A search for retrovirus infection in systemic lupus erythematosus and rheumatoid arthritis

B K PELTON,¹ MARGARET NORTH,² R G PALMER,¹ WINSOME HYLTON,¹ CAROLINE SMITH-BURCHNELL,¹ ANNE L SINCLAIR,² M MALKOVSKY,² A G DALGLEISH,² AND A M DENMAN¹

From the ¹Connective Tissue Diseases Research Group, and the ²Division of Immunological Medicine, Clinical Research Centre, Watford Road, Harrow, Middlesex

SUMMARY Evidence for retroviral infection in general and human immunodeficiency virus (HIV) infection in particular was sought in freshly isolated peripheral blood T cells, B cells, and monocyte-macrophages from patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) and also in T cell and B cell lines established from the same source. Similar cells isolated from rheumatoid synovial membrane were also examined. The strategy used for the detection of virus was cocultivation with susceptible cell lines looking for syncytia formation, reverse transcriptase production, and nucleic acid hybridisation with HIV cDNA probes. No evidence for infection was obtained.

Retroviral infection has often been suggested as the aetiology for rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) but without experimental support. Interest in this possibility, however, is sustained by the many immunopathological similarities between these diseases and those caused by lentiviruses in animals.¹ In particular, caprine arthritis encephalitis virus induces synovitis in goats with many of the characteristic features of RA.²

We, therefore, sought evidence for retrovirus infection in patients with these diseases using sensitive methods of proved efficacy in known retroviral diseases, notably HIV infections.

Patients and methods

PATIENTS

Material was obtained from 25 patients with SLE, all of whom fulfilled the American Rheumatism Association criteria for diagnosis of this disease³ and who had active disease, i.e., activity scores >10 by criteria of Hollingworth *et al*.⁴ Their clinical features are summarised in Table 1. Twenty three patients were women and their age range was 18-53 years (mean 34 years). Material was also obtained from

one patient with systemic juvenile chronic arthritis, eight patients with classical RA, and five normal laboratory staff. In addition, mononuclear cells were isolated from two synovial effusions and two synovectomy specimens from patients with classical RA. None of these patients was receiving steroids or cytotoxic drugs. The cell preparations tested are listed in Table 2. Unseparated blood mononuclear cells from each donor were screened on three or more occasions. Where T cell or B cell colonies were tested, no less than 10 colonies a patient were tested.

Table 1 Clinical features of the patients with SLE (n=25)

Disease duration	3 mths-6 yrs (mean 3.2 yrs)
Vasculitis	17
Photosensitivity	12
Arthritis	17
Serositis	11
Respiratory involvement	19
Renal disease	19
CNS* disease	3
ANA* positive	25
DNA antibody positive	15
Hypergammaglobulinaemia	13
Hypocomplementaemia	9
Anaemia (Hb<100 g/l)*	24
Coombs' test positive	10
Thrombocytopenia	8

*CNS=central nervous system; ANA=antinuclear antibody; Hb=haemoglobin.

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Correspondence to Dr B K Pelton, Connective Tissue Diseases Research Group, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ.

Table 2 Indicator cells for screening different cell populations

Cell population	Indicator cells
T cells	} JM, H4, H9, CEM
T cell lines	
B cells	} Raji, JM, CEM
B cell colonies	
Macrophages	} JM, CEM
Macrophage colonies	

For details of the CD4 positive lines see Ref. 21.

PREPARATION OF MONONUCLEAR CELL POPULATIONS

Mononuclear cells were isolated from heparinised blood samples by density sedimentation on Ficoll-Hypaque. Mononuclear cells were isolated from synovectomy samples by an established technique⁵ and from synovial fluids by density gradient centrifugation.⁶ All cells were cultured in RPMI 1640 medium (Flow Laboratories Ltd) supplemented with 20% fetal calf serum.

T lymphocytes

T lymphocytes for short term culture were isolated by rosette formation with neuraminidase treated sheep red blood cells. These cells were stimulated with recombinant interleukin 2 (Biogen).

T cell colonies

T cell colonies were established using phytohaemagglutinin and interleukin 2 (Biotest Diagnostics) in a limiting dilution technique with a lymphoblastoid cell line as feeder cells. Clonality was verified using monoclonal T cell antibodies and analysis by fluorescence activated cell sorting. Cell lines were cocultured 20 days or more after they were established.

B lymphocytes

B cells for short term culture were obtained by depleting non-T cells of macrophages. The cells were stimulated with B cell growth factor.⁷ Their B cell nature was verified with anti-B cell and immunoglobulin monoclonal antibodies and fluorescence activated cell sorting analysis.

B cell colonies

B cell colonies were obtained by a modification of the technique of Trudgett *et al*⁸ using 10 µg/ml staphylococcal protein A (Pharmacia, Sweden), 5×10⁻⁵ mg/ml 2-mercaptoethanol (BDH Ltd, Poole) and growth factor derived from HMY2 cells⁸ in a limiting dilution technique. Their nature and

clonality was verified by the same technique as that used for short term B cell cultures.

When aggregates of more than 50 cells were observed morphologically, after about 20 or more days, they were removed by micropipetting for cocultivation.

Macrophages

Macrophages for short term culture were obtained from the T cell depleted preparation by adherence to plastic. Their identity was confirmed by characteristic morphology and phenotype determination.

Macrophage cell lines

Macrophage preparations were stimulated with a macrophage growth factor.⁹

ADDITIONAL TREATMENT OF SCREENED CELLS

At initiation cultures received bromodeoxyuridine (BUDR) (30 µmol) or monoclonal antihuman interferon-α (Wellcome Research Laboratories), or both, added at a neutralising titre of 1500 units of interferon. Polybrene (4 µg/ml) was also added when some cultures were established. BUDR induces retroviral gag gene expression.¹⁰ Anti-interferon antibody suppresses virus induced interferon production, which inhibits viral replication. Polybrene decreases cell surface negative charge, thereby aiding the release of negatively charged virus particles from the cell surface. These techniques have been successively used to assist viral detection.¹¹

VIRUS DETECTION

Cocultivation

Different cell populations were cultured for periods of up to 34 days in the presence of the appropriate indicator cells (Table 1).

Throughout this period the cocultures were examined morphologically and for the presence of reverse transcriptase activity at two to three day intervals.

Reverse transcriptase

Reverse transcriptase was measured by the method of Hoffman *et al*¹² using enzyme extracted from HIV infected cells as a positive control. This assay used poly (rA) oligo (dT) as template and Mg⁺⁺ divalent cation and is the most sensitive assay for detecting HIV induced enzyme. Unseparated mononuclear cells, T cells, B cells, or macrophages from six patients with SLE were cocultivated with the appropriate cell line (Table 1) before being assessed for the presence of reverse transcriptase enzyme.

Hybridisation

High molecular weight DNA was prepared from mononuclear cells, and restriction endonuclease digests were performed at 37°C for 16 hours on five µg DNA samples with Bgl II. Digested DNA was electrophoresed through a 0.7% agarose gel and partially hydrolysed by acid depurination before alkaline denaturation.¹³ The treated DNA was transferred to a nitrocellulose filter (Schleicher and Schull BA85 0.45 µm) by blotting with 20×SSC (0.15 M NaCl+0.015 M sodium citrate=1×SSC).

Hybridisations were performed at 68°C for 18 hours using ³²P labelled nick-translated HIV 9kb SacI/SacI insert from λBH-10.¹⁴ The probe was used at 1×10⁶ cpm/ml (specific activity approximately 2×10⁷ cpm/µg). Filters were washed three times for 30 min on each occasion at 65°C in 3×SSC containing 0.1% sodium dodecyl sulphate (SDS) followed by three washes in 1×SSC containing 0.1% SDS. Under these conditions the probe hybridises with HTLV-I and HTLV-II DNA and with most HIV isolates but not with the HTLV-I variant HTLV-Ib

Blots were exposed to Kodak x AR-5 film for seven days at -70°C. DNA from the cell line infected with HIV was used as a positive control.

Results

COCULTIVATION

Retrovirus sensitive cell lines cocultivated with mononuclear cells from these patients failed to show signs of syncytia formation consistent with infection by retroviruses or other viruses. Nor were any cytopathic effects seen after treatment with BUDR, anti-interferon antibody, or polybrene.

NUCLEIC ACID HYBRIDISATION

Proviral DNA sequences in SLE lymphocytes were sought by hybridisation with a cDNA probe for HIV, but none was detected (Fig. 1).

REVERSE TRANSCRIPTASE ASSAYS

Assays for reverse transcriptase were consistently negative, i.e., <800 cpm, whereas the positive control value always exceeded 11 000 cpm.

Discussion

Retroviruses induce neoplastic and immunodeficiency diseases in man and other species,¹⁵ whereas some lentiviruses, known to belong to this group, cause slow, immunopathological disorders in sheep and goats.² One such disorder induced by the lentivirus caprine arthritis encephalitis virus is of particular rheumatological interest,² and it is a reasonable postulate that analogous agents cause

Fig. 1 Hybridisation assay using HIV cDNA probe. Lane 1 shows molecular weight markers; lanes 2 and 10 show a typical restriction enzyme pattern of DNA from CEM HIV infected cells reactive with the HIV probe; lanes 3–9 show negative results with DNA isolated from lymphocytes of seven different patients with SLE.

similar diseases in man. In addition, the many immunopathological features in common between acquired immune deficiency syndrome (AIDS) and SLE,—namely, autoimmunity to red cells and platelets and polyclonal hypergammaglobulinaemia,¹⁶ encourage speculation that HIV or a closely related agent may be involved in the pathogenesis of SLE. So far, however, most attempts to detect retroviral infections in RA and SLE have proved negative. In particular, anti-HIV and anti-HTLV-I antibodies have not been found in patients with SLE.¹⁷ In contrast, Olsen *et al* have reported that the sera of 12 African patients with SLE were positive for anti-HTLV-I antibodies in titres ranging from 1/20 to 1/80 detected by immunofluorescence.¹⁸ Also five of seven sera from North American patients with SLE reacted with the gp 46 antigen of HTLV-I and gp 41 antigen of HIV by immunoblotting. Moreover, these authors reported that lymphocyte culture

supernatants from all five patients with SLE tested contained reverse transcriptase. Our experiments, however, have not confirmed these claims as culture supernatants did not contain significant amounts of reverse transcriptase. In additional experiments not described in this report we have also failed to detect DNA sequences of HTLV-I in SLE lymphocytes with cDNA probes. This latter negative result is in accord with the findings of Boumpas *et al.*¹⁷ As retroviral expression is restricted in cultures of human and other primate cells we used several methods of increasing viral expression but without success.

Our experiments so far have failed to incriminate retroviruses in the pathogenesis of RA and SLE. This hypothesis should not be lightly discarded, however, as the threshold of detection with probing techniques is one proviral DNA copy per 10 cells,¹⁹ and virus infection in these diseases may be below that level of detection²⁰ if only a minority of circulating lymphocytes or synovial lymphocytes are infected. Preliminary experiments may be needed to select and expand infected cells. We have attempted to address this point by cloning T and B lymphocytes and macrophages for screening. Furthermore, the putative retroviral infection may not be detected by the HIV cDNA probes used because, as indicated, not all known human retroviruses hybridise with these probes. There is incomplete homology between probes for lentiviruses in other species with different pathological consequences so that it is unreasonable to assume that probes for a human immunodeficiency virus would necessarily be appropriate for detecting viruses inducing prolonged immunopathological disorders.

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