Dear Editor,

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with complex pathogenesis which is yet to be completely understood. Since the original discovery of anti-double-stranded deoxyribonucleic acid (dsDNA) antibodies, the general result from intense research has been that native mammalian dsDNA is not immunogenic, even if presented with complete Freund’s adjuvant.\(^1,3\) This is despite an antigen-driven response as evidenced by the clonality of anti-dsDNA antibodies and patterns of random somatic mutations in both patients and murine models of SLE to suggest that DNA is the selecting antigen.\(^2,4\) These studies demonstrate that mechanisms for the production of anti-dsDNA antibodies and their affinity maturation toward anti-dsDNA specificity are operational in SLE patients.\(^4\) The question that arises is what transforms DNA to the immunogenic form in patients with SLE. Microparticles (MPs) represent a heterogeneous population of membrane-bound vesicles with a diameter of 0.1 μm to 1 μm that are released by the budding of plasma membrane and express antigens specific of their parental origin.\(^5\) The release of MPs by various cell types is a ubiquitous process that gets accelerated during cellular activation and apoptosis.\(^6\) Upon initiation, translocation of phosphatidylserine from the inner to outer surface leaflet of the plasma membrane results in loss of normal phospholipid asymmetry.\(^5\) As shown in cell lines undergoing in vitro apoptosis, DNA is sequestered into granules and then packaged into separate apoptotic bodies.\(^5\) Whereas the mechanism mediating this cellular rearrangement is not known, the end result is the repositioning of nuclear constituents in a form that may be more accessible to the immune system.\(^7\) We hypothesise that in this model, MPs may provide a framework to intensify the immunogenicity of the component DNA to induce anti-dsDNA antibody production and therefore, the aim of the present study was to evaluate the putative role of DNA within MPs (MP DNA) as the circulating antigenic target.

The study was cross-sectional in design. Fourteen unselected patients (12 women and 2 men) fulfilling at least 4 of the American College of Rheumatology 1997 revised classification criteria for SLE were included.\(^8\) Disease activity was assessed using the Safety of Estrogens in Lupus Erythematosus National Assessment (SELENA)-modified SLE Disease Activity Index (SLEDAI).\(^9\) Eight healthy female individuals were included as control subjects. Anti-dsDNA immunoglobulin G (IgG) titers, measured using indirect enzyme-linked immunosorbent assay (Bio-Rad, Hercules, CA, US), and other disease markers were performed by the clinical laboratory of National University Hospital. Circulating MPs were obtained from platelet-free plasma (PFP) obtained by successive centrifugations of venous blood. PicoGreen (Invitrogen, Waltham, MA, US), a membrane-permeable dsDNA specific dye, was used to label the MP samples. We analysed the samples on FACSCalibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, US). An initial MP-size gate was set with the help of calibrating fluorescent 0.22 μm, 0.45 μm, 0.88 μm and 1.34 μm polystyrene beads (Spherotech, Lake Forest, IL, US). MP DNA were enumerated on the SSC/FL1 plot and defined as events that were sensitive to differential detergent lysis using 0.05% Triton X-100. Therefore, each detergent-sensitive FL1-positive event on the SSC/FL1 plot was considered as 1 MP DNA. Plasma concentrations (MP DNA/μL) were calculated according to the actual flow rate of the flow cytometer, MP DNA count per unit of time and net dilution during sample preparation of the analysed sample. No distinct population of detergent-sensitive MPs on the SSC/FSC plot could be gated to measure total MP numbers. Some samples were treated with 1 U of RNAse-free DNase (Promega, Fitchburg, WI, US) for 20 min at 37°C to assess sensitivity of MP DNA to DNase I.

We identified MP DNA in PFP, based on fluorescence and detergent sensitivity (Figs. 1a and 1b). With this approach, extracellular DNA (plasma DNA) not associated with MPs were also detected in PFP. Importantly, although plasma DNA remained intact after detergent treatment, MPs containing DNA were detergent-soluble, establishing their phospholipid composition. To evaluate if MP DNA is resistant to DNase activity, MPs were treated with DNase I, counterstained with PicoGreen and then analysed using flow cytometry. There was a reduction of MP DNA count following DNase I treatment (Fig. 1c). Further treatment with 0.05% Triton X-100 led to complete solubilisation of MP DNA by detergent (Fig. 1d). Thus, DNA packaged within MPs is protected from DNase activity. The median concentration of MP DNA/μL in PFP of the 14 SLE patients was significantly higher than the 8 healthy controls\(^{[2]} 2460.28 [Q1; Q3 1010.91; 3416.26] \text{ vs } 403.73 [Q1; Q3 222.26; 1801.88] \text{ [P} = 0.020\text{]} \text{ (Fig. 2). The median age of the SLE patients was 39.5 (Q1; Q3 25.0; 54.8), with a median disease |
duration of 24.5 years (Q1; Q3 17.0; 42.5) and a median SELENA-SLEDAI score of 7.0 (Q1; Q3 5.3; 15.3). The median age of healthy controls was 33.5 years (Q1; Q3 25.0; 43.5). The concentration of MP DNA positively correlated with anti-dsDNA IgG (r [10] = 0.806, \( P = 0.005 \)) (Fig. 3) after exclusion of outliers, which may be consistent with MP DNA as an antigenic source for anti-dsDNA IgG formation. However, MP DNA did not correlate with complement levels or SELENA-SLEDAI scores.

The results from this study provide new perspectives on the presence of extracellular DNA in SLE patients and healthy controls. Using flow cytometry, MP DNA were visualised and enumerated in the PFP of SLE patients and healthy controls. In the form of MPs, DNA likely exists on both the surface and interior.\(^{10}\) This would account for the observation of DNA packaged within MPs being protected from DNase activity.\(^{11}\) Further, plasma DNA not associated with MPs were detected in both SLE patients and healthy controls. Experiments to assess the origins of plasma DNA and its sensitivity to DNase I were not performed in the current study. The most striking finding in this study is the marked difference in the concentration of MP DNA in SLE patients compared to healthy controls. Although the increased MP numbers suggest a role in SLE immunopathogenesis, elucidating this role is difficult at present. Ullal et al showed some increase in the total number of MP and increase in IgG positive MPs in SLE patients compared to healthy controls.\(^{12}\) Nielsen et al showed that MP populations that do not bind annexin V are increased whereas total number of MP and annexin V-binding MPs are decreased in SLE patients compared to healthy controls.\(^{13}\) We showed a correlation between MP DNA concentration and anti-dsDNA IgG titers. Ullal et al and Nielsen et al showed a correlation between the number of IgG-positive MPs and anti-DNA levels in SLE patients.\(^{12,14}\) However, their findings did not demonstrate that the bound IgG antibody was anti-dsDNA IgG. In this regard, IgG positive MPs can also occur in the synovial fluid of rheumatoid arthritis patients.\(^{12,15}\) Hence, to our knowledge, this is the first study to demonstrate the presence of MP DNA definitively in the
plasma of SLE patients. The correlation with anti-dsDNA IgG suggests that MP DNA may be a source of immunogenic autoantigen for the production of anti-dsDNA antibodies and our results support this idea. Further studies will refine the role of MP DNA in the pathogenesis, perpetuation and modulation of disease activity in SLE.

REFERENCES