Typical Western blotting pattern of human immunodeficiency virus type 1 (HIV-1) antibody of controls and patients (R-F: reference serum positive for anti-HIV-1 antibody, R-N: reference serum negative for anti-HIV-1 antibody, H-F: healthy control positive for anti-P25 antibody, SY: synovial patient (S-6) positive for anti-P25 and anti-P55 antibodies, SLE-1: SLE patient (L/ACL negative) (N-9) positive for anti-P52 and anti-P68 antibodies, SLE-2: SLE patient with aCL (C-2), positive for anti-P25 and anti-P52 antibodies).

and 4/23 (17%) aCL/LA-negative). None of the subjects’ sera that was reactive to HIV-1 and/or HTLV-1 viral proteins showed a typical reactive pattern diagnostic of true HIV-1 and/or HTLV-1.

Talal et al. reported that 22/61 patients with SLE were positive for p24 (sympathetic with p25 in this paper) antibody and suggested that there was a close relation between HIV-1 and conserved idiotype antibody. They also found that anti-Sm antibody in this clinical setting showed cross-reaction with p24. Bick et al. confirmed that HIV-1 hybridising sequences are frequently present in patients with SLE. Furthermore, Ranki et al. reported that a few patients with connective tissue diseases, including SLE, had antibodies reactive to the gag protein (p24, p55) of HIV-1.

These reports prompted us to investigate whether or not there was a connection between the prevalence of aCL and/or LA and retrovirus infection in patients with SLE. Contrary to our hypothesis, however, the HIV-1 antibodies were more frequently detected in aCL/LA-negative SLE patients than in aCL/LA-positive SLE patients. These findings indicated that there may be pathogens and/or factors other than retroviruses acting in the aetiology of SLE® along with aCL/LA production, and this point needs to be clarified further.

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Studies on small numbers of subjects revealed a frequency of 0·119 of the mutant allele in white populations. About 1–4% of the population therefore is assumed to be homogeneous for that mutation. Several asymptomatic hemagglutinates were identified in these studies. This finding raises questions about the pathogenic significance of MAD deficiency. Determination of allele frequency from larger numbers of subjects with various clinical conditions is required to provide specific conditions concerning the clinical importance of MAD deficiency. The C34-T mutation which seems to be predominant in black populations and mutations of any gene in white populations is also of ethnologic interest since it has not yet been found in the Asian gene pool.

The MaeII restriction analysis faces two major disadvantages: first of all, this enzyme is expensive. Secondly, it is only sufficiently active under specific assay conditions. The reaction sample after PCR cannot be boiled to allow the digestion with MaeII. Instead, the PCR product must be precipitated and resuspended in the MaeII assay buffer.

Therefore, a new method for detection of the homoduplex mutation is presented that is both less expensive and less time consuming. DNA is extracted from blood by standard procedures. Exon 2 and part of intron 2 of the AMPD1 gene are amplified by PCR using 18mer primers. The PCR product starts in intron 1 and ends at the second nucleotide of exon 2. It corresponds to the wild type AMPD1 gene with the deletion of the second to last nucleotide, which is C instead of A (5’-CATACTGCTGAAAGAGACA-3’). After amplification of the mutant allele, the PCR product contains the sequence CATATG at the exon 2–intron 2 boundary which represents a Nsp1 restriction site. This Nsp1 site is not present after amplification of the wild type allele. The lower primer P2 starts at the 83rd nucleotide of intron 2 (5’-AACACTGCTGACATATGAAAGAGACA-3’). The PCR product has a size of 118 base pairs. Only the C34-T mutant allele is cut by Nsp1 into two fragments of 20 and 99 base pairs. Nsp1 digestion of heterozygous DNA results in three fragments: two fragments of the mutant allele and one of the unrestricted band of the wild type allele.

The PCR is carried out in a DNA thermal cycler (Perkin Elmer Cetus Instruments) for 30 cycles at 94°C denaturing temperature, 44°C annealing temperature, and 72°C extension temperature, each for one minute. The 100 µl PCR reaction volume contains 10 mM Tris-HCl, 50 mM KCL, 1·5 mM MgCl2, 0·001% (w/v) gelatin (Perkin Elmer Cetus PCR reaction buffer), 200 µM each of dNTP, 150 ng of each primer, 2·5 U AmpliTaq DNA polymerase (Perkin Elmer Cetus) and 10 ng of genomic DNA.

After PCR, 1/10 volume of 100 mM Tris-HCl pH 7·5, 100 mM MgCl2 and 10 mM diithiothreitol (L-buffer from USB, Cleveland, Ohio) are added to the reaction mixture and incubated at 37°C for one hour. After incubation, the DNA is then separated by electrophoresis in a 2·5% agarose gel followed by ethidium bromide staining. The 2 base pair fragment is hardly detectable. Only the bands of 99 and 119 base pairs are seen clearly (figure).
DNA of 11 patients with MAD deficiency and 100 randomly selected subjects were analysed for the C34-T mutation using both methods and always demonstrated identical results. The mismatch in primer 1 did not prevent sufficient PCR amplification in any experiment.

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