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Production of a monoclonal antibody to a membrane antigen of human T-cell leukaemia virus (HTLV1/ATLV)-infected cell lines from a systemic lupus erythematosus (SLE) patient: serological analyses for HTLV1 infections in SLE patients

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SUMMARY

Human T-cell leukaemia virus (HTLV1/ATLV), which causes adult T cell leukaemia (ATL), is an infectious, lymphotrophic retrovirus unique for humans. The present study was undertaken to determine whether HTLV1 had any pathogenetic role for systemic lupus erythematosus (SLE). The incidence of antibodies to ATL cell-associated antigens (ATLA) in sera from patients with SLE and other collagen diseases was investigated by an indirect immunofluorescent cytoplasmic staining of an HTLV1-infected cell line (MT-1). A radioimmunoassay was also performed to detect antibodies to HTLV1 protein and crude membrane faction derived from an HTLV1-producing cell line MT-2. Furthermore, an Epstein-Barr virus (EBV)-transformed B cell line (ES-1) was constructed from an SLE patient, which produced a monoclonal antibody (IgG,λ) reactive to an HTLV1-related cell-membrane antigen expressed on MT-1 and MT-2 cells. The specific reactivity of the monoclonal antibody was analysed by an indirect immunofluorescent cell-membrane staining and a microcytotoxicity test. The incidence of anti-ATLA antibodies was not different among SLE and other collagen diseases. The monoclonal antibody produced by ES-1 stained and killed HTLV1-infected cell lines specifically, but did not react with other human lymphoid cell lines. This monoclonal antibody failed to react with peripheral blood mononuclear cells (PBMC), mitogen-induced T cell blasts, and iododeoxyuridine-treated T cells from SLE patients. Thus, a possible role of HTLV1 in the aetiology of SLE was not established.

Keywords human T cell leukaemia viruses systemic lupus erythematosus human monoclonal antibody

INTRODUCTION

A possible role of viruses in the aetiology of systemic lupus erythematosus (SLE) has been regarded as an attractive hypothesis (Taral, 1971; Ziff, 1971; Philips, 1975; Schwartz, 1975; Pincus, 1982). Most clinical findings in SLE as well as the associated immunological abnormalities of impaired T cell functions and increased humoral immunity can be seen in known viral infections (Notkins, Mergnehagen & Haward 1970), and elevated titres of antibodies in SLE sera to many viruses, e.g. cytomegalovirus, herpes simplex, and measles virus, have been reported (Pincus, 1982). Furthermore, renal biopsies of SLE patients sometimes reveal tubuloreticular structure, which are not a

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specific viral structure, but are associated with many viral infections (Gyorkey, Min & Sinkovics, 1969; Hurd *et al.*, 1969; Norton, 1969; Pincus *et al.*, 1970). The rationale for the possible role of viruses in SLE was strengthened further by the expression of infectious retroviruses in New Zealand mice with features of SLE (East, Prossor & Holborrow, 1967; Mellors & Huany, 1966). These mice show high levels of immune complexes containing the retroviral structural protein gp70 which are associated with lupus nephritis (Yoshiki *et al.*, 1974; Lerner *et al.*, 1976). Extensive efforts have been undertaken to identify a specific retrovirus in SLE on the basis of clinical, histological, and immunological features described above. However, no evidence for a particular virus infection in SLE was revealed by studies using serology, electron microscopy, and isolation techniques of viruses.

Recently, three species of human retroviruses, HTLV1/ATLV, HTLV2, and HTLV3, were isolated from patients with adult T cell leukaemia by Yoshida, Miyoshi & Hinuma (1982), and hair cell leukaemia by Kalyanaraman *et al.* (1982), and acquired immune deficiency syndrome (AIDS) by Popovic *et al.* (1984), respectively. In this study, the possible involvement of HTLV1 in the aetiology of SLE was investigated.

MATERIALS AND METHODS

Sera. Sera were obtained from 10 patients with SLE, 14 with rheumatoid arthritis (RA), four with mixed connective tissue disease (MCTD), three with Behçet's disease, four Sjögren's syndrome, and two with dermatomyositis (DM) in the Nagasaki area. All patients with SLE and RA included in this study fulfilled the criteria for SLE and RA defined by the American Rheumatism Association (Ropes *et al.*, 1958; Tan *et al.*, 1982).

Tumour cell lines. Two cell lines, MT-1 and MT-2, infected with HTLV1 were used primarily. The MT-1 cell line was derived from peripheral blood leukaemia cells from a patient with ATL (Hinuma *et al.*, 1981), and the MT-2 cell line was established from cord blood lymphocytes co-cultivated with leukaemia cells from a patient with ATL (Miyoshi *et al.*, 1981). Both of these cell lines express HLA-DR antigen and Tac antigen (Uchiyama, Border & Waldmann, 1981) on the surface and ATLA in the cytoplasm. The other human lymphoid cell lines used were CCRF-CEM, MOLT-3, MOLT-4, Raji, Daudi, and K562. Their characteristics are listed in Table 3. All of these cell lines were cultured in PRMI 1640 (GIBCO, Grand Island, New York, USA) medium with 10% fetal calf serum (FCS: GIBCO).

Indirect immunofluorescent cytoplasmic staining. Indirect immunofluorescent staining of MT-1 cytoplasm with the patients' sera was performed in accordance with the procedure described by Hinuma *et al.* (1981). Briefly, MT-1 cells were smeared on glass slides, fixed with acetone, and treated with the patients' sera diluted at 1/10 in phosphate buffered saline (PBS), 0·01 M, pH 7·2. After washing, smears were treated with fluorescence-conjugated goat anti-human immunoglobulin G (IgG) (Miles Laboraties, Inc., Elkhart, Indiana, USA) and examined with a Zeiss microscope.

Indirect immunofluorescent cell-membrane staining. MT-1 cells (5×10^5) were incubated with the patients' sera (1/10 dilution in PBS) for 30 min on ice. The cells were then stained with fluorescence-conjugated goat anti-human IgG antibodies for another 30 min on ice. After washing, the cells were analysed on the Fluorecence-activated cell-sorter (FACS IV, Beckton-Dickinson, Mountain View, California, USA).

Preparation of solubilized HTLV protein and MT-2 cell membranes. HTLV1 particles in MT-2 culture supernatant were precipitated with 50% ammonium sulphate saturation and purified by centrifugation through a 25–60% sucrose gradient. The virus particles were then disrupted in lysis buffer (10 mM Tris-HCl, pH 8·0, 0·5% Triton X-100, 0·5% sodium deoxycholate, 1 mM EDTA, 1 mM phenylmethyl sulfonyl fluoride). MT-2 cells were also disrupted in the lysis buffer, and the supernatant containing the membrane fraction was collected by centrifugation at 10,000 **g** for 15 min. These solubilized materials were then diluted with PBS and adjusted at the concentrations of 1 μg/ml and 1 mg/ml, respectively.

Solid-phase radioimmunassay. A modified version of a previously published solid-phase radioimmunoassay (Rosenthal, Hayashi & Notkins, 1972) was employed. Ninety-six-well flexible polystyrene plates (Cooke Laboratory Products Division, Dynatech Laboratories, Inc., Alexan-

dria, Virginia, USA) were coated with the solubilized viral protein (50 ng/well) and cell-lysate (50 μg/well), respectively. 40 μl of each serum sample (1/10 dilution in PBS) were added in triplicate to wells of antigen-coated plates and incubated for 1 h at room temperature. After washing the wells with 1% BSA in PBS, 40 μl of ^{125}I-labelled goat anti-human IgG was applied to each well for 1 h at room temperature. Unbound radiolabelled detector was aspirated and washed three times with 1% BSA in PBS. The wells were then cut apart and counted in a gamma counter. The goat anti-human IgG (Cappel Laboratories, Downington, PA) was radiolabelled by the chloramine T method as described elsewhere (Kurata *et al.*, 1984).

Preparation of peripheral blood T-cells and non-T cells. Six patients with SLE and five normal volunteers were used as cell donors. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Conray gradient centrifugation. PBMC were pelleted with neuraminidase-treated sheep erythrocytes and the rosette-forming T cells in the pellet were separated from the non-T cell fraction by second-density gradient. Sheep erythrocytes were lysed with 0·83% ammonium chloride in Tris buffer (0·05 M, pH 7·2).

Generation of mitogen-induced T cell blasts and treatment of T cells with 5-iodo-2′-deoxyuridine. Cell suspensions of peripheral blood T cells at a density of 2×10^6 cells/ml were cultured for 72 h with 10 μg/ml of concanavalin A (Con A: DIFCO Laboratories, Detroit, Michigan, USA), 1 μg/ml of phytohaemoagglutinin (PHA: DIFCO), or 50 μg/ml of 5-iodo-2′-deoxyuridine (IdUrd: Sigma Chemical Company, St. Louis, Missouri, USA). All cells were then washed three times with PPMI 1640 containing 2% FCS.

Epstein-Barr virus (EBV) infection and culture conditions. The transforming B95-8 substrain of EBV was used to immortalize the lymphocytes (Steinitz *et al.*, 1977). The PBMC (2×10^7 cells) from an SLE patient were incubated for 2 h at 37°C in 20 ml of supernatant from the EBV-producing cell line (B95-8) cultures. Infected cells were cultured at a density of 1×10^5 cells/0·1 ml in Linbro 96-well microtitre plates (Flow Laboratories, Inc., McLean, Virginia, USA) in RPMI 1640 supplemented with 10% heat-inactivated FCS (GIBCO), 40 mM L-GLUTAMINE, 100 U/ml penicillin and 100 μg/ml streptomycin. The cells were nourished by replacing half of the culture medium twice a week.

Screening and cloning. Screening was performed by indirect immunofluorescent cell-membrane staining as described above. Instead of patients' sera, 50 μl of supernatant from cultures was added to 1×10^6 MT-1 cells. EBV-transformed cells from positive wells were cloned by limiting dilution on Linbro 96-well microtitre plates on feeder layers consisting of human PBMC (5×10^5/well) treated with mitomycin C (25 μg/ml) for 30 min at 37°C. The cloning procedure was performed twice in each positive well, and finally, a clone (ES-1) was established. The immunoglobulin isotype of the monoclonal antibody secreted by ES-1 was determined by Ouchterlony's technique using the culture supernatant of ES-1 and class-specific rabbit anti-human immunoglobulin antisera (Miles Laboratories).

Analysis of specific reactivity of the ES-1 culture supernatant against HTLV1-infected cells. The reactivity of the ES-1 culture supernatant against variously prepared cells was analysed by the following three procedures: the culture supernatant of ES-1, instead of patients' sera, was reacted with acetone-fixed smears of MT-1 and MT-2 cells and analysed by the same manner as described above; indirect immunofluorescent cell-membrane staining and a microcytotoxicity test were also performed using the human lymphoid cell lines, PBMC, mitogen-induced T cell blasts IdUrd-treated T-cells from SLE patients and normal donors as targets.

Microcytotoxicity test. 20 μl of the ES-1 culture supernatant were mixed with the same volume of the cell suspensions (1×10^6/ml) and incubated in 96-well V-bottom Linbro plates (Flow Laboratories) for 30 min at room temperature. After washing, the cells were reacted with 20 μl of selected rabbit complement for 30 min at 37°C, and then stained by trypan blue. Percentage lysis was calculated using the following formula:

$$\text{Percentage lysis} = \left(a \sqrt{b}\right) \times \left(\frac{1}{1000-b}\right) \times 100$$

where a = percentage of cells stained by trypan blue after incubation with the culture supernatant and complement.

b = percentage of cells stained after incubation with complement alone.

RESULTS

The incidence of HTLV1 infection in patients with SLE and other collagen diseases

Sera from 10 patients with SLE and patients with other collagen diseases were screened of the presence of antibodies to cytoplasmic and surface antigens of MT-1 cells by indirect immunofluorescent methods (Tables 1 and 2). The serum from an SLE patient, two sera from RA patients, and serum from a patient with Sjögren's syndrome stained the cytoplasmic antigen(s) in a small portion (2–5%) of MT-1 cells. There was no difference in the fluorescent pattern and the percentage of stained cells among these three sera. Furthermore, these characteristics in the staining of MT-1 cytoplasm are quite similar to the staining pattern of ATL cell-associated antigen (ATLA) with sera from ATL patients described by Hinuma *et al.* (1981). Thus, it is reasonable to consider that these four patients with SLE, RA, or Sjögren's syndrome were infected with HTLV1 and had anti-ATLA antibodies in their sera. The sera from seven SLE patients, including a patient with anti-ATLA antibodies, stained the membrane of MT-1 cells as shown in Table 1, whereas the only two sera from RA patients, who also had anti-ATLA antibodies, reacted as shown in Table 2. In the other collagen diseases, three patients with MCTD, and a patient with Sjögren's syndrome also had membrane-reactive (cytophilic) antibodies, but patients with Behçet's disease and DM did not. The positivity rate for cytophilic antibodies in SLE was significantly higher than healthy adults ($\chi^2=5{\cdot}80$, $P<0{\cdot}05$) in the sign test (Mainland, 1963). The right columns of Tables 1 and 2 show the results of the solid-phase radioimmunoassay detecting antibodies against viral protein and membrane lysates derived from MT-2 cells. In the radioimmunoassay, sera were judged to be positive when the counts were greater than mean values plus 2 s.d. of the counts of 32 normal sera. These controls did not have any antibodies reactive to ATLA and membrane antigens of MT-1 cells in the indirect immunofluorescent staining analyses. Four sera from patients with SLE, RA, or Sjögren's syndrome, who had anti-ATLA antibodies, reacted with viral protein. Furthermore, the sera from seven SLE, two RA, and three MCTD patients, who had cytophilic antibodies against MT-1 cells, reacted with the membrane fraction of MT-2 cells. Thus, no difference was observed between the

Table 1. Reactivity of SLE sera with HTLV1, ATLA, and membrane antigens in HTLV1-infected cell lines

		Staining*		Radioimmunoassay†	
Patient	Disease	ATLA	Membrane	HTLV1	Membrane
M.S.	SLE	−	+	1,598‡	4,328
H.I.	SLE	−	+	1,534	2,916
R.W.	SLE	−	+	1,131	3,105
Y.M.	SLE	−	−	1,539	2,000
T.S.	SLE	−	−	1,335	1,630
N.S.	SLE	−	−	1,409	1,815
U.I.	SLE	−	+	1,066	2,731
M.M.	SLE	+	+	3,652	2,919
T.I.	SLE	−	+	1,330	1,334
Y.S.	SLE	−	+	1,306	2,598
Normal ($n=32$)		(mean ± s.d.)		1,420 ± 410	1,360 ± 350

* Indirect immunofluorescent staining for cytoplasmic antigen and membrane antigen of MT-1 cells.

† Radioimmunoassay for reactivity of sera with HTLV1 and membrane fraction derived from MT-2 cells.

‡ Count per minute: the counts greater than mean values plus 2 s.d. of the control counts were judged as positive.

Table 2. Reactivity of sera from patients with collagen diseases, except for SLE, with ATLV, ATLA, and membrane antigens of ATLV-infected cell lines.

		Staining		Radioimmunoassay*	
Patient	Diseases	ATLA	Membrane	ATLV	Membrane
Y.O.	RA	−	−	−	−
M.W.	RA	−	−	−	−
W.U.	RA	+	+	+	+
M.M.	RA	−	−	−	−
T.F.	RA	−	−	−	−
H.F.	RA	−	−	−	−
E.T.	RA	−	−	−	−
K.Y.	RA	−	−	−	−
M.F.	RA	+	+	+	+
F.I.	RA	−	−	−	−
N.A.	RA	−	−	−	−
S.K.	RA	−	−	−	−
K.Y.	RA	−	−	−	−
E.O.	RA	−	−	−	−
S.T.	Sjögren	−	−	−	−
M.E.	Sjögren	+	+	+	+
K.I.	Sjögren	−	−	−	−
A.N.	Sjögren	−	−	−	−
Y.S.	MCTD	−	+	−	+
U.I.	MCTD	−	+	−	+
K.M.	MCTD	−	+	−	+
T.K.	MCTD	−	−	−	−
M.M.	Behçet	−	−	−	−
H.H.	Behçet	−	−	−	−
H.M.	Behçet	−	−	−	−
K.T.	DM	−	−	−	−
S.K.	DM	−	−	−	−

* + Represents positive binding when counts exceeded the mean value plus 2 s.d. (shown in Table 1).

results of indirect immunofluorescent methods and solid-phase radioimmunoassay. Hinuma *et al.* (1981) reported that the incidence of anti-ATLA antibodies in healthy adults was 10–30% in an ATL-endemic area where Nagasaki is located (Tajima *et al.*, 1979; Hinuma *et al.*, 1981). We also studied for the presence of anti-ATLA antibodies about more than 200 healthy adults by the indirect immunofluorescent methods, and the incidence was around 13%. In this study, anti-ATLA antibodies were detected in 1 of 10 SLE patients (10%) and 2 of 14 RA patients (14%) in the Nagasaki area. There appeared to be no difference in the incidence of ATLV infections among SLE patients, RA patients and healthy adults, although only a limited number of sera have been tested.

Establishment of an EBV-transformed cell line from an SLE patient

An EBV-transfomed line (ES-1) was established from an SLE patient (H.I. in Table 1) who had antibodies reactive with membranes of HTLV1-infected cells, but did not have anti-ATLA antibodies in her serum. It was shown that almost all of ATL patients have anti-ATLA antibodies, but there is no evidence at present that HTLV1-infected individuals always have anti-ATLA in their

sera with sufficient titres for ordinary detection. The patient, H.I., was selected as the donor of PBMC, considering the possibility of a latent infection of HTLV1 in SLE which was not sufficient to generate high titres of anti-ATLA antibodies. Alternately, SLE patients might be genetically restricted and respond to HTLV1-infections in a different manner from ATL patients and normal individuals.

ES-1 secreted the monoclonal antibody (IgG,λ) which recognizes a surface antigen of HTLV1-infected cell lines (MT-1 and MT-2) as shown below.

The specific reactivity of the monoclonal antibody from ES-1 against HTLV1-infected cell lines
The reactivity of the monoclonal antibody produced by ES-1 against various human lymphoid cell lines was tested by indirect immunofluorescent cell-membrane staining and microcytotoxicity tests. The results are shown in Table 3. The monoclonal antibody stained surface membranes of HTLV1-infected cell lines (MT-1 and MT-2), but not other T-cell lines (MOLT 3, MOLT 4, and CCRF-CEM), B-cell lines (Raji, Daudi), and a myeloid line (K562). The supernatant of ES-1 was also specifically reactive with an HTLV1-infected line (MT-1) in the microcytotoxicity assay. 23% of MT-1 cells were lysed by the monoclonal antibody, whereas other ATLA-negative cell lines were lysed less than 5%. Figure 1 shows fluorescent profiles of lymphoid cell lines analysed by FACS IV. Both of the HTLV1-infected cell lines, MT-1 and MT-2, were stained by the culture supernatant of ES-1. MT-2 cells, which were actually secreting HTLV1 into the culture supernatant, appeared to show stronger immunofluorescence than MT-1 cells. Thus, it was considered that the monocolonal antibody, produced by ES-1, was reactive with an HTLV1-related antigen expressed on the membrane of HTLV1-infected cell lines.

The reactivity of the monoclonal antibody produced by ES-1 to variously prepared PBMC
The reactivity of the monoclonal antibody was tested for T cells, non-T cell, mitogen (Con A and PHA)-induced T cell blasts, and IdUrd-treated T cells from five SLE patients and five normal donors by indirect immunofluorescent cell-membrane staining and microcytotoxicity tests. Three of the five SLE patients (M.S., R.W., and Y.S.) had antibodies reactive to membrane antigens of HTLV1-infected cell lines, and the others (Y.M. and N.S.), did not as shown in Table 1. Unfortunately, we could not study the patient (M.M.) who had antibodies reactive to HTLV1-protein and membrane of infected cells in her serum, because she dropped out of our follow-up. IdUrd (Lowy *et al.*, 1971) and some lectins (Moroni & Shumann, 1975) were reported to be

Table 3. Specificity of monoclonal antibody produced by EBV-transformed cell line ES-1 for ATLV-infected cells by immunofluorescence and microcytotoxity tests.

Cell lines	Characters*	Staining†	Cytotoxicity‡
CCRF-CEM	T (ATLA$^-$,Ia$^-$,EBNA$^-$)	−	<5
MOLT3	T (ATLA$^-$,Ia$^-$,EBNA$^-$)	−	<5
MOLT 4	T (ATLA$^-$,Ia$^-$,EBNA$^-$)	−	<5
MT1	T (ATLA$^+$,Ia$^+$,EBNA$^-$)	+	23
MT2	T (ATLA$^+$,Ia$^+$,EBNA$^-$)	+	ND
Raji	B (ATLA$^-$,Ia$^+$,EBNA$^+$)	−	<5
Daudi	B (ATLA$^-$,Ia$^+$,EBNA$^+$)	−	<5
K562	M (ATLA$^-$,Ia$^-$,EBNA$^-$)	−	<5

* T: T-cell line, B: B-cell line, M: myeloid cell line, ATLA: ATL-associated antigen, Ia: HAL-DR gene product, EBNA: EBV-nuclear antigen.

† Indirect immunofluorescent membrane-staining of various human lymphoid cell lines.

‡ Percentage cytotoxicity.

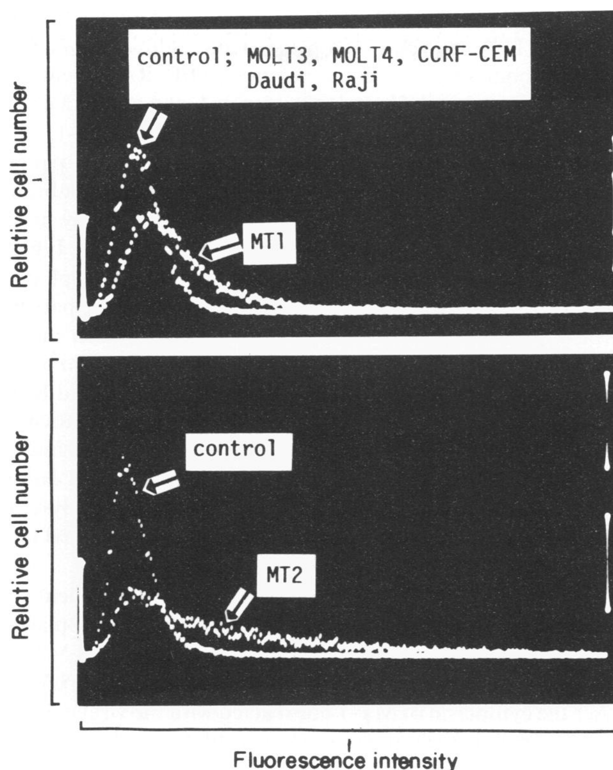

Fig. 1. Flow fluorocytometric analysis of MT-1 and MT-2 cells stained with the monoclonal antibody produced by ES-1 using indirect immunofluorescence (FITC-labelled goat anti-human IgG); *x*-axis: green fluorescence intensity; *y*-axis: relative number of cells. Both cells were stained, and MT-2 showed stronger immunofluorescence than MT-1.

chemical compounds which could induce endogenous infectious retroviruses in murine lymphocytes. It was further shown that IdUrd could induce HTLV1 in MT-1 and increase the expression of ATLA in the cytoplasm (Hinuma *et al.*, 1981). The culture supernatant of ES-1 did not react with IdUrd-treated T cells and mitogen-induced T cell blasts nor with non-treated T cells and non-T cells from SLE patients and normal donors.

DISCUSSION

HTLV1-infected cells with ATLA in cytoplasm expressed surface markers (OKT 1, 3, 4, 10, and 11) of inducer-T cells and showed suppressor activity in an *in vitro* antibody production system co-cultivating with normal PBMC (Uchiyama *et al.*, 1978). Furthermore, Morimoto *et al.* (personal communication) stated that leukaemia cells from ATL patients could actually induce suppressor T-cells from normal donors *in vitro*. Therefore, it appeared possible that HTLV1 preferentialy infects T-cells in suppressor lineage and elicits the production of lymphocytotoxic antibodies which recognize membrane antigens of infected T-cells in SLE patients. Thus, the immunological abnormalities in SLE, e.g. decreased T cell numbers, impaired T cell function, and existence of lymphocytotoxic antibodies against suppressor T cells (Sakane *et al.*, 1979; Morimoto *et al.*, 1980), could be explained. On the basis of this hypothesis, we examined the reactivity of SLE sera with MT-1 cells using indirect immunofluorescent methods and radioimmunoassay.

Seven sera out of 10 from SLE patients stained membranes of MT-1, whereas serum from only one patient reacted with ATLA in MT-1 cytoplasm with indirect immunofluorescence. The incidence of anti-ATLA antibodies was not different among SLE, RA patients, and normal adults in the Nagasaki area which located in ATL-endemic area in Japan as alluded to earlier. However, four SLE sera without anti-ATLA antibodies stained membranes of MT-1, whereas no RA sera without anti-ATLA antibodies reacted. Furthermore, sera from ATL patients and HTLV1-carriers, who had antibodies reactive to ATLA, failed to react with membranes of MT-1 cells, although these sera reacted with membranes of MT-2 cells which actually produced viruses and expressed a lot of viral envelope antigens on their surface (data not shown). It has been reported by several investigators that RA patients as well as SLE had lymphocytotrophic antibodies in their sera, which could play a role in modulating the immune responses of these patients (Terasaki *et al.*, 1970; Browning *et al.*, 1977). However, it has not yet been defined whether the loss of tolerance to lymphocyte antigen in patients with SLE and RA is due to the modification of the antigens by any specific agent such as viruses, or due to spontaneously occurring polyclonal B-cell activation. The cytotrophic antibodies against HTLV1-infected cells in the seven SLE patients might have been generated by polyclonal activation of B-cells unrelated to specific HTLV1 infections. However, it might be possible that the SLE patients were genetically restricted and had different immune responses against HTLV1 infections than ATL patients. In order to analyse this possibility, we established a B-cell line from an SLE patient, which secreted a monoclonal antibody reactive to HTLV1-infected cells.

An EBV-transformed cell line (ES-1) was established from an SLE patient who had antibodies reactive to membranes of HTLV1-infected cells but not to ATLA in the cytoplasm. The monoclonal antibody (IgG,λ) produced by ES-1 reacted with membranes of MT-1 and MT-2, but did not react with other human lymphoid cell lines which were not infected with HTLV1. This monoclonal antibody stained neither the cytoplasm of MT-1 nor reacted with the viral protein derived from the culture supernatant of MT-2 cells in the radioimmunoassay (data not shown). MT-1 and MT-2 cells express various antigens on their surface, e.g. differentiation antigens of human T-cells, HLA-DR products and Tac-antigen. The monoclonal antibody did not react with peripheral T-cells which express T-cell differentiation antigens, non-T cells which express HLA-DR products, nor mitogen-induced T-cell blasts which express both HLA-DR products and Tac antigen. Thus, the monoclonal antibody specifically reacted with an HTLV1-related antigen expressed on surface membranes of HTLV1-infected cell lines. It was not demonstrated here whether the antigens, recognized by the monoclonal antibody, on MT-1 and MT-2 cells were identical. However, it should be reasonable to consider that these cells expressed same antigen related to HTLV1 and the monoclonal antibody reacted with an antigenic determinant of the antigen, because the antigenic determinant expressed more on MT-2 cells, i.e. actual virus-producer, than on MT-1 cells, i.e. non-producer, as shown in Fig. 1. There are many studies about anti-viral antibodies in SLE sera, however, this is the first report demonstrating the existence of a B-cell clone in SLE, which produced an antibody against a retrovirus-related membrane antigen in infected cells. Lee *et al.* (1984) showed that specific antibodies to cell membrane antigens found on HTLV-infected cells, included two glycoproteins of 61 and 45 kDa, have been detected in ATL patients and in asymptomatic carriers, using a live cell-membrane immunofluorescence assay and radioimmunoprecipitation. It is not defined at present which antigen the monoclonal antibody recognizes, because it is difficult to get sufficient amount of antibody from the EBV-transformed cell line for an immunoprecipitation analysis.

The reactivity of the monoclonal antibody was tested against PBMC treated or non-treated with non-specific mitogens (Con A, PHA) or IdUrd. Con A is a mitogen which can generate suppressor T cells *in vitro*, and IdUrd is a chemical which can induce endogenous retroviruses in lymphocytes. The monoclonal antibody did not react with non-treated PBMC nor with mitogen- or IdUrd-treated T cells from SLE patients. These results seem to indicate that HTLV1 does not play a pathogeneic role for SLE. However, the SLE patients could have been infected with HTLV1, but the T-cell subsets which was infected could have eliminated. Or, the patients could have lost the virus while the pathophysiologic consequence of the infection remained. This is true in AIDS where there is a lower incidence of isolation of HTLV3 in seropositive patients. A failure to induce

HTLV1-associated membrane antigens in the IdUrd-treated lymphocytes of SLE patients is not the same as the absence of past infection and subsequent loss of the virus. It would be important to continue studies that analyse possible infections of known retroviruses including HTLV1 in SLE patients.

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REFERENCES

BROWNING J.D., DICK H.M., EL-GHOBEREY A. & DICK W.C. (1977) Levamisole, rheumatoid arthritis and cold lymphocytotoxic antibodies (letter. *Lancet*, **11**, 820.

EAST J., PROSSOR P.R. & HOLBORROW E.J. (1976) Autoimmune reactions and viirus-like particles in germ-free NZB mice. *Lancet*, **i**, 755.

GYORKEY F., MIN K.W. & SINKOVICS J.G. (1969) Systemic lupus erythematosus and myxovirus. *New Engl. J. Med.* **280**, 333.

HINUMA Y., NAGATA K., HANAOKA M., NAKAI M., MATSUMOTO T., KINOSHITA K.I., SHIRAKAWA S. & MIYOSHI I. (1981) Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. natn. Acad. Sci. USA.* **78**, 6476.

HURD E.R., EIGENBRODT E., ZIFF M. & STRUNK S.W. (1969) Cytoplasmic tubular structures in kidney biopsies in systemic lupus erythematosus. *Arth. Rheum.* **12**, 541.

KALYANARAMAN V.S., SARNGADHARAN M.G., MIYOSHI I., BLAYNEY D., GOLDE D. & GALLO R.C. (1982) A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science*, **218**, 571.

KURATA A., OHTA K., MINE M., FUKUDA T., IKARI N., KANAZAWA H., MATSUNAGA M., IZUMI M. & NAGATAKI S. (1984) Monoclonal antihuman thyroglobulin antibodies. *J. Clin. Endoc. Metab.* **59**, 573.

LEE T.H., COLIGAN J.E., HOMMA T., MCLANE M.F., TACHIBANA N. & ESSEX M. (1984) Human T-cell leukemia virus-associated membrane antigens: Identity of the major antigens recognized after virus infection. *Proc. natn. Acad. Sci. USA.* **81**, 3856.

LERNER R.A., WILSON C.B., DELVILLANO B.C., MCCONAHEY P.J. & DIXON F.J. (1976) Endogenous oncornaviral gene expression in adult and fetal mice: quantitative, histologic and physiologic studies of the major viral glycoprotein, gp70. *J. exp. Med.* **143**, 151.

LOWY D.R., ROWE W.P., TEICH N. & HARTLEY J.W. (1971) Murine Leukemia virus: high-frequency activation *in vitro* by 5′-iododeoxyuridine and 5′-bromodioxyuridine. *Science*, **174**, 155.

MAINLAND D. (1963) In *Elementary Medical Statistics* (ed. by D. Mainland) pp. 204–206. W. B. Saunders, Philadelphia and London.

MELLORS R.C. & HUANG R.J. (1966) Immunopathology of NZB/Bl mice. V. Virus-like (filtrable) agent separable from lymphoma cells and identifiable by electron microscopy. *J. exp. Med.* **124**, 1031.

MIYOSHI I., KUBONISHI I., YOSHIMOTO S., AKAGI T., OHTSUKI Y., SHIRAISHI Y., NAGATA K. & HINUMA Y. (1981) Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukemic T-cells. *Nature*, **294**, 770.

MORIMOTO C., REINHERTZ E.L., ABE T., HOMMA M. & SCHLOSSMAN S.F. (1980) Characteristics of anti-T cell antibodies in systemic lupus erythematosus: Evidence for selective reactivity with normal suppressor cells defined by monoclonal antibodies. *Clin. Immunol. Immunopathol.* **16**, 474.

MORONI C. & SCHUMANN G. (1975) Lipopolysaccharide induced C-type virus in short term cultures of BALB/C spleen cells. *Nature*, **254**, 60.

NORTON W.L. (1969) Endothelial inclusions in active lesions of systemic lupus erythematosus. *J. Lab. Clin. Med.* **74**, 369.

NOTKINS A.L., MERGNEHAGEN S.E. & HAWARD R.J. (1970) Effect of virus infection on the function of the immune system. *Ann. Rev. Microbiol.* **24**, 525.

PHILLIPS P.E. (1975) The role of viruses in systemic lupus erythematosus. *Clin. Rheum. Dis.* **1**, 505.

PINCUS T. (1982) Studies regarding a possible function for viruses in the pathogenesis of systemic lupus erythematosus. *Arth. Rheum.* **25**, 847.

PINCUS T., BLACKLOW N.R., GRIMLEY P.M. & BELLANTI J.A. (1970) Glomerlular microtubles of systemic lupus erythematosus. *Lancet*, **ii**, 1059.

POPOVIC M., SARNGADHARAN M.G., READ E. & GALLO R.C. (1984) Detection, isolation, and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. *Science*, **224**, 497.

ROPES M.W., BENNET G.A., COBB S., JACOX R. & JESSAR R.A. (1958) revision of diagnostic criteria for rheumatoid arthritis. *Bull. Rheum. Dis.* **9**, 175.

ROSENTHAL J.D., HAYASHI K. & NOTKINS A.L. (1972) Rapid micro-radioimmunoassay for the measurement of antiviral antibody. *J. Immunol.* **109**, 171.

SAKANE T., STEINBERG A.D., REEVES J.P. & GREEN I. (1979) Studies of immune functions of patients with systemic lupus erythematosus. Complement-dependent immunoglobulin M anti-thymus-derived cell antibodies preferentially inactivate suppressor cells. *J. clin. Invest.* **63**, 954.

SCHWARTZ R.S. (1975) Viruses and systemic lupus erythematosus. *New Engl. J. Med.* **293**, 132.

STEINITZ M., KLEIN G., KOSKIMIES S. & MÄKELÄ O. (1977) EB virus-induced B lymphocyte cell lines producing specific antibody. *Nature*, **269**, 420.

TAJIMA K., TOMINAGA S., KUROISHI T., SHIMIZU H. &

SUCHI T. (1979) Geographic features and epidemiological approach to endemic T-cell leukemia/lymphoma in Japan. *Jpn. J. Clin. Oncol.* **9,** 495.

TAN E.M. COHEN A.S., ERIES J.F., MASI A.T., MCSHANE D.J., ROTHFIELD N.F., SHALLER J.G., TARAL N. & WINCHESTER R.J. (1982) The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arth. Rheum.* **25,** 1271.

TARAL N. (1971) Immunologic and viral factors in the pathogenesis of systemic lupus erythematosus. *Arth. Rheum.* **13,** 887.

TERASAKI P.I., MOTTIRONI V.D. & BARNETT E.V. (1970) Cytotoxins in disease. Autocytotoxin in lupus. *New Engl. J. Med.* **283,** 724.

UCHIYAMA T., BORDER S. & WALDMANN T.A. (1981) A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T-cells. I. Production of anti-Tac monoclonal antibody and distribution of Tac(+) cells. *J. Immunol.* **126,** 1393.

UCHIYAMA T., SAGAWA K., TAKATSUKI K. & UCHINO H. (1978) Effect of adult T-cell leukemia cells on pokeweed mitogen-induced normal B-cell differentiation. *Clin. Immunol. Immunopathol.* **10,** 24.

YOSHIDA M., MIYOSHI I. & HINUMA I. (1982) Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc. natn. Acad. Sci. USA.* **79,** 2031.

YOSHIKI T., MELLORS R.C., STRAND M. & AUGUST J.T. (1974) The viral envelope glycoprotein of murine leukemia virus and the pathogenesis of immune complex glomerulonephritis of New Zealand mice. *J. exp. Med.* **140,** 1011.

ZIFF J. (1971) Viruses and the connective tissue diseases. *Ann. Intern. Med.* **75,** 951.