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Interpretation of the Raji cell assay in sera containing anti-nuclear antibodies and immune complexes

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SUMMARY

The Raji cell assay is regarded as a test for the detection and quantitation of immune complexes. It is frequently positive in sera from patients with SLE. We have demonstrated a relationship between Raji cell binding and antibodies to DNA and soluble cellular antigens. In five sera containing high titres of antibodies of known single specificity, most of the Raji cell binding occurred in the 7S IgG fraction where the majority of anti-nuclear antibody was also found. When each of these sera was incubated with its specific antigen, Raji cell binding increased. Subsequent fractionation showed that this binding was in the high molecular weight fraction (> 200,000 daltons) and that Raji cell binding and antibody activity were abolished in the 7S fraction. These data confirm that Raji cells bind immune complexes but also indicate that 7S anti-nuclear antibodies may interact directly with Raji cells by an unknown mechanism. Therefore, in sera of patients with anti-nuclear antibodies, binding to Raji cells does not necessarily imply the presence of immune complexes alone.

INTRODUCTION

Raji cells are a continuous lymphoblastoid cell line derived from Burkitt's lymphoma (Pulvertaft, 1965) and possess certain B cell characteristics, such as low-avidity IgG Fc receptors and C3–C3b, C3d and C1q receptors, but are devoid of membrane-bound immunoglobulin. These receptors make the cells potentially useful in detecting complement-fixing immune complexes which may be quantitated in pathological sera by reference to a standard curve of aggregated human gammaglobulin (Theofilopoulos, Wilson & Dixon, 1976; Theofilopoulos & Dixon, 1976).

In our laboratory we have found that the Raji cell assay (RCA) is frequently positive in sera of patients with systemic lupus erythematosus (SLE) and that there is a significant correlation between anti-nuclear antibodies (ANA) and Raji cell binding. This raises the possibility that the RCA is positive either because these sera contain immune complexes (IC) or because ANA are being detected directly.

Abbreviations: AAHG = alkaline-aggregated human gammaglobulin, ALA = anti-lymphocyte antibodies, ANA = anti-nuclear antibodies, BSA = bovine serum albumin, C1qBA = C1q-binding assay, ENA = extractable nuclear antigens, HA = haemagglutination, HMW = high molecular weight, IC = immune complexes, NHS = normal human serum, PBS = phosphate-buffered saline, RA = rheumatoid arthritis, RCA = Raji cell assay, RCE = Raji cell extract, RTE = rabbit thymus extract, SLE = systemic lupus erythematosus.

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To answer this question, sera of known ANA specificity were separated into putative IC and antibody-rich 7S fractions and their binding to Raji cells compared.

MATERIALS AND METHODS

Source of sera

Sera (200) from 54 SLE patients were examined for the correlation between DNA and Raji cell binding. For the fractionation studies, six pathological sera and serum from a normal donor were selected on the basis of known ANA specificity. Each serum was tested for antibodies to dsDNA, Sm, RNP, SS-A and SS-B (Table 1).

In addition, 39 RA and 94 SLE sera with DNA antibodies less than 50 units/ml were tested in the RCA and screened for antibodies to extractable nuclear antigens (ENA) using counter immunoelectrophoresis (Venables, Erhardt & Maini, 1980). The six normal sera used in the RCA were ANA-negative. All sera were stored at $-70°C$.

Raji cell assay

Cell culture. Raji cells were maintained in continuous suspension culture in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum supplemented with glutamine, penicillin and streptomycin. Cell number was determined by haemocytometer count and viability was assessed by trypan blue exclusion.

Alkaline-aggregated human gammaglobulin (AAHG). For the purpose of a standard curve, human IgG (Cohn fraction II) in aqueous solution was aggregated by the addition of an equal volume of 0·2 N NaOH. The solution was mixed and dialysed against phosphate-buffered saline (PBS), pH 7·2, at room temperature for 6 hr. The buffer was replaced and dialysis continued overnight at room temperature. Aliquots (100 μl, 10 mg/ml) of the aggregates were stored at $-70°C$ and the protein content was determined by the method of Lowry *et al.* (1951).

Radioiodination of protein A. Staphylococcal protein A (Pharmacia; 5 mg/ml) was stored at $-70°C$ as 15-μl aliquots in 0·1 M phosphate buffer, pH 7·3. Protein A was radiolabelled with ^{125}I using the chloramine T method (McConahey & Dixon, 1966). PBS (10 μl), pH 7·2, 5 μl chloramine T (0·8 mg/ml) and 5 μl ^{125}I (equivalent to 500 μCi) were added to 10 μl protein A (50 μg).

After 1 min at room temperature, 10 μl sodium bisulphite (1 mg/ml) were added to stop the reaction. The reaction mixture was diluted with 1 ml PBS containing 5 mg bovine serum albumin (BSA). The reaction tube was washed out with a further 1 ml of PBS containing BSA to give a total of 2 ml radiolabelled protein A solution. This was dialysed overnight at 4°C in PBS, pH 7·2, and then stored at $-70°C$ in 100-μl aliquots. Each aliquot was diluted with 1 ml calcium- and magnesium-free Hanks' solution containing 0·1% BSA (Hanks/BSA) for use in the RCA.

Determination of Raji cell binding. Cells were removed from culture, counted and assessed for viability. The initial suspension was centrifuged at room temperature, supernatants aspirated and the cell pellet resuspended to a concentration of 5×10^6 cells/ml in Hanks/BSA. The cells were washed once more and the resuspended pellet (10×10^6 cells/ml) was aliquoted into U-well microtitre plates (200 μl/well) and centrifuged. Supernatants were removed by inversion and draining on absorbent tissue. All centrifugation was performed at 400 ***g*** for 10 min.

Preparation of standard aggregate curve. An aggregate standard curve was prepared by serially diluting 50 μl AAHG in 17 two-fold dilutions in Hanks/BSA. Normal human serum (NHS) diluted 1:4 in Hanks/BSA was used as a source of complement and 50 μl were added to each AAHG dilution. A baseline of radioactive uptake was established on 100 μl of a 1:8 dilution of the same serum. G-200 fractions (75 μl) were diluted with either 75 μl of a 1:4 dilution of NHS (the same complement source used in a standard curve) or 75 μl Hanks/BSA.

The AAHG standards, G-200 fractions and 1:8 dilutions of normal and test sera were incubated at 37°C for 30 min. Subsequently, 25 μl of each AAHG dilution or serum and 50 μl of G-200 fractions were incubated with 2×10^6 Raji cells per well at 37°C for 30 min with shaking every 10 min. All measurements were peformed in duplicate. The cells were washed three times at room temperature with 200 μl Hanks/BSA per well. Radiolabelled protein A (20 μl) was added to each

well and the plate was incubated at 4°C for 30 min with shaking every 10 min. The cells were washed three times at 4°C with 200 μl Hanks/BSA per well. The cell pellets were washed into tubes with 2 × 40 μl Hanks/BSA and counted for 1 min on a gamma counter.

Expression of results. By reference to the standard AAHG curve the amount of uptake of radiolabelled protein A (mean of duplicate c.p.m.) by test sera could be expressed in terms of μg equivalents of AAHG/ml serum (μgEq/ml). The results of uptake of protein A by G-200 fractions took into account the elution volumes and the volume of serum applied to the column and were expressed as μgEq/ml of neat serum. The alternative method of expression of results of sera was using a test/control ratio, i.e. mean duplicate c.p.m. test serum : geometric mean c.p.m. 6 NHS. This was necessary because Raji cell binding of normal sera was frequently below the lowest concentration of AAHG used and because results expressed in this way showed less interassay variation than results expressed in relation to the standard curve. The geometric mean of 6 NHS was used because a normal distribution of sera could not be assumed. The upper limit of normal for each assay was derived by dividing the duplicate mean for each of the 6 NHS by their geometric mean. The arithmetic mean plus two standard deviations of these six ratios was taken as the upper limit of normal (1:80).

Gel filtration and ultracentrifugation

Sera (2 ml) for fractionation experiments were applied to a 1·5 × 100 cm column containing Sephadex G-200 in PBS, pH 7·2. Fractions were collected every 15 min by downwards elution at a flow rate of 6 ml/hr and extinction at 280 nm was plotted. An aliquot of the IgG peak (fraction numbers 42–51 inclusive) was ultracentrifuged at 150,000 ***g*** for 90 min at 8°C. The upper third of this solution (7S IgG) was removed and tested immediately in the RCA. The high molecular weight fraction (HMW) containing putative ICs comprised fractions 33–39 inclusive.

Measurement of anti-nuclear antibodies and preparation of antigen–antibody complexes in vitro.

Antibodies to dsDNA were measured by the Farr assay (Holian *et al.*, 1975) using an anti-DNA kit supplied by the Radiochemical Centre, Amersham, England. Results were expressed either in units/ml by reference to a standard serum or as c.p.m. for fractionated sera. Serum from a patient (F.C.) with SLE and a high level of DNA binding (410 units/ml) was treated with dsDNA (calf thymus, Sigma) in order to establish the effect on ^{125}I-DNA and Raji cell binding. Serum from patient M.E. (Table 1) was treated with dsDNA and subsequently fractionated on G-200. Aliquots of serum (50 μl) were incubated with 50 μl dsDNA at 1, 10, 100 and 1,000 μg/ml in PBS, pH 7·2, at 37°C for 1 hr and overnight at 4°C. The volume of each sample was made up to 400 μl with Hanks/BSA. The treated sera (25 μl) were tested in the Raji cell- and DNA-binding assays. DNA, 50 μl (10 mg/ml in PBS, pH 7·2), was incubated with 500 μl serum at 37°C for 1 hr and at 4°C for 24 hr. Five hundred microlitres of DNA-treated serum were fractionated on Sephadex G-200 as described above. Pooled fractions were tested in the Raji cell- and DNA-binding assays. Antibodies to the soluble nuclear antigens Sm and RNP were quantitated using the haemagglutination technique of Tan & Peebles (1976) and their identity was checked by observing lines of identity with precipitins from reference sera on double diffusion. The sera of two patients (C.O.M. and K.T.) and a control serum (NHS. Table 1) were treated with rabbit thymus extract (Pelfreeze Biologicals, Rogers, Arkansas) as a source of Sm and RNP. Aliquots of serum (50 μl) were incubated with 1, 10, 100 and 250 μl extract (protein content, 10 mg/ml) at 37°C for 1 hr and overnight at 4°C. The volume of each sample was made up to 400 μl with Hanks/BSA. The treated sera (25 μl) were tested in the RCA and antibody titres measured by haemagglutination. Antibodies to SS-A and SS-B were quantitated by serial dilution of serum D.N. (Table 1) on double diffusion against Raji cell extract (RCE) prepared by a previously published method (Venables *et al.*, 1980). The titre was the highest dilution at which a precipitin was observed. Antibody specificity of the serum has been confirmed by Dr Eng Tan, Denver, Colorado, by immunodiffusion. Aliquots of this serum (25 μl) were incubated with RCE at 0·12, 1·2, 12, 60, 120 and 198 mg soluble protein/ml serum for 30 min at 37°C and 18 hr at 4°C in a total volume of 200 μl adjusted with Hanks/BSA. The treated sera (25 μl) were tested in the RCA and for anti-SS-A and SS-B antibodies by immunodiffusion.

Table 1. ANA specificity and quantity in seven sera used for fractionation experiments

Patient	Diagnosis	dsDNA (units/ml)†	Sm HA titre	RNP HA titre	SS-A titre	SS-B titre
M.E.	SLE	1,800	–	–	–	–
N.A.	SLE	620	–	–	4	–
C.O.M.	SLE	28	1,600,000	*	–	–
K.T.	Raynaud's phenomenon	14	–	160,000	–	–
D.N.	Sicca syndrome	13	–	–	256	16
D.W.	Reiter's syndrome	20	–	–	–	–
NHS	Healthy	0	–	–	–	–

* Anti-RNP HA titre not possible to determine because of high-titre anti-Sm, anti-RNP also not detectable by counter immunoelectrophoresis or double diffusion.
† Normal range 0–25 units/ml. (–) Indicates negative.

RESULTS

In 200 sera from 54 patients with SLE the ratio of test/control in the RCA was greater than 1·80 (upper limit of normal) in 172 sera (86%). When the T/C ratio was plotted against DNA binding (units/ml) for each of these sera (Fig. 1), a statistically significant correlation was found ($r = 0{\cdot}57$, $P < 0{\cdot}001$).

Raji cell binding and anti-DNA antibodies in sera and their fractions

The possibility was raised that the correlation was due to direct interaction of anti-DNA antibodies with the Raji cells. This was investigated in sera from two patients with SLE (N.A. and M.E., Table 1), both of which had high Raji cell binding (816 and 480 μgEq/ml) and DNA binding of 620 and 1,800 units/ml respectively. After sera were fractionated on Sephadex G-200, Raji cell and DNA binding were measured in the HMW and 7S fractions (Fig. 2a, b). In the HMW fractions of both sera, Raji cell binding was positive (80 and 30 μgEq/ml respectively) and low-level DNA binding was also detected (4 and 2×10^3 c.p.m. respectively).

In contrast, the 7S fractions of both sera showed much higher levels of Raji cell binding (370 and

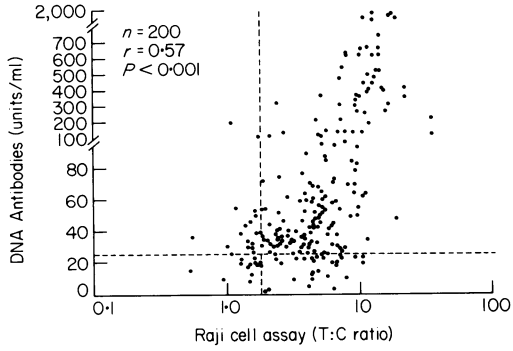

Fig. 1. Relationship between anti-DNA antibodies and Raji cell binding. Each point represents the result on a single serum sample. The 200 sera were derived from 54 patients with SLE. The broken lines represent the upper limit of normal for each assay (arithmetic mean + 2 s.d.). In this and Figs 1–6, a T/C ratio > 1·8 in the Raji cell assay indicates a positive result.

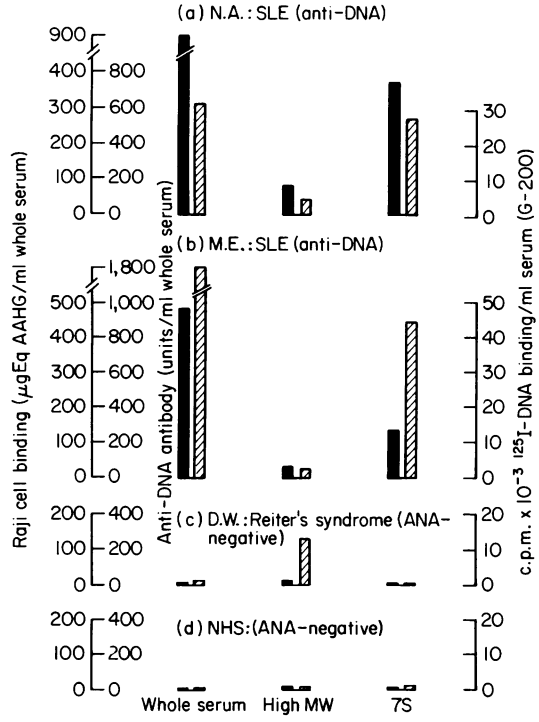

Fig. 2. Raji cell binding and anti-DNA antibodies in whole sera and G-200 fractions. Raji cell binding (solid bars) is expressed as μgEq AAHG/ml serum. In whole serum, anti-DNA antibodies (hatched bars) are expressed as units/ml and for G-200 fractions as c.p.m. $\times 10^{-3}$ ^{125}I-DNA bound/ml serum applied to the column. All values for G-200 fractions were corrected for dilution; (a) and (b) were serum samples from two patients with SLE and anti-DNA antibodies; (c) was a serum sample from an ANA-negative patient with Reiter's syndrome and (d) normal human serum from an ANA-negative healthy donor.

132 μgEq/ml respectively) and this fraction contained the majority of the DNA binding (27 and 44×10^3 c.p.m. respectively). The 7S binding appeared to be complement-independent. For example, in serum N.A. binding of 7S material to Raji cells was 68,057 c.p.m. in the presence and 60,416 c.p.m. in the absence of fresh normal serum. In contrast, the Raji cell binding of a single concentration of AAHG was markedly complement-dependent, i.e. 119,093 c.p.m. in the presence and 46,279 c.p.m. in the absence of fresh normal serum.

Raji cell binding and antibodies to Sm and RNP in sera and their fractions

During our study of SLE patients we observed that sera with high Raji cell but low DNA binding frequently contained antibodies to soluble nuclear antigens (Fig. 3). This raised the possibility that ANA binding to Raji cells was not restricted to anti-DNA antibodies. To investigate this further, sera from patients with high antibody titres to Sm (1:1,600,000) and RNP (1:160,000) were fractionated on Sephadex G-200 and compared with similarly fractionated serum from a patient with Reiter's disease (D.W.) and an ANA-negative control serum from a healthy donor (NHS, Table 1).

The results of Raji cell binding and antibody levels both in whole and fractionated sera are shown in Fig. 4.

Anti-Sm and anti-RNP antibody-containing sera had high ENA haemagglutination (HA) titres of 1:1,600,000 and 1:160,000 respectively (Fig. 4a, b), and therefore the HA test was expressed as the number of doubling dilutions, e.g. 21 and 17 $\log_2$ respectively.

In whole (C.O.M.) serum with anti-Sm activity (Fig. 4a), Raji cell binding was 132 μgEq/ml and following fractionation, most of the binding was present in the 7S fraction (50 μgEq/ml) with less in

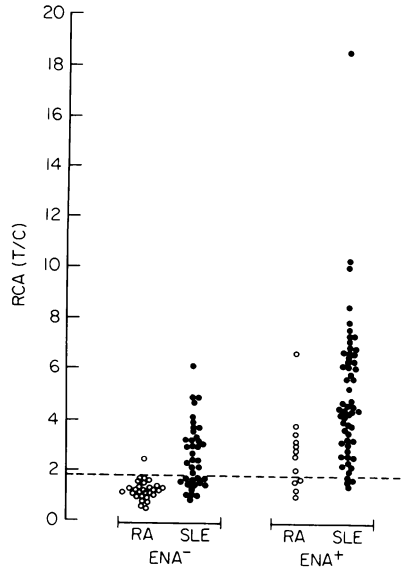

Fig. 3. Raji cell binding in rheumatoid arthritis (RA) and SLE sera and relationship to antibodies to extractable soluble nuclear antigens (ENA). RA sera (o) and SLE sera (•) were selected from patients with anti-DNA antibodies of less than 50 units/ml. The broken line represents the upper limit of normal.

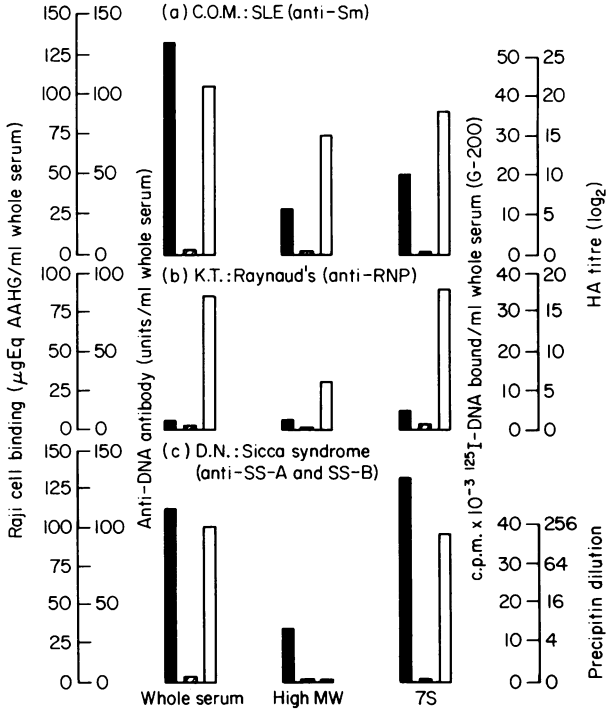

Fig. 4. Raji cell binding and antibodies in whole sera and G-200 fractions. Raji cell binding and anti-DNA antibodies are expressed as in Fig. 2. Other antibodies (open bars) are (a) anti-Sm, (b) anti-RNP expressed as $\log_2$ haemagglutination titre and (c) anti-SS-A and -SS-B expressed as the highest dilution at which a precipitin was observed.

the HMW fraction (28 μgEq/ml). Most of the antibody was also in the 7S fraction (18 $\log_2$) compared to the HMW fraction (15 $\log_2$). A similar finding was observed in the serum with anti-RNP activity (Fig. 4b) although Raji cell binding of the neat serum was much lower (4·8 μgEq/ml). Nevertheless, the 7S fraction bound 10 μgEq/ml which was greater than the HMW fraction which bound 5 μgEq/ml. The observation that the HA titre in the 7S fraction was 18 $\log_2$ compared to the HMW fraction of only 6 $\log_2$ confirmed that most of the anti-RNP antibody was 7S material.

Raji cell binding and antibodies to SS-A and SS-B in a serum and its fractions

A serum (D.N., Table 1) containing antibodies to the soluble nuclear antigens SS-A and SS-B and normal levels of DNA binding was positive in the Raji cell assay (110 μgEq/ml). Fig. 4c illustrates that much of this Raji cell binding was located in the 7S fraction (131 μgEq/ml) where all the detectable antibody was found (titre 1:82). However, Raji cell binding was also present to a lesser extent in the HMW fraction (32 μgEq/ml) in the absence of detectable antibody.

Raji cell binding to ANA-negative sera and fractions

Two sera which were ANA-negative and also did not bind to Raji cells were fractionated. One of these, D.W. (Table 1), from a patient with Reiter's disease (Fig. 2c) showed low levels of Raji cell and DNA binding in the HMW fraction (10 μgEq/ml and $6{\cdot}5 \times 10^3$ c.p.m. respectively). However, the 7S fraction was negative with respect to anti-DNA antibodies, other ANA and Raji cell binding. The serum from a healthy donor (Fig. 2d) was negative in all assays in each fraction.

Effects on Raji cell binding of adding nuclear antigens to sera containing ANA

These data suggest that the presence of certain anti-nuclear antibodies such as anti-DNA, anti-Sm,

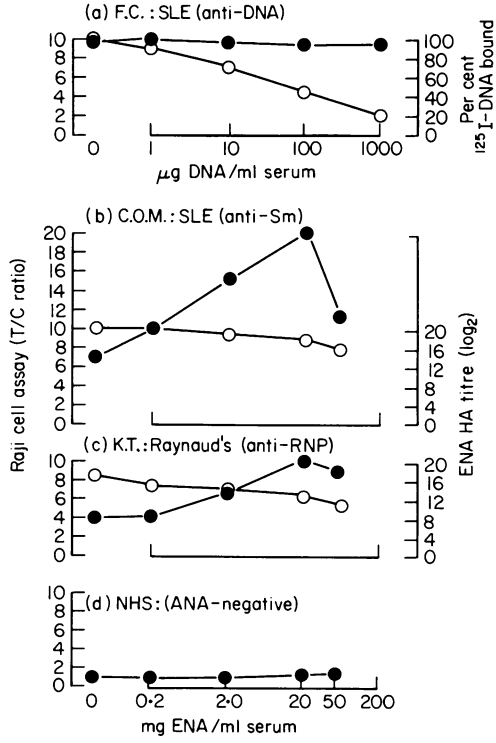

Fig. 5. Effect of addition of increasing amounts of nuclear antigens on Raji cell binding (•) and anti-nuclear antibody titres (o). (a) Addition of calf thymus DNA to serum containing anti-DNA antibodies. Rabbit thymus nuclear extract as a source of ENA was incubated with serum containing antibodies to (b) Sm and (c) RNP and with (d) normal human serum from an ANA-negative healthy donor.

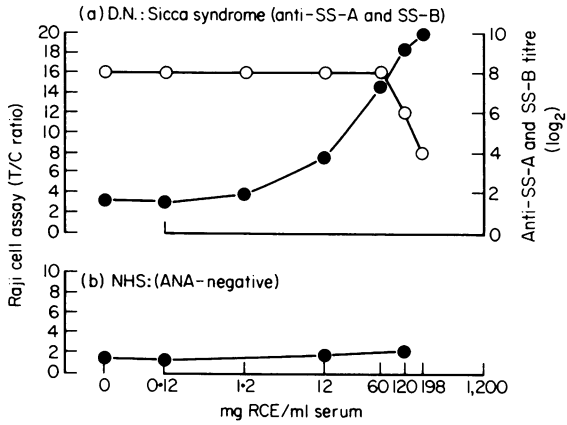

Fig. 6. Effect of addition of soluble antigen extract of Raji cells (RCE) on Raji cell binding (•) and anti-SS-A and -SS-B antibody titres (o).

anti-RNP and anti-SS-A and SS-B in sera results in increased Raji cell binding. To confirm that Raji cells did bind immune complexes in addition to ANA, the same sera were incubated with their appropriate antigens to form *in vitro* immune complexes and then re-tested with the Raji cell assay.

Fig. 5 shows the results of adding increasing amounts of (a) dsDNA, (b) Sm, (c) RNP to patients' sera and (d) the effect of adding RTE (ENA) antigens to NHS.

Addition of increasing amounts of DNA to the serum of a patient with SLE and high DNA-binding levels resulted in a decrease in DNA binding but did not significantly alter the Raji cell binding (Fig. 5a).

In contrast, the addition of increasing amounts of RTE antigens to sera from patients with anti-Sm and anti-RNP antibodies (Fig. 5b & c respectively) resulted in an increase in Raji cell binding and a decrease in ENA HA titre. Addition of RTE antigens to NHS did not affect Raji cell binding (Fig. 5d).

The addition of increasing amounts of RCE to serum with anti-SS-A and -SS-B antibodies also produced an increase in Raji cell binding with a decrease in antibody titre (Fig. 6a). Raji cell binding of normal human serum was only slightly increased on addition of RCE (Fig. 6b).

It was noted that addition of dsDNA to a serum containing anti-DNA antibodies showed unaltered Raji cell binding. In contrast, the addition of soluble nuclear antigens to sera containing antibodies to Sm, RNP, SS-A and SS-B showed increased binding to Raji cells as immune complexes were formed *in vitro*.

Raji cell binding of nuclear antigen–antibody complexes formed in vitro

As further evidence that Raji cells could detect immune complexes, two sera were treated with the appropriate antigen to form immune complexes *in vitro* and then fractionated on Sephadex G-200.

When RCE was added to serum containing anti-SS-A and -SS-B antibodies, no free antibodies could be detected in the 7S fraction and Raji cell-binding material had moved to the HMW fraction.

Table 2. Abolition of 7S IgG binding by incubation of ANA-positive serum with specific antigen

	Raji cell assay (μgEq AAHG/ml)		DNA binding (c.p.m.)	
Patient M.E.(SLE)	>200,000 MW	7S	>200,000 MW	7S
Untreated serum	31	128	2,268	44,408
Serum + DNA	25	<0·7	0	2,286

Similarly, when DNA was added to serum containing anti-DNA antibodies, no free antibodies could be detected in the 7S fraction in which Raji cell binding was also abolished. In contrast, there was no significant change in Raji cell binding in the HMW fraction suggesting that the DNA-anti-DNA complexes formed *in vitro* were not detected (Table 2).

DISCUSSION

Our data have shown that the Raji cell assay detects not only circulating immune complexes but also 7S material from sera with known ANA specificity. The observation that the RCA is frequently positive in SLE has been noted in previous studies (Theofilopoulos *et al.*, 1976; Robinson *et al.*, 1979; Woodroffe *et al.*, 1977; Halla, Volanakis & Schrohenloher, 1979). Initially, the correlation of anti-DNA and anti-ENA antibody levels with Raji cell binding which we observed was thought to be due to immune complexes in the ANA-positive sera. However, our data show that direct binding of certain ANA in the sera tested may explain the relationship between ANA and Raji cell binding. This is supported by the observation that fractionated 7S material showed higher levels of binding than the HMW fraction containing putative endogenous immune complexes.

The sera chosen for fractionation were carefully selected on the basis of high-titre antibodies reacting with a single nuclear antigen. Such sera are atypical since SLE patients commonly have antibodies to a wide spectrum of nuclear antigens (Notman, Kurata & Tan, 1975) and also contain anti-lymphocyte antibodies (ALA) (Winchester *et al.*, 1974; Winfield *et al.*, 1975). Our results show that 7S material from sera with anti-DNA or anti-Sm or anti-SS-A antibodies has a strong avidity for Raji cells. Evidence that it is ANA rather than ALA that bind is provided by two observations: firstly, almost all the ANA was in the 7S fraction and secondly, incubation of two sera, M.E. and D.N., with extracts containing specific antigen (DNA and SS-A respectively) and subsequent fractionation depleted both antibody activity and Raji cell binding of 7S material. Further evidence was sought by screening all the sera in Table 1 for ALA. Although serum M.E. contained ALA, 7S binding was completely abolished by purified DNA. Serum D.N. had no ALA activity against lymphocytes from a panel of 20 donors.

As the IgG fraction of ANA-positive sera apparently binds to Raji cells, the question then arises: Do the cells also detect immune complexes? Certainly they detect aggregated IgG which is the basis of the standard curve used to quantitate binding in the RCA and in others such as the C1q-binding assay (C1qBA). Previous work has shown that the RCA measures immune complexes formed *in vitro* (Theofilopoulos, Dixon & Bokisch, 1974; Theofilopoulos, Eisenberg & Dixon, 1978). Our own experiments confirm this by demonstrating an increase in binding in some of the ANA-positive sera when incubated with extracts containing the appropriate antigens, with the notable exception of DNA. The addition of DNA to serum containing anti-DNA antibodies removes 'free' antibody and Raji cell binding from the 7S fraction. However, in the serum of patient F.C. (Fig. 5a) not all the *in vitro* DNA–anti-DNA complexes were detected by the Raji cells whereas with patient M.E. (Table 2) none of the *in vitro* complexes were detected in the HMW fraction. This difference may be attributable to the spontaneous precipitation of such complexes during the incubation stage (Griffiths *et al.*, 1977) which effectively removes them from the aliquots subsequently tested.

If ANA do bind to Raji cells, then the mechanism remains to be elucidated and is currently under investigation. Failure of 7S material from normal sera to show high levels of binding to Raji cells excludes the involvement of Fc receptors. Furthermore, the 7S binding is not to complement receptors since this was unaltered by the addition of fresh normal human serum. One intriguing possibility is that certain ANA penetrate the cell wall and nucleus of viable cells by a mechanism involving active transport as described by Alarcón-Segovia *et al.* (1978, 1979). This is unlikely: firstly, Raji cells are a B cell line whereas 'antibody penetration' was described in T cells. Secondly, it would be necessary to postulate another active transport mechanism for the penetration of radiolabelled protein A used for detection of the IgG. Alternative explanations include the possibility that in the course of the assay non-viable Raji cells become permeable and permit the interaction of ANA and nuclear antigens by passive diffusion through the cell. Furthermore, ANA binding to viable cells may occur if sufficient nuclear antigenic material was expressed on the cell

membrane. In another lymphoblastoid B cell line (Wil_2), small amounts of DNA have been demonstrated on the cell membrane (Hall *et al.*, 1971).

Reference has been made to the existence of immune complexes of 7S size (Lewis & Roberts, 1980; Roberts, Robinson & Lewis, 1979) and 6·5 and 7S material from sucrose density-gradient-fractionated sera has been shown to bind to Raji cells (Robinson *et al.*, 1979; Woodroffe *et al.*, 1977). If our 7S material were an immune complex then it should have bound to Raji cells through complement receptors only if it contained bound complement components. Assuming the antigen is a nucleotide and such an immune complex was bound to C4b2a3b or C4b2a3d, the calculated molecular weights would be in excess of 660,000 and 340,000 daltons respectively and the corresponding sedimentation coefficients would exceed 7S. In our experiments, the 7S material bound to Raji cells whether or not additional normal human serum as a source of complement was added.

This study confirms that the RCA binds immune complexes but also binds 7S material with ANA activity. If this binding is directly due to ANA the usefulness of the assay as a measurement of immune complexes may be limited. However, it should be noted that other so-called immune complex assays such as the C1qBA may also bind materials non-specifically such as rheumatoid factors (Erhardt, Mumford & Maini, 1979), DNA (Agnello, Winchester & Kunkel, 1970) and bacterial endotoxins (Sobel, Bokisch & Mueller-Eberhard, 1975). Our data do not challenge the use of the assay in clinical practice and studies are in progress to assess its potential usefulness for diagnosis, response to treatment and the prediction of flares in rheumatoid arthritis and SLE.

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Note added in proof

Since this manuscript was submitted we have shown that removal of DNA-binding material from two sera by treatment with DNA-cellulose significantly decreased but did not abolish Raji cell binding. Furthermore, when recovered, this material bound to Raji cells and gave intense fluorescent staining of Crithidiae kinetoplasts. This provides additional evidence for the binding of anti-DNA antibodies to Raji cells.