

MNCs reported by other investigators (2-3) suggests there might be a subgroup of MS patients with HTLV-I-like sequences in MNCs. Such sequences may also occur in subjects without MS, and the significance of the observation will require the investigation of larger numbers of MS patients and control subjects. Our studies do not exclude the presence of a distantly related retrovirus or some other infectious agent that cannot be detected with the six primer pairs we used. However, it appears that HTLV-I proviral genome is not commonly found in patients with definite MS.

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peripheral blood MNCs. PCR reactions were done in a 50- μ l volume with 1 μ g of DNA, 1 unit of *Taq* polymerase (Cetus Corporation, Norwalk, Connecticut), and 0.5 μ g of each primer. The reaction buffer was 50 mM KCl, 1.5 mM MgCl₂, 200 μ M of each dNTP, 10 mM tris-HCl, pH 8.3; and 0.01% gelatin. Samples were denatured for 10 min at 95°C and then given 35 cycles of amplification (93°C for 0.1 min, 60°C for 0.1 min, and 72°C for 0.5 min). Ten microliters of the reaction products were separated by electrophoresis in 2.5% agarose and transferred to Zetaprobe membrane (Bio-Rad) in 0.4M NaOH. Filters were incubated for 1 hour at 50°C in 6 \times SSC, 1% SDS, and 0.5% dried skimmed milk, and then for 2 hours in the same solution containing 5 ng/ml oligonucleotide probe end-labeled with ³²P and T4 kinase to a specific activity of 5 \times 10⁸ dpm/ μ g/min. Filters were washed in 6 \times SSC/1% SDS for 10 min at room temperature and then for 10 min at 50°C and autoradiographed for 0.5 to 48 hours at -70°C.

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Response: One argument that Bangham *et al.* make in their comment is that we (1) have found an identical *env* sequence between nucleotides 5684 and 6151 in blood samples from all the six MS patients and that this is virtually impossible because, in their opinion, sequences of this region *must* vary between individual isolates. In support of their contention, they quote their unpublished data of sequence analysis of polymerase chain reaction (PCR)-amplified material, which I have not had an opportunity to examine. This argument reflects the opinion of Bangham *et al.* and is not supported by the published data on sequence analysis of

several cloned proviral HTLV-I genes. In support of our argument that a majority of HTLV-I proviruses have identical sequences in the region we amplified, Fig. 1 (2) shows a comparison of the published sequences of three different isolates of HTLV-I. One of the sequences is that of ATK-1 isolated from a patient with adult T cell leukemia (ATL) in Japan in 1982 (3). The MT-2 isolate is also from a patient with ATL (4). We determined the sequence of the *env* region of this clone. The third isolate, recently isolated and sequenced by Tsujimoto *et al.* (5), is from a patient with HAM (HTLV-I-associated myelopathy). The sequence of the *env* region that we have used for amplification is identical in these three isolates, which were obtained at different times and from different disease phenotypes. In addition, we have recently cloned and sequenced the HTLV-I proviral genome from the peripheral lymphocytes of a TSP patient of Caribbean origin (6). The *env* region is unaltered in this clone also (7). (In fact the sequence of the *env* region amplified from the six MS patients differs from that of these four different isolates from ATL, HAM, and TSP patients). If the argument of Bangham *et al.* were correct, all these published sequences of HTLV-I are incorrect, which is unlikely.

Bangham *et al.* state they cannot find amplification of HTLV-I sequences in the nine MS patients they studied. Richardson *et al.* report that they examined the DNA of several MS patients for the presence of HTLV-I sequences, including those of two DNAs originally found to be positive by Greenberg *et al.* (8) with the use of PCR assay; they report that they cannot find amplification of HTLV-I sequences in the MS patients they studied, but they do find

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9. Primers used for PCR amplification were as follows. (i) *Gag* primers [nucleotides (nt) 1795 to 2030]: 5'-GTCAGACCTGGACCCCAAGA-3', 3'-GTC-TGGTCTCCCTTCTACGGGAG-5'; probe: 5'-CCCCAAATCAGCCGTGCTTC-3'; (ii) X-region primers (nt 6824 to nt 7066): 5'-CCTTCTCAGC-CCCTTGTCTCCAC-3', 3'-GCGACGGCTAGT-GCTACGCAAAG-5'; probe: 5'-TACTCAGCGG-TCTGCTTTTC-3'; (iii) *gag* region primers (nt 842 to nt 1376) (2, 12); (iv) *pol* region primers (nt 2801 to nt 3018) (3, 12); (v) *env* region primers (nt 5799 to nt 6106) (3, 12); (vi) *env* region primers (nt 5684 to nt 6151) (2, 12).
10. DNA was prepared from Ficoll-Paque separated

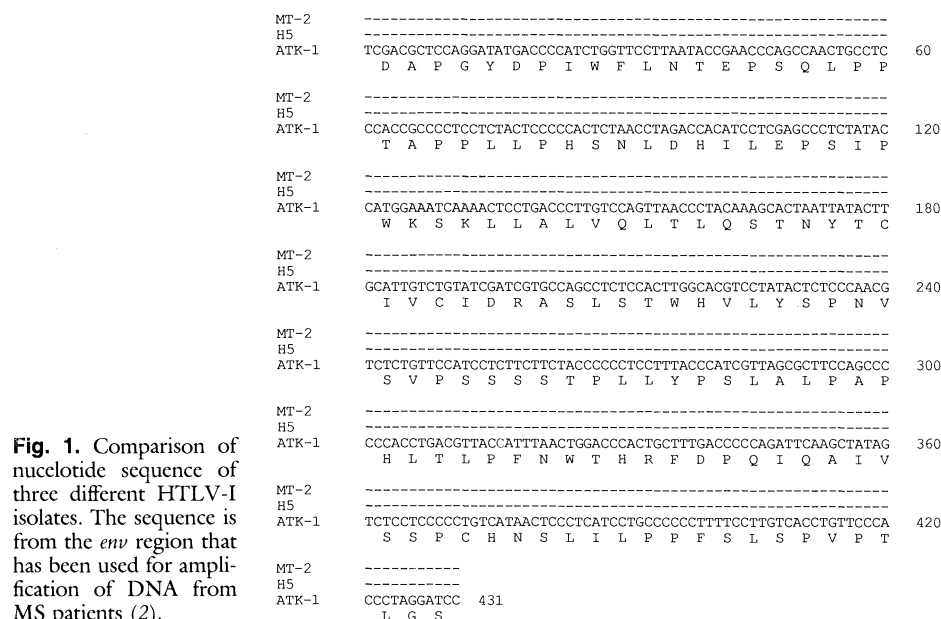


Fig. 1. Comparison of nucleotide sequence of three different HTLV-I isolates. The sequence is from the *env* region that has been used for amplification of DNA from MS patients (2).

that DNA obtained from two MS patients of Greenberg *et al.* (8) are positive for HTLV-I sequences. There are several possible explanations for these findings. The amplification of HTLV-I sequences in their positive controls appears to be considerably lower than that seen with our positive controls. It is possible that they will find weak hybridization, as we have observed, when they expose their autoradiograms longer. In our paper, we pointed out that, in some instances, we could not detect amplification of HTLV-I sequences by Southern blotting of amplified material. We circumvented this problem by subcloning the amplified material and screening the libraries generated from the amplified material, which made detection of HTLV-I sequences at least 10-

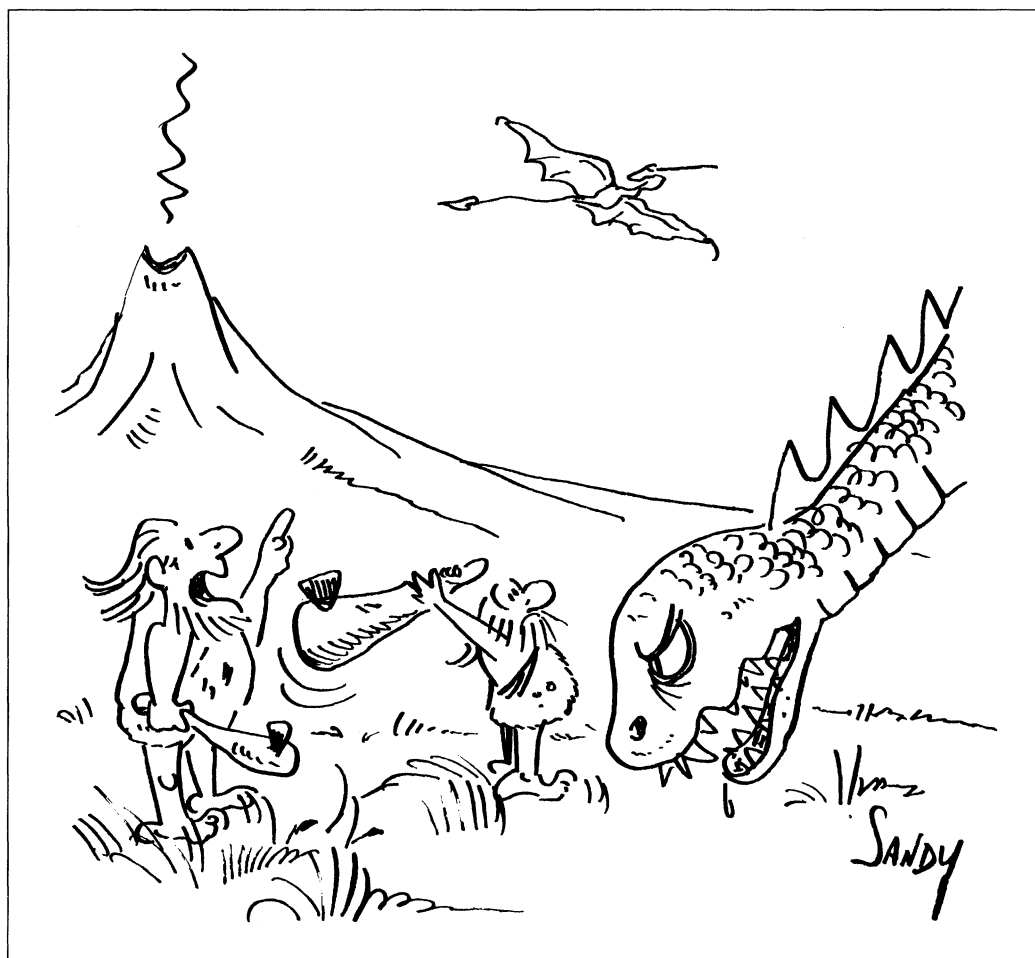
to 100-fold more sensitive. This does not appear to have been done by Bangham *et al.* or by Richardson *et al.* We did not state in our paper that all MS patients should harbor HTLV-I sequences. Recent work indicates that even in the case of patients with TSP or HAM, only a fraction are positive for HTLV-I antibodies. Our recent data (7) indicate that sequences from some MS patients are related to HTLV-II and that they do not amplify with HTLV-I-specific primers; this leads us to conclude that different patients may harbor different retroviral sequences. Further investigation is needed to determine whether sequences of HTLV-I or other retroviruses can be found by PCR assay in those patients negative for HTLV-I antibodies.

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2. Single letter abbreviations for the amino acid residues are as follows. A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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" Hold it! Hold it! That's a doe !"