and 423 (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 (synergistic 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.

New method for detection of C34-T mutation in the AMPD1 gene causing myoadenylate deaminase deficiency

Myoadenylate deaminase (MAD) is the muscle isozyme of AMP deaminase (EC 3.5.4.6). MAD deficiency is the most common enzyme defect in human skeletal muscle and seems to cause a metabolic myopathy which is found in 2–5% of all muscle biopsies. It therefore might be one of the most common causes of exercise-induced myalgias and early fatigue.

MAD is encoded by the AMPD1 gene. Recently, the nonsense mutation C34-T (a transition at the 83rd nucleotide in exon 2) in this gene was reported to cause all cases of MAD deficiency studied so far. This mutation affects the last nucleotide of exon 2 and destroys the MAD protein expression site at the exon 2–intron 2 boundary. This mutation can therefore be detected by MaelII restriction analysis of the AMPD1 gene after PCR of exon 2 and the adjacent intron sequences.

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 heterozygous for that mutation. Several asymptomatic myozymyoses were identified in these studies. This finding raises questions about the pathogenic significance of MAD deficiency. Determination of allele frequency in larger numbers of subjects with various clinical conditions is required to provide specifics concerning the clinical importance of MAD deficiency. The C34-T mutation seems to be one of the first mutations of any gene in white populations. It is also of ethnological interest since it has not yet been found in the Asian gene pool.

The MaelII 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 amplified to allow the use of MaelII. Instead, the PCR product must be precipitated and resuspended in the MaelII assay buffer.

Therefore, a new method for detection of the C34-T 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 exon 1 and ends at the second nucleotide of exon 2. It corresponds to the wild type AMPD1 gene with the modification of the second to last nucleotide, which is C instead of A (5'-CATACGCTG-AAGAGACA-3'). After amplification of the mutant allele, the PCR product contains the sequence CATGTT at the exon 2–intron 2 boundary which represents a NspI restriction site. This nonpI site is not present after amplification of the wild type allele. The lower primer P2 starts at the 83rd nucleotide of intron 2 (5'-AACACTGCTGAAAATAAG-3'). The PCR product has 119 bp allele pairs. Only the C34-T mutant allele is cut by NspI into two fragments of 20 and 99 base pairs. NspI 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 of each 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 diethoether (L-buffer from USB, Cleveland, Ohio) are added to the reaction volume. The PCR product is mixed with 10 U NspI (USB, Cleveland, Ohio) and incubated at 37°C for one hour. After incubation, the DNA is cut separately by electrophoresis in a 2.5% agarose gel followed by ethidium bromide staining. The 20 base pair fragment is hardly detectable. Only the bands of 99 and 119 base pairs are seen clearly (figure).
Ethyldium bromide staining of an agarose gel after NspI digestion of PCR product. The PCR amplified exon 2 and part of intron 2 of the AMPD1 gene using P1 and P2 primers. 40 μl of digested PCR product was loaded into a 2-5% gel. (1, 4) homozygous patients with MAD deficiency; (2) heterozygous mother of patient (1); (3) PCR size marker with band of 50, 100, 200, 300, 400, 500, 700 and 1000 bp; (5) wild type DNA.

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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