期SLE 26例において有意に($p < 0.01$)高かった。しかし、Background比は、UV-ELISA法において $1.0 \pm 0.9\%$ にすぎなかった。これはPLL-ELISA法における $16.8 \pm 10.8\%$ に比べ有意に($p < 0.01$)低かった。以上の成績から、抗DNA抗体測定のためのELISAとして、UV-ELISA法は、既報のモノクローナル抗一本鎖(ss)DNA抗体の測定のみならず、ヒト血清中抗dsDNA抗体の測定にも有用であることが考えられた。

I. Introduction

One of the important serological features of patients with systemic lupus erythematosus (SLE) is the detection of anti-double stranded (ds) DNA antibodies. The presence of anti-dsDNA antibodies has been adopted as one of the revised criteria for the classification of SLE¹⁾. High titers of anti-dsDNA antibodies are associated with the disease activity²⁾. Anti-dsDNA antibodies are also related to the development of nephritis through the formation of immune complexes³⁾, and they are predictive of relapses⁴⁾. Many methods of detecting serum anti-dsDNA antibodies have been reported, and the enzyme-linked immunosorbent assay (ELISA) is widely accepted as a convenient method^{2,5-7)}. To perform ELISA, microtiter plates have been precoated to immobilize dsDNA with poly-L-lysine^{2,5)}. However, another rapid ELISA with ultraviolet (UV)-treated polystyrene microtiter plates (UV-ELISA) has been reported for the measurement of murine and human monoclonal anti-single stranded (ss) DNA antibody⁶⁾.

In this report, we reevaluate the efficacy of UV-ELISA with UV-treated polystyrene microtiter plates for the detection of human serum polyclonal anti-dsDNA antibodies, comparing it with conventional ELISA with poly-L-lysine coated microtiter plates (PLL-ELISA).

II. Patients and methods

The subjects consisted of 38 patients with SLE, according to the revised criteria for the classification of SLE¹⁾. Their clinical activity was determined by the criteria of Schur and Sandson⁸⁾. Sera from 25 healthy controls were also examined.

IgG anti-dsDNA antibodies were examined by UV-ELISA and PLL-ELISA, according to the methods described previously⁵⁻⁷⁾. Briefly, calf thymus DNA (Sigma Chemical Company, USA) was purified by a combination of S1 nuclease treatment and BNDC (benzoylated naphthoylated DEAE cellulose) column chromatography⁷⁾. Polystyrene microtiter plates (Immulon I, Dynatech Laboratories, USA) were pretreated with UV for 12 hours at room temperature in UV-ELISA. These plates were first precoated with fifty $\mu\text{g/ml}$ poly-L-lysine (Sigma Chemical Company, USA), and they were incubated for 30 minutes at room temperature in PLL-ELISA. Polystyrene microtiter plates were coated with one hundred μl of 10 $\mu\text{g/ml}$ dsDNA in both UV-ELISA and PLL-ELISA. The plates were then blocked with PBS containing 3% bovine serum albumin (BSA). Sera were diluted to 1:200, and bound antibodies to dsDNA were detected with alkaline phosphatase-labeled goat anti-human IgG. The results were expressed as absorbances at 405 nm. Absorbances for the same serum sample were read in both wells with dsDNA and wells

without dsDNA. The titers of IgG anti-dsDNA antibodies were obtained by subtracting absorbances in wells without dsDNA from those in wells with dsDNA in both UV-ELISA and PLL-ELISA. The cutoff point was determined by calculating the mean value plus three times the standard deviation in sera from healthy controls.

The background ratio was calculated as follows in sera from 26 clinically active patients :

$$\text{Background ratio} = \frac{\text{A 405 of wells without dsDNA}}{\text{A 405 of wells with dsDNA}} \times 100 (\%)$$

III. Results

The titers of IgG anti-dsDNA antibodies according to the results of both UV-ELISA and PLL-ELISA were significantly ($p < 0.01$) higher in sera

from 26 clinically active patients than in those from 12 clinically inactive patients (Figure 1). All sera from clinically active patients and three sera from clinically inactive patients were determined to be positive.

The background ratio was significantly ($p < 0.01$) lower in UV-ELISA ($1.0 \pm 0.9\%$) than in PLL-ELISA ($16.8 \pm 10.8\%$) (Figure 2). Absorbances in wells with dsDNA and wells without dsDNA gave 0.784 ± 0.518 and 0.004 ± 0.002 , respectively, in UV-ELISA. However, absorbances in wells with dsDNA and wells without dsDNA was 0.729 ± 0.469 and 0.105 ± 0.068 , respectively, in PLL-ELISA. In this case, the background ratio was studied in sera from 26 clinically active patients, as the absorbances in most of sera from clinically inactive patients were too low to be calculated.

Figure 1 Titers of IgG anti-dsDNA antibodies

A. Results of UV-treated polystyrene microtiter plates (UV-ELISA) are shown.

The titers of IgG anti-dsDNA antibodies were significantly higher in sera from 26 clinically active patients (0.780 ± 0.260) with SLE than in those from 12 clinically inactive patients (0.023 ± 0.017). The cutoff point was 0.030.

B. Results of ELISA with poly-L-lysine coated polystyrene microtiter plates (PLL-ELISA) are shown.

The titers of IgG anti-dsDNA antibodies were significantly higher in sera from 26 clinically active patients (0.625 ± 0.432) than in those from 12 clinically inactive patients (0.056 ± 0.032). The cutoff point was 0.090.

Figure 2 Background ratio in ELISA with UV-treated polystyrene microtiter plates (UV-ELISA) and ELISA with poly-L-lysine coated polystyrene microtiter plates (PLL-ELISA)

The background ratio (A 405 of wells without DNA/A 405 of wells with DNA $\times$ 100) was significantly lower in UV-ELISA than in PLL-ELISA. Sera from 26 clinically active patients with SLE were examined.

IV. Discussion

Zouali and Stollar described a rapid and convenient ELISA with UV-treated polystyrene microtiter plates for the measurement of murine and human monoclonal anti-ssDNA antibody⁶⁾. We have already reported that serum polyclonal anti-ssDNA antibodies and anti-dsDNA antibodies in SLE patients can be detected by this UV-ELISA⁷⁾.

In this study, we extended our observation. The titers of serum anti-dsDNA antibodies examined by UV-ELISA were correlated with disease activity in patients with SLE, and PLL-ELISA revealed the similar results. This finding is compatible with the results of other studies²⁻⁴⁾. However, the production of many autoantibodies with different specificities by polyclonal B cell activation is a serological feature in patients with SLE³⁾. Thus, sera from patients with SLE can show reactivity against poly-L-lysine, which leads to the high absorbances in poly-L-lysine precoated wells without DNA in conventional PLL-ELISA. We confirmed a significantly lower background in UV-ELISA. These results indicate that ELISA with UV-treated polystyrene microtiter plates (UV-ELISA) is convenient and accurate for the detection of serum anti-dsDNA antibodies in patients with SLE.

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