# ABSENCE OF RETROVIRAL ANTIBODIES IN THE SERA OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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*ABSTRACT*

***Retroviruses have been postulated as environmental triggers in the aetiopathogenesis of systemic lupus erythematosus. Sera from 100 lupus patients were screened for the presence of antibodies against recombinant HIV-1 core and envelope, and HIV-2 envelope antigens by an enzyme immunoassay. This will detect antibodies resulting from direct HIV-1 or HIV-2 infections or those generated as a result of antigenic similarities by other human retroviruses. The sera were obtained from 11 male and 89 female lupus patients. Retroviral antibodies were not detected in the sera of these lupus patients, thus contradicting published findings that up to 30% of lupus patients have antibodies against the p24 gag protein of HIV-1.***

***Keywords:*** *HIV-1, HIV-2 retrovirus*

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## INTRODUCTION

The aetiopathogenesis of systemic lupus erythematosus (SLE) involves genetic[1], hormonal[2] and environmental factors. A viral trigger has been postulated to be the initiating factor for the autoimmune process in patients who are genetically predisposed. Retroviral infections have been implicated circumstantially in the aetiology of rheumatic diseases[3-5]. In mice, a subclass of Type C retroviruses, the mink cell focus forming (MCF) virus, has been implicated in the aetiopathogenesis of murine lupus[6,7] while the caprine arthritis-encephalitis virus[8] and the bovine lentivirus[9] can cause progressive arthritis similar to rheumatoid arthritis (RA). More specifically, an RNase-insensitive RNA was isolated from plasmapheresis fluids of active lupus patients and demonstrated to have homologies as well as similar actions to that of human retroviruses[10]. It has also been demonstrated that antibodies to the p24 gag of HIV-1 was present in up to 30% of lupus patients[11]. There was postulation that an endogenous retrovirus with antigenic similarities to the gag regions of HIV stimulated the immune response. However the evidence is not conclusive as other workers have been unable to demonstrate evidence of retroviral infections in SLE[12,13]. With this background of equivocal evidence for a retroviral aetiology, we screened the sera of our lupus patients for the presence of retroviral antibodies using recombinant HIV antigens containing the core and envelope proteins.

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## METHODS

### Patient selection

One hundred patients who satisfied the ARA criteria for the classification of SLE[14] and followed up in our department were entered into the study. The sera were obtained from lupus patients during the period January 1991 to December 1992. They were randomly obtained and not pre-selected according to any particular organ involvement.

### Sera collection

5 mL of peripheral venous blood were obtained under aseptic conditions using disposable syringes timed to coincide with the routine venepuncture. Sera were separated after centrifugation of the whole blood at 2,000 rpm for 10 mins. They were labelled and stored in a -70°C freezer until being assayed. Badly haemolysed or contaminated specimens were excluded.

### Retroviral antibody assay

The assay, performed in duplicate for each sample, is a solid phase enzyme immunoassay using polystyrene beads coated with recombinant proteins *(E. Coli)* representing HIV-1 core and envelope and HIV-2 envelope antigens (ABBOTT Diagnostics Division, West Germany). Briefly, if antibodies to the recombinant HIV-1 and HIV-2 antigens are present, they will be captured on the solid phase. After a period of incubation, unbound materials were aspirated and the beads washed. Goat anti-human immunoglobulin antibody conjugated with horseradish peroxidase was then incubated with the beads. Unbound materials were again aspirated and the beads washed, followed by addition of O-phenylenediamine (OPD) solution containing hydrogen peroxide. The resultant colour changes were read by a spectrophotometer at 492nm after addition of 1 N sulphuric acid. The presence or absence of antibodies against recombinant HIV-1 and/or HIV-2 antigens was determined by relating the absorbance of the specimen to the cut-off value. The cut-off value was defined as the Negative Control Mean absorbance plus 0.15 times the Positive Control Mean absorbance. Positive results will be those with absorbances higher than the cut-off value. Equivocal or positive results will be reconfirmed by repeat screening and Western blotting.

### Data collection

The biodata, clinical features and presence of rheumatoid factors

were obtained by chart review.

## RESULTS

The 100 lupus patients comprise 89 female and 11 male patients. Table I shows the sex and racial composition, and the presence of rheumatoid factors. Only 4 of the lupus patients, all females, had low titers of rheumatoid factors (1/20, 1/40, 1/40, 1/40) and these should not interfere with the assays. None of the patients had any features suggestive of the acquired immunodeficiency syndrome (AIDS) ie Kaposi's sarcoma and *Pneumocystis carinii* infection, although many had episodes of viral, bacterial or fungal infections resulting from their immunosuppressive therapy. None of the patients had pre-existing immunodeficient states. Assays for presence of antibodies against the recombinant HIV-1 (core and envelope) and HIV-2 (envelope) antigens were negative in all patients (Table II). Western blotting for confirmation was not performed as there was no positive or equivocal results.

**Table I – Biodata and presence of rheumatoid factor**

| | Female | Male |
|---|---|---|
| No. of patient | 89 | 11 |
| Race | C : 76 | C : 7 |
| | M : 10 | M : 2 |
| | I : 3 | I : 2 |
| Rheumatoid factor | 4 | 0 |

C : Chinese, M : Malays, I : Indians.

**Table II – Results of the retroviral antibodies assays**

| Retroviral assay | Positive* | Negative |
|---|---|---|
| Female (89 patients) | 0 | 89 |
| Male (11 patients) | 0 | 11 |

* A positive result is defined as absorbance above the cut-off value (ie. negative control mean absorbance plus 0.15 times the positive control mean absorbance).

## DISCUSSION

Circumstantial evidence for the involvement of retroviruses in the aetiopathogenesis of SLE has been reported since the 1980s. The demonstration of reverse transcriptase activity in supernatants of lymphocyte cultures of SLE patients[3], the detection of atypical interferon being produced[15] and the isolation of a DNA from active SLE patients' plasma that has homology to HIV-1 suggest a possible aetiopathogenetic role for human retroviruses. In addition, slow viruses or lentiviruses[16], which include the human retroviruses (HIV-1 and HIV-2), have several characteristics that make them ideal candidates as environmental triggers in SLE. They have a long variable incubation time, cofactors are needed in the establishment and perpetuation of the infection and the fact that a small percentage of infected individuals develop the full blown disease suggest the involvement of additional genetic factors. Other studies have however shown no evidence of retroviral infection in SLE[12,13,17,18]. Differing laboratory methods or techniques could have accounted for some of the differences as well as the possibility that the small groups of patients studied could have coincidental retroviral infection. In our lupus patients, the screening of a large number of sera for antibodies against recombinant HIV-1 core and envelope and HIV-2 envelope antigens serve as a simple and quick way of determining whether human retroviral infection may have a role in the aetiopathogenesis of SLE. The use of recombinant antigens eliminates many of the false positive results that have been reported when whole virus or virus lysates are used as antigens in ELISA tests[19]. It also allows the detection of antibodies due to direct HIV infections or cross reactions due to antigenic similarities with other human retroviruses. Western blots, though useful when used as confirmatory tests, can also give rise to indeterminate results in approximately a third of healthy individuals[20]. The absence of retroviral antibodies in the sera of our lupus patients argues against a major role for human retroviruses in the aetiopathogenesis of SLE. However, it does not preclude other viruses which may be involved and not detected by this EIA test. It is also possible that autoimmunity could be triggered by a virus without an abnormal host humoral response to the viral antigens or that viruses could be implicated only in a proportion of patients with the disease[21]. Although this study does not conclusively exclude a viral trigger, it suggests that human retroviruses with antigenic similarities to HIV-1 or HIV-2 or direct HIV-1 or HIV-2 infections do not play a major role in the causation of SLE. After more than 10 years of intensive search, the postulated viral trigger still remains elusive. Future studies and technological advances in virus detection may throw more light on the precise role/roles of virus agents in the development of SLE.

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