Dear Editor,

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease in which the immune system attacks the patient’s tissues, resulting in inflammation and damage (Tan et al., 1982). SLE is associated with the presence of abnormally elevated titers of a variety of antibodies, including antibodies to double-stranded (ds) DNA, in the serum (Egner, 2000). The presence of anti-dsDNA antibodies in abnormal titers has become the serological hallmark of the disease, and it is widely acknowledged that deposition of IgG into the tissues damage that are characteristics of SLE (Riboldi et al., 2005). Yet the perfect method for detecting anti-dsDNA remains controversial, whether regarding the antigen source or the technique used (Egner, 2000; Riboldi et al., 2005; McCloskey et al., 2010). Here we describe a simplified method for the isolation and purification of DNA associated with the plasma membrane of cultured human lymphocytes (Wil2), hereafter designated ‘pmDNA’ that is shown, by electrophoretic mobility shift assay (EMSA), to form a complex with IgG isolated from SLE sera but not with IgG isolated from healthy individuals’ sera. We also show that Wil2 pmDNA is a choice antigen for the detection of anti-dsDNA in the serum of SLE patients by an indirect immunofluorescence in vivo assay.

Wil2 lymphocytes incubated with untreated SLE patients’ sera and then with fluorescein-conjugated goat anti-human IgG exhibited a ring-like peripheral membrane fluorescence (Figure 1A, a) while no fluorescence was observed with healthy sera (Figure 1A, b). In addition to cells exhibiting a continuous distribution of the fluorescence, others exhibiting patching or capping were also seen with SLE sera (Figure 1A, c–e). This reflected a ligand-induced movement of lymphocyte membrane macromolecules (Cohen and Gilbertsen, 1975; Pavan et al., 1990), indicating that the fluorescent anti-human IgG bound to an IgG–plasma-membrane-antigen complex. Fluorescence was also observed when Wil2 cells were pre-treated with RNase (Figure 1A, f), pronase E (Figure 1A, g), or phospholipase C (Figure 1A, h), but not with DNase (Figure 1A, i), indicating that the plasma membrane antigen that bound the SLE patients’ IgG was DNA. Sera of patients with other autoimmune diseases were also submitted to the indirect immunofluorescence assay using Wil2 lymphocytes. While at dilution 1/2, fluorescence was observed with 4 of 15 Sjögren’s syndrome sera, 2 of 10 spondylarthritis sera, and 5 of 15 rheumatoid arthritis sera, no fluorescence was observed at dilution 1/40, regardless of the serum’s origin. In contrast, fluorescence was observed with SLE sera, regardless of the dilution. When all sera were tested for the presence of anti-dsDNA using a routine enzyme-linked immunosorbent assay (ELISA) with calf-thymus DNA as antigen, results showed that the indirect immunofluorescence test described here was more sensitive and more specific in detecting anti-dsDNA IgG in the sera of SLE patients than the routine ELISA. Previous investigations indicated that the use of cultured B-lymphocytes (Wil2) in an indirect immunofluorescence test allowed for the detection of specific IgG in SLE patients with a higher specificity and sensitivity than other assays (Keyhani et al., 1995), but the adequacy of the test for anti-dsDNA detection was not unequivocally shown.

IgG fractions were isolated from the sera of healthy individuals (Figure 1B, a and untreated SLE patients (Figure 1B, a, inset) by affinity chromatography on protein G-Sepharose. The second peak, corresponding to IgG (see Supplementary data), exhibited two bands (identified, respectively, as the heavy and light chains of immunoglobulins) after SDS–polyacrylamide gel electrophoresis (Figure 1B, b).

The pmDNA isolated from Wil2 plasma membrane (see Supplementary data) revealed a single band upon electrophoresis in agarose gel. The band was eluted from the gel so as to remove residual RNA (Figure 1C, a), giving a pmDNA ~17000 bp-long, free of RNA or protein contamination (Figure 1C, b): the band corresponding to the purified pmDNA was no longer detectable after pmDNA treatment with DNase (lane 2), but was unaltered after pmDNA treatment with RNase (lane 3) or pronase E (lane 4).

pmDNA mobility in agarose gel was remarkably reduced after incubation with IgG from SLE sera (Figure 1D, a and c) but remained unaffected after incubation with IgG from control sera (Figure 1D, b). The reduction in pmDNA mobility was dose dependent, with complete inhibition in the presence of 10 μg SLE IgG and various extents of inhibition, depending on the patient, at lower amounts of SLE IgG (Figure 1D, a, lane 4 vs. c, lane 3). These results indicated that a complex was formed between pmDNA and IgG isolated from SLE sera but that no complex was formed between pmDNA and control IgG, confirming the immunofluorescence test results. Formation of an IgG–pmDNA complex was also examined by co-incubating pmDNA with SLE IgG and pronase (pronase alone does not alter pmDNA migration as shown in Figure 1C, b). In the absence of pronase, an IgG–pmDNA complex was
formed as evidenced by the progressive shift in pmDNA electrophoretic mobility after incubation with increasing amounts of SLE IgG (Figure 1E, lanes 2, 4, 6); however, when pronase was added to the mixture of pmDNA and SLE IgG, formation of the IgG–pmDNA complex was prevented as a consequence of IgG digestion by pronase and pmDNA electrophoretic mobility remained unaltered (Figure 1E, lane 3) or quasi-unaltered (Figure 1E, lanes 5, 7). The EMSA results also suggest that SLE IgG affinity for Wil2 pmDNA was higher than that for mammalian DNA from other sources such as calf thymus (see Keyhani et al., 1998a, b; Supplementary data).

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References