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La/SSB ribonucleoprotein levels increased in transformed cells

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SUMMARY

Autoantibodies to the La/SSB ribonucleoprotein are commonly found in patients with Sjögren's syndrome. Previous studies have shown that La/SSB accumulates in cells shortly after viral infection. We have extended these studies by investigating levels of the La/SSB antigen in virally and spontaneously transformed cell lines (contact-insensitive and tumourigenic) relative to their non-transformed counterpart cell lines (contact-sensitive and non-tumourigenic). Transformed BALB/3T12-3 and KNRK fibroblasts were visibly brighter by immunofluorescence assay than non-transformed BALB/3T3 and NRK fibroblasts respectively, when reacted with anti-La/SSB specific sera. This was confirmed by flow cytometry, as La/SSB levels were elevated in the transformed counterparts of the same cell lines. An anti-Sm monoclonal antibody and normal human serum reacted with these cell lines failed to show a significant increase by flow cytometry. Finally, a two-fold increase in the La/SSB antigen was demonstrated in cell lysates of these cell lines by a capture ELISA. These data show that La/SSB is elevated in transformed cell lines compared with non-transformed counterpart cell lines and suggest that this increase is not restricted to viral transformation.

Keywords La/SSB Sjögren's syndrome

INTRODUCTION

Circulating autoantibodies to conserved cellular antigens are common in patients with systemic rheumatic diseases. These autoantibodies have been useful for diagnosis and have contributed to our understanding of certain cellular molecules (Tan, 1982). One such antibody is directed against a 45–50-kD protein belonging to an RNA–protein complex called La/SSB (Mattioli & Reichlin, 1974; Lerner *et al.*, 1981b; Francoeur & Mathews, 1982; Habets *et al.*, 1983). Anti-La/SSB antibodies have been reported in 40–87% of patients with Sjögren's syndrome, and at lower frequencies in other connective tissue diseases (Reichlin, 1986). The La/SSB antigen was first shown to be transiently associated with RNA polymerase III transcripts by Hendrick *et al.* (1981). More recently, La/SSB has been shown to be intimately involved with the regulation and termination of RNA polymerase III transcript production (Gottlieb & Steitz, 1989a, 1989b).

La/SSB also binds the small viral transcripts of Epstein–Barr virus (EBV) and adenovirus (Lerner *et al.*, 1981a; Rosa *et al.*, 1981). It has been demonstrated that infection of HEp-2, HeLa and HEL cell lines with adenovirus or cytomegalovirus causes a two- to 13-fold increase in La/SSB (Baboonian *et al.*, 1989). The increase in La/SSB was shown to peak 3–7 days after viral infection before quickly returning to a level near that of the uninfected cultures. In another study, La/SSB levels were shown to increase 15-fold in Vero cells 8 h after infection with herpes simplex virus (HSV) (Bachmann *et al.*, 1986). These data indicate an elevation of La/SSB directly after an acute viral infection.

Here, a comparison of the La/SSB levels in transformed and non-transformed immortal cell lines derived from the same sources was undertaken. The counterpart cell lines were selected based on similarities in size, DNA content and doubling time. We show that La/SSB is elevated in spontaneously transformed mouse fibroblasts (BALB/3T12-3) and murine sarcoma virus-transformed rat fibroblasts (KNRK) relative to the non-transformed counterpart cell lines (BALB/3T3 and NRK, respectively) using immunofluorescence assay, flow cytometry and quantitative ELISA.

MATERIALS AND METHODS

Patient sera and anti-Sm monoclonal antibody

The anti-La/SSB reference serum was generously provided by Dr Joan Steitz, Yale University. All other autoimmune reference sera were provided by Dr Stan Sumeda, Virginia Mason Clinic. CB, RB and FJ patient sera were selected from the serum bank of the Arthritis/Immunology Section at the Oklahoma Medical Research Foundation, courtesy of Dr Morris Reichlin and Dr John Harley. The immunologic specificity of sera used in this study was determined by immunodiffusion against bovine spleen extract (Clark, Reichlin & Tomasi, 1969) or by immuno-

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precipitation and gel analysis of the RNAs (Lerner *et al.*, 1981a). Patient sera were also tested for anti-dsDNA antibody by *Crithidia* assay. The anti-La/SSB patient sera, CB and RB, show a single line of identity with La/SSB by immunodiffusion and were anti-dsDNA negative. Immunoprecipitation and gel analysis of the RNAs showed that the anti-Ro/SSA reference serum precipitated Ro/SSA RNAs and the anti-La/SSB reference serum precipitated La/SSB RNAs only. FJ scleroderma patient serum has an anti-nucleolar staining pattern and has been shown to react with fibrillarin (Lischwe *et al.*, 1985). The anti-Sm monoclonal antibody was derived from a MRL/*lpr* mouse and was shown to immunoprecipitate U1, U2, U4, U5 and U6 RNAs (unpublished data).

Cells

BALB/3T12-3, BALB/3T3, KNRK and NRK fibroblast cell lines, purchased from ATCC (Rockville, MD) were maintained in minimal essential media (MEM) with 10% fetal calf serum (FCS), 1% L-glutamine and 1% penicillin/streptomycin. BALB/3T3 and NRK cell lines are contact-sensitive and non-tumourigenic, and BALB/3T12-3 and KNRK cell lines are contact-insensitive and tumourigenic.

Immunologic reagents

FITC-conjugated anti-human IgG (Antibodies Incorporated, Davis, CA) was used at 1/100 in phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) in the slide assay and flow cytometry. Alkaline phosphatase (AP) conjugated goat anti-human IgG ($F(ab')_2$ specific) or horseradish peroxidase (HRP) conjugated goat anti-human IgG (Fc specific) were purchased from Jackson Immunoresearch Laboratories (Avondale, PA). They were used at 1/2500–1/10000 in PBS with 0·1% BSA and 0·05% Tween 20 in the ELISA. The Superose 12 HR 10/30 column was obtained from Pharmacia (Piscataway, NJ). The peroxidase substrate solutions A and B (Kirkegaard & Perry Laboratories, Gaithersburg, MD) were combined 1:1 before use. Immunoprecipitin was obtained from Bethesda Research Laboratories (Gaithersburg, MD). Affinity-purified La/SSB was a generous gift from Dr John Harley, Oklahoma Medical Research Foundation. All other immunologic reagents used were purchased from Sigma Chemical Co. (St Louis, MO).

Indirect immunofluorescence microscopy and photography

BALB/3T12-3 and BALB/3T3 cells were seeded onto 12-well glass slides (Cell Line Associates, Newfield, NJ) at 3000 cells/well and cultured at 37°C for 16–20 h. Cells were fixed on slides with 1% paraformaldehyde (E. M. Grade, Polysciences, Warrington, PA) in PBS for 20 min followed by 0·2% Triton X-100 in PBS for 5 min. Sera or monoclonal antibody were diluted in PBS with 1% BSA and 30 μl were added to each well for 20 min. After washing in PBS, 30 μl of FITC-conjugated anti-human IgG were added for 20 min. After a final wash with PBS, slides were mounted in nine parts glycerol and one part bicarbonate buffer, pH 8·0, containing 1 mg/ml *p*-phenylenediamine. Slides were photographed at the same exposure time using Tri-X pan 400 at ASA 1600 and developed with HC-110, dilution B (Eastman Kodak, Rochester, NY). Both u.v. and phase-contrast illuminations were taken of each field (Nikon Optiphot).

Indirect immunofluorescence staining for flow cytometry

Fibroblast cells (BALB/3T12-3, BALB/3T3, KNRK and NRK) were removed from culture flasks by trypsinization and fixed (1×10^7 cells/tube) with 5 ml of 1% paraformaldehyde in PBS for 20 min. Cells were permeabilized with 5 ml of 0·2% Triton X-100 or 95% ethanol in PBS for 10 min. Although the use of either Triton X-100 or ethanol gave identical results, the ethanol-treated cells gave cleaner profiles by flow cytometry and were subsequently used. Patient sera were diluted in PBS with 1% BSA and added to 1×10^6 fixed cells for 20 min. The optimal patient sera dilutions (saturating dilutions) were determined using titration curves from flow cytometry (data not shown). This was followed by a 20-min incubation with FITC-conjugated anti-IgG. Washes between steps were performed with PBS and cells pelleted each time at 500 *g* for 5 min. All reactions were carried out at room temperature.

Flow cytometry analysis

Cells reacted with sera as described above were suspended in 0·25 ml of PBS and analysed (1×10^4 cells/sample) using a Coulter Epics V flow cytometer (Coulter Electronics, Hialeah, FL) at 488 nm. Using forward-angle light scatter (FALS) as a gating criterion, log-integrated green fluorescence was measured. Normal human serum was used at the same IgG concentration to determine non-specific binding.

Lysate preparation

BALB/3T12-3, BALB/3T3, KNRK and NRK cells were trypsinized in log phase and counted. Aliquots of 5×10^6 cells from each line were suspended in 2 ml of 10 mM Tris buffer, pH 7·4, containing 1 mM PMSF. The cells were then sonicated on a Branson Sonifier 185 at a setting of 5 with 10-secs bursts until lysis was complete. The lysates were kept on ice during preparation.

Preparation of $F(ab')_2$ from anti-La/SSB-specific serum

Immunoglobulin was isolated from serum (CB) with high titers of anti-La/SSB by 33% ammonium sulphate precipitation followed by dialysis in 0·2 M sodium acetate buffer. A pepsin digest was performed by adding 3 mg of pepsin/100 mg of immunoglobulin and the reaction was neutralized with 2 *N* NaOH. The digest was fractionated by FPLC using a Superose 12 HR 10/30 column, and the $F(ab')_2$ peak identified using anti-human $F(ab')_2$ by ELISA. Selected fractions were passed over a Protein A–Sepharose CL-4B column to eliminate contaminating whole IgG.

Immunoprecipitation of ^{32}P-labelled RNAs

Mouse L-M cells were pretreated for 30 min with MEM-based phosphate-free media (Gibco Laboratories, Grand Island, NY). The starvation media was removed and replaced with phosphate-free MEM with addition of 2% FCS and 1 mCi (^{32}P)orthophosphate (New England Nuclear, Boston, MA) per 75-cm^2 flask (2×10^7 cells) and the cells incubated for 16–18 h. Cell lysates were prepared and immune complexes precipitated using immunoprecipitin, essentially as described (Lerner *et al.*, 1981b). RNA was isolated from immunoprecipitates treated with 0·1 mg/ml proteinase K (Bethesda Research Laboratories) for 1 h at 37°C followed by phenol/chloroform extraction and ethanol precipitation. RNAs were denatured and fractionated

on 8% polyacrylamide gels containing 8 M urea, 0·2 M Tris, 0·083 M boric acid and 1 mM EDTA.

ELISA capture assay

The ELISA capture assay was performed as described by Radar *et al.* (1989). Costar U-bottomed vinyl microtitration plates (Costar, Cambridge, MA) were coated with $F(ab')_2$ from anti-La/SSB specific serum (50 μl/well at 10 μg/ml) in carbonate buffer, pH 9·4, overnight at 4°C. After blocking the plates with Blotto (5% dry skimmed milk, 0·01% Thimerosal and 0·01% Antifoam A concentrate in PBS) for 1 h, dilutions of the four cell lysates were added and incubated for 2 h. In the next step, anti-La/SSB or anti-Ro/SSA serum was added at a concentration of 1/1000 in PBS with 0·1% BSA for 1 h, followed by the addition of HRP-conjugated anti-human IgG (Fc specific) for 1 h. The plates were then developed using peroxidase substrate. Plates were washed between steps with PBS containing 0·05% Tween 20. Absorbance values were measured on a MR 580 Auto Plate Reader (Dynatek Laboratories, Alexandria, VA) at 405 nm, against a substrate solution blank. La/SSB standard curves were obtained using affinity purified La/SSB antigen.

Statistical analysis

The Student's *t*-test was used to test for differences between means using the Epistat software package for PC-based computer systems (T. Gustafson, Round Rock, TX).

RESULTS

Indirect immunofluorescence slide assay of transformed versus *non-transformed fibroblast cell lines*

To test whether an increase in La/SSB could be demonstrated between transformed and non-transformed cell lines that were derived from the same sources, BALB/3T12-3 and BALB/3T3 fibroblasts were fixed onto slides and reacted with an anti-La/SSB patient serum (RB; Fig. 1). These cell lines are both rapidly dividing and are comparable in size and DNA content. The transformed BALB/3T12-3 cells (Fig. 1A) were visibly brighter than the non-transformed BALB/3T3 cells (Fig. 1B) indicating an increase of La/SSB in the former. Cells from the two cell lines were indistinguishable by phase contrast illumination (Fig. 1C,D). An increase in La/SSB was also demonstrated in BALB/3T12-3 and KNRK cells relative to their non-transformed counterpart cells (BALB/3T3 and NRK, respectively) using another anti-La/SSB serum (CB). There were no differences seen between the transformed and non-transformed cells using an anti-Sm monoclonal antibody or normal human serum (data not shown).

Flow cytometric analysis of La/SSB levels in transformed and non-transformed fibroblast cell lines

KNRK and NRK cells were fixed, permeabilized and reacted with anti-La/SSB sera, an anti-Sm monoclonal antibody or normal human serum, and their fluorescence compared by flow cytometry. Figure 2 (a and b) shows the shift in fluorescence between the transformed KNRK cells and non-transformed NRK cells reacted with two different anti-La/SSB sera (CB and RB, respectively). Figure 2c depicts the negligible shift seen when cells were reacted with an anti-Sm monoclonal antibody. Figure 2d demonstrates the background fluorescence with normal human serum showing no shift between the transformed

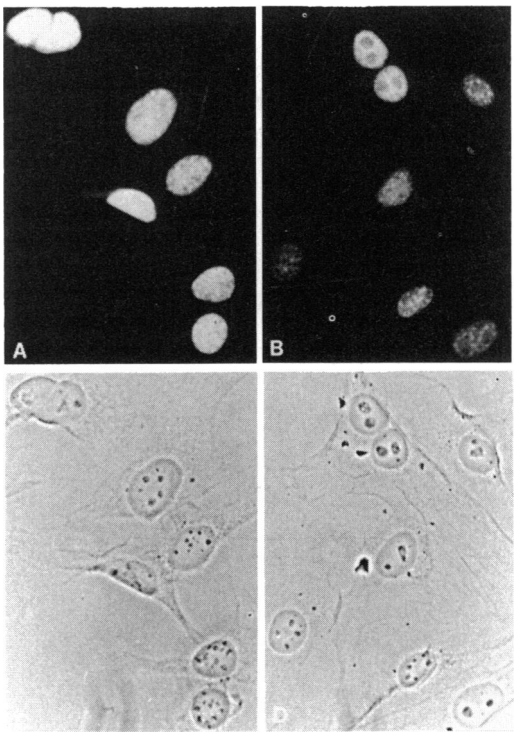

Fig. 1. Transformed BALB/3T12-3 and non-transformed BALB/3T3 fibroblast cells were compared by an immunofluorescence slide assay using anti-La serum. Cells were fixed on slides and reacted with RB serum as described in Materials and Methods and photographed at identical time exposures. Immunofluorescence of BALB/3T12-3 cells (A) and BALB/3T3 cells (B) and phase contrast micrographs of the same fields (C and D, respectively) are shown. The BALB/3T12-3 cells were visibly brighter than BALB/3T3 cells stained with anti-La/SSB serum.

KNRK cells and non-transformed NRK cells. BALB/3T12-3 and BALB/3T3 cells were reacted with the same anti-La sera, the anti-Sm monoclonal antibody and an anti-fibrillarin serum (FJ) (data not shown). The anti-La/SSB sera alone demonstrated a significant fluorescence increase on the BALB/3T12-3 transformed cells.

Quantification of La/SSB protein in transformed and non-transformed cell lysates by an ELISA capture assay

The La/SSB protein level in lysates of BALB/3T12-3, BALB/3T3, KNRK and NRK cell lines was quantitated by ELISA. Lysates were made from the same number of cells for each cell line. In the linear region of a La/SSB standard curve, BALB/3T12-3 and KNRK cells contained at least two-fold more La/SSB than BALB/3T3 and NRK cells, respectively (Fig. 3). Using the 1/8 dilution of cell lysates as an example, the differences in La/SSB concentrations were statistically significant ($P < 0.0001$ for BALB/3T12-3/BALB/3T3 and $P < 0.01$ for KNRK/NRK).

Analysis of anti-La/SSB serum (CB) specificity

The La/SSB-specific antisera used in this study were shown to be void of other commonly occurring autoantibodies by conventional methods (see Materials and Methods). To rule out that anti-Ro/SSA (which usually occurs with anti-La/SSB) was possibly contributing to the increase in La/SSB levels, the

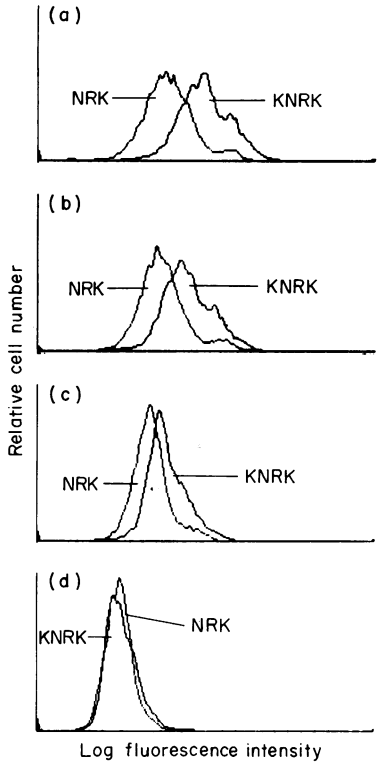

Fig. 2. Levels of La/SSB antigen in transformed KNRK and non-transformed NRK fibroblasts were quantified by flow cytometry. Cells were detached by trypsinization, fixed and reacted with anti-La patient sera an anti-Sm monoclonal antibody or normal human serum as described in Materials and Methods. (a) and (b), fluorescence of cells reacted with anti-La/SSB sera (CB and RB, respectively); (c), the fluorescence of cells reacted with anti-Sm monoclonal antibody; (d), the staining with normal human serum (NHS). The anti-La/SSB sera demonstrated a significant increase in fluorescence intensity on transformed versus non-transformed cells.

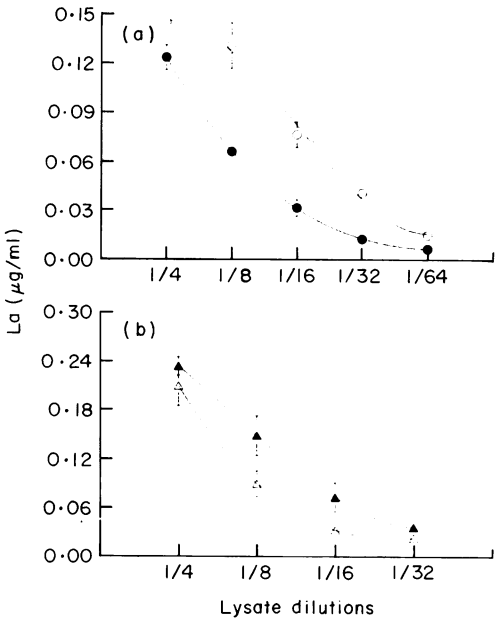

Fig. 3. Levels of La/SSB antigen in transformed and non-transformed cell lysates were quantified by an ELISA capture assay. Levels of La/SSB antigen were quantified using ELISA plates coated with $F(ab')_2$ fragments prepared from anti-La/SSB serum (CB) as described in Materials and Methods. (a), The levels of La/SSB in BALB/3T3 (closed circles) *versus* BALB/3T12-3 (open circles) cell lysates; (b), the levels of La/SSB in NRK (open triangles) *versus* KNRK (closed triangles) cell lysates. Each lysate was prepared from the same number of cells (5×10^6 cells) and the level of La/SSB antigen was quantified over a range of lysate dilutions using a standard curve of purified La/SSB. The level of La/SSB antigen was two-fold higher in both transformed cell lysates compared with the non-transformed cell lysates.

specificity of CB serum for rodent La/SSB was analysed by ELISA using anti-La/SSB and anti-Ro/SSA autoimmune reference sera. The reference sera were shown to be specific for rodent La/SSB or Ro/SSA by RNA immunoprecipitation on mouse L-M cell lysates (Fig. 4). The anti-La/SSB reference serum and CB serum reacted with La/SSB antigen captured from cell lysates by anti-La/SSB serum $F(ab')_2$ fragments, while the anti-Ro/SSA reference serum and normal human serum did not react (Fig. 5). Therefore, this assay was specific for La/SSB, and the Ro/SSA antigen did not contribute to these results.

DISCUSSION

In this study, transformed and non-transformed immortal cell lines derived from the same sources were compared for levels of La/SSB. By utilizing counterpart cell lines of comparable sizes and proliferation rates, we were able to show by immunofluorescence assay and flow cytometry that La/SSB was consistently increased in the transformed cell lines while the Sm antigen and fibrillarin were not increased.

In order to verify that the increased fluorescence observed with anti-La/SSB sera by immunofluorescence assay and flow cytometry was due to an increase in La/SSB antigen, an ELISA capture assay was performed. Lysates prepared from transformed BALB/3T12-3 and KNRK cells exhibited a two-fold higher level of La/SSB compared with lysates from the non-transformed counterpart cells (BALB/3T3 and NRK, respectively) on a per cell basis. To confirm that the serum used in the ELISA was specific for La/SSB it was characterized further. As anti-human Ro/SSA antibody usually occurs in patient sera with anti-La/SSB antibody (Harley, Yamagata & Reichlin, 1984), the question arises whether anti-Ro/SSA might have contributed to the increase in autoantigen measured. It has been shown that anti-Ro/SSA patient sera react less effectively with mouse and rat Ro/SSA compared to human Ro/SSA by ELISA (Reichlin & Wolfson-Reichlin, 1989; Reichlin, Rader & Harley, 1989). The cell lines used in our study were all derived from a mouse or rat source. We also demonstrated that a reference serum monospecific for Ro/SSA by RNA immunoprecipitation did not react with captured La/SSB in the ELISA while anti-La/SSB reference serum did react. In addition, over 65% of the reactivity to the mouse cell lysate in the ELISA capture assay was blocked by preincubation of CB serum with heterologous purified bovine La/SSB (data not shown). These data indicate that the increase in autoantigen levels measured with two anti-La/SSB sera was due to an increase in the La/SSB antigen.

The increase in La/SSB cannot be explained by the increase in proliferation rate due to transformation. A study by Aaronson & Todaro (1968) showed that the growth rates of BALB/3T12-3 and BALB/3T3 were almost identical with doubling

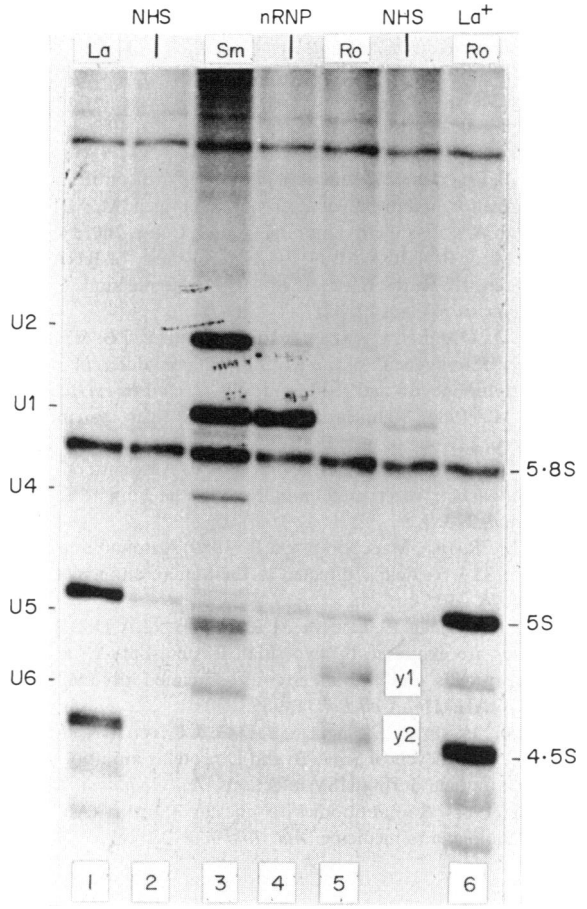

Fig. 4. Anti-La/SSB and anti-Ro/SSA autoimmune reference sera were shown to be specific for La/SSB and Ro/SSA respectively by RNA immunoprecipitation and gel analysis of RNA. IgG from sera was immobilized on Protein A/Sepharose and incubated with ^{32}P-labelled mouse L-M cell sonicates. RNAs were extracted from the immunoprecipitates and fractionated on 8% polyacrylamide gels. Shown are RNAs immunoprecipitated by: (1) anti-La/SSB reference serum; (2) normal human serum (also shown in the unnumbered lane between lanes 5 and 6); (3) anti-Sm reference serum; (4) anti-nRNP reference serum; (5) anti-Ro/SSA reference serum; and (6) lupus patient serum with anti-La/SSB and anti-Ro/SSA antibodies. The major small RNAs precipitated by the autoimmune control sera are as follows: anti-La/SSB (5S and mouse 4·5S), anti-Sm (U1, U2, U4, U5 and U6), anti-nRNP (U1) and anti-Ro/SSA (mouse Y1 and Y2). Anti-La/SSB and anti-Ro/SSA reference sera were specific for immunoprecipitating only La/SSB or Ro/SSA RNAs, respectively.

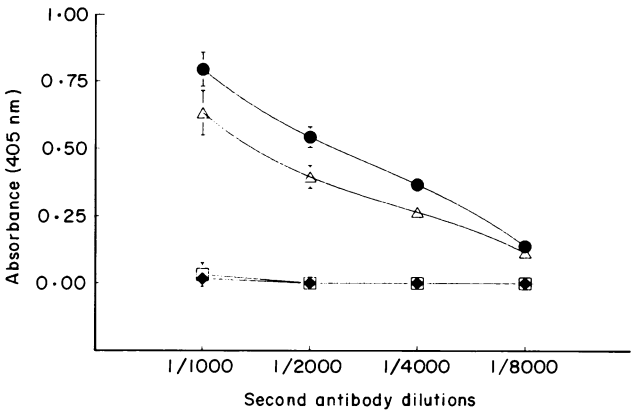

Fig. 5. The specificity of the ELISA capture assay for La/SSB antigen was analysed using the autoimmune reference sera in Fig. 4. Plates were coated with anti-La/SSB F(ab')$_2$ from CB serum and incubated with a 1/8 dilution of the NRK cell lysate, followed by incubation with either CB anti-La/SSB serum (circles), used for preparation of the F(ab')$_2$, anti-La/SSB reference serum (triangles), anti-Ro/SSA reference serum (squares) or normal human serum (diamonds) as described in Materials and Methods. Only La/SSB antigen was measured by the ELISA capture assay.

times of approximately 22 h. Our experiments confirm that the BALB/3T12-3 and BALB/3T3 cells had similar doubling times as did the KNRK and NRK cells (data not shown).

The role that increased levels of La/SSB play in autoantibody induction is unclear. Recent studies have indicated that autoantigen presentation to the immune system could be involved in eliciting an immune response. Sharpe *et al.* (1989) proposed that the abnormally high levels of Sm antigen during a lytic HSV infection may cause autoantibody production. Le Feber *et al.* (1984) suggested that membrane expression of Ro/SSA, RNP and the Sm antigen following exposure to ultraviolet light could similarly be involved in an autoimmune response to these antigens. Baboonian *et al.* (1989) showed that La/SSB increased and relocated to the cell membrane following acute infection with adenovirus. Here we determined that La/SSB was increased in transformed cell lines compared with non-transformed cell lines but failed to see La/SSB surface expression. The absence of La/SSB surface expression in our studies could reflect a steady state of La/SSB in transformed cells following a transient accumulation and relocation during earlier events in the transformation process. This is supported in part by the fact that La/SSB levels increase rapidly during early viral infection but soon decline to a lower level similar to what we report here (Baboonian *et al.*, 1989).

The increase in La/SSB was found for murine sarcoma virus-transformed NRK cells (KNRK) as well as spontaneously transformed BALB/3T12-3 cells. Moreover, we have recently found that La/SSB is elevated above other autoantigens in mouse hemopoietic cell lines whether transformed virally (WEHI-3), chemically (70Z/3) or by irradiation (WEHI-279) suggesting that this result is not restricted to cells transformed by virus (manuscript in preparation). The exact nature of the increase in La/SSB in transformed cell is the subject of further investigation.

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REFERENCES

AARONSON, S.A. & TODARO, G.J. (1968) Development of 3T3-like lines from Balb/c mouse embryo cultures: transformation susceptibility to SV40. *J. cell. Physiol.* **72,** 141.

BABOONIAN, C., VENABLES, P.J.W., BOOTH, J., WILLIAMS, D.G., ROFFE, L.M. & MAINI, R.N. (1989) Virus infection induces redistribution and membrane localization of the nuclear antigen La (SS-B): a possible mechanism for autoimmunity. *Clin. exp. Immunol.* **78,** 454.

BACHMANN, M., FALKE, D., PREUHS, J., SCHRODER, H.C., PFEIFER, K. & MULLER, W.E.G. (1986) Occurrence of novel small RNAs with concomitant inhibition of host cellular U small nuclear RNA synthesis in Vero cells infected with herpes simplex virus type 1. *J. gen. Virol.* **67,** 2587.

FRANCOEUR, A.M. & MATHEWS, M.B. (1982) Interaction between VA RNA and the lupus antigen La: formation of a ribonucleoprotein particle *in vitro*. *Proc. natl Acad. sci. USA*, **79,** 6772.

GOTTLIEB, E. & STEITZ, J.A. (1989a) The RNA binding protein La influences both the accuracy and the efficiency of RNA polymerase III transcription *in vitro*. *EMBO J.* **8,** 841.

GOTTLIEB, E. & STEITZ, J.A. (1989b) Function of the mammalian La protein: evidence for its action in transcription termination by RNA polymerase III. *EMBO J.* **8,** 851.

HABETS, N.J., DEN BROK, J.H., BOERBOOMS, A.M.TH., VAN DE PUTTE, L.B. & VAN VENROOIJ, W.J. (1983) Characterization of the SS-B (La) antigen in adenovirus infected and uninfected HeLa cells. *EMBO J.* **2,** 1625.

HARLEY, J.B., YAMAGATA, H. & REICHLIN, M. (1984) Anti-La/SSB antibody is present in some normal sera and is coincident with anti-Ro/SSA precipitins in systemic lupus erythematosus. *J. Rheumatol.* **11,** 309.

HENDRICK, J.P., WOLIN, S.L., RINKE, J., LERNER, M.R. & STEITZ, J.A. (1981) Ro small cytoplasmic ribonucleoproteins are a subclass of La ribonucleoproteins: Further characterization of the Ro and La small ribonucleoproteins from uninfected mammalian cells. *Mol. Cell Biol.* **1,** 1138.

LE FEBER, W.P., NORRIS, D.A., RYAN, S.R., HUFF, J.C., LEE, L.A., KUBO, M., BOYCE, S.T., KOTZIN, B.L. & WESTON, W.L. (1984) Ultraviolet light induces binding of antibodies to selected nuclear antigens on cultured human keratinocytes. *J. clin. Invest.* **74,** 1545.

LERNER, M.R., ANDREWS, N.C., MILLER, G. & STEITZ, J.A. (1981a) Two small RNAs encoded by Epstein-Barr virus and complexed with protein are precipitated by antibodies from patients with systemic lupus erythematosus. *Proc. natl Acad. Sci. USA*, **78,** 805.

LERNER, M.R., BOYLE, J.A., HARDIN, J.A. & STEITZ, J.A. (1981b) Two novel classes of small ribonucleoproteins detected by antibodies associated with lupus erythematosus. *Science*, **211,** 400.

LISCHWE, M.A., OCHS, R.L., REDDY, R., COOK, R.G., YEOMAN, L.C., TAN, E.M., REICHLIN, M. & BUSCH, H. (1985) Purification and partial characterization of a nucleolar scleroderma antigen ($M_r = 34{,}000$; pI, 8·5) rich in N^G, N^G-dimethylarginine. *J. biol. Chem.* **260,** 14304.

MATTIOLI, M. & REICHLIN, M. (1974) Heterogeneity of RNA protein antigens reactive with sera of patients with systemic lupus erythematosus. *Arthritis Rheum.* **17,** 421.

RADAR, M.D., O'BRIEN, C., YUNSHANG, L., HARLEY, J.B. & REICHLIN, M. (1989) Heterogeneity of the Ro/SSA antigen: different molecular forms in lymphocytes and red blood cells. *J. clin. Invest.* **83,** 1293.

REICHLIN, M. (1986) Significance of the Ro antigen system. *J. clin. Immunol.* **6,** 339.

REICHLIN, M. & WOLFSON-REICHLIN, M. (1989) Autoantibodies to the Ro/SS-A particle react preferentially with the human antigen. *J. Autoimmun.* **2,** 359.

REICHLIN, M., RADER, M. & HARLEY, J.B. (1989) Autoimmune response to the Ro/SSA particle is directed to the human antigen. *Clin. exp. Immunol.* **76,** 373.

ROSA, M.D., GOTTLIEB, E., LERNER, M.R. & STEITZ, J.A. (1981) Striking similarities are exhibited by two small Epstein-Barr virus-encoded ribonucleic acids and the adenovirus-associated ribonucleic acids VAI and VAII. *Mol. Cell Biol.* **1,** 785.

SHARPE, N.G., ISENBERG, D., WILLIAMS, D.G. & LATCHMAN, D.S. (1989) Elevation of the levels of SmB, B′ and D proteins but not that of the La (SS-B) antigen during HSV infections. *J. Autoimmun.* **2,** 701.

TAN, E.M. (1982) Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. *Adv. Immunol.* **33,** 167.