A DNA PROBE CONTAINING ELEMENTS OF THE HIV-1 SURFACE GLYCOPROTEIN GENE: AN EFFECTIVE MOLECULAR MARKER FOR MAPPING THE HUMAN GENOME

E. I. Rogaev and A. B. Shlenskii

UDC 616-079.3:375

KEY WORDS: HIV-I, genotype mapping, glycoprotein.

The gene of the surface glycoprote in (envelope) is the most variable component of the genome of the HIV-I retrovirus. The hypervariable region of this germ contains several short straight DNA repeating sequences, organized into small clusters (not more than three copies), which are characterized by variation in the number of copies in different strains of the HIV-I virus [8, 10]. Families of tandem repeats of DNA characterized by allelic hypervariability for number of copies also have been found in the human genome [1, 2, 4-6, 9].

The question accordingly has arisen: is there a genome "parasite" in the HIV-I retrovirus genome, and in its host, the human genome, are there DNA elements with similar structure, namely recombination "hot" spots, inducing rapid changes in segments of the genome.

To examine this problem several varying repeating regions of the envelope gene of HIV-I were synthesized chemically. These regions were used as probes in blot-hybridization with human genome DNA. One probe unexpectedly showed intensive hybridization with many regions of the human genome, characterized by extraordinarily high interindividual variability of the DNA restriction fragments.

EXPERIMENTAL METIIOD

Blot-Hybridization of DNA. Human nuclear DNA was isolated from cellular tissues as described in [7]. The DNA (10 μg) was hydrolyzed with HaeIII restriction endonuclease, fractionated by electrophoresis in 0.7% agarose gel (16 cm), and transferred to a nylon filter (HyBond) by Southern's method. ^{32}P-labeling of the DNA probes was carried out as described in [3]. Hybridization took place in a solution of 0.5 M sodium phosphate, 8% sarcosyl, 10 mM EDTA, and 0.1% polyvinylpyrrolicione (60°C, 16-18 h). The material was washed in a solution of 1 × SSC, 0.1 SDS (60°C, 3 h).

Cloning of the elements of DNA of the env-gene of HIV. Chemically synthesized complementary strands of the red site of the env-gene, 24 base pairs long, were annealed ard ligated and the fraction was applied to 15% polyacrylamide gel. The fraction corresponding to fragments more than 60 base pairs long was elated from the gel, denatured, annealed, again ligated, and cloned into plasmid pUC-13.

Transformation was carried out in recA cells of strain Jm109 of *E. coli.*

EXPERIMENTAL RESULTS

One region at the beginning of the env-gene of HIV-I, 24 base pairs long, which we called red-1 (repeat envelope deletion), consists of two short tandem monomers, one of which was deleted in several strains of HIV-I. This region we amplified in vitro to 10 DNA copies in the fragment and cloned it in pUC-19.

Institute of Clinical Psychiatry, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR M. E. Vartanyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 110, No. 12, pp. 646-647, December, 1990. Original article submitted December 28, 1989.

 0007-4888/90/0012-1712$12.50

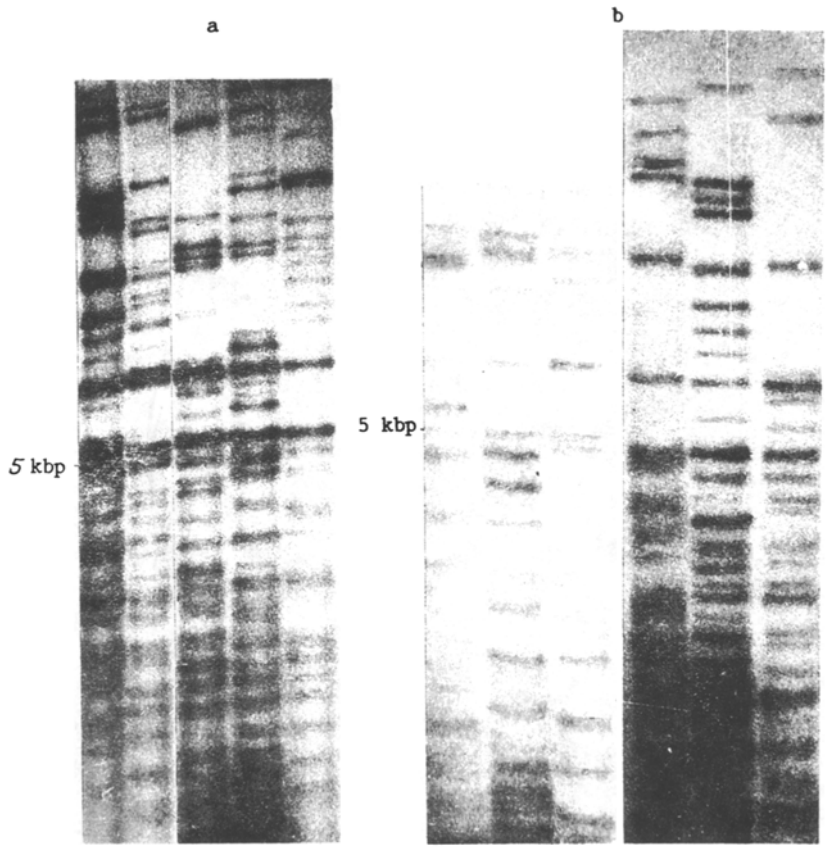

Fig. 1. Identification of discrete fragments of human chromosomal DNA, hybridized with elements of DNA of the HIV-I-env-gene in the human genome. Hybridization of HaeIII-restriction fragments of human DNA: a) with HIV-red-1 double-stranded insert, labeled with the aid of a Klenow fragment and scattered priming, b) with HIV-red-1 single-stranded insert, labeled with Klenow fragment and sequenced primer. DNA from different individuals is represented on all lanes.

The HIV-red-1-insert was labeled with ^{32}P and blot-hybridized with HaeIII-digests of DNA of unrelated individuals. The result was the discovery of a set of discrete hypervariable fragments of human DNA hybridized with the HIV-red-1 DNA probe (Fig. 1). The number of polymorphic fragments discovered was not less than 15 per person. Analysis of about 20 individuals shows that the probability (p) of random coincidence of the sets of polymorphic DNA fragments in unrelated individuals is close to 0 ($p < 10^{-10}$).

Analysis of one family and different tissues of two individuals showed that polymorphic fragments, inherited by Mendel's laws, are identical in monozygotic twins and in different tissues of the same individual.

The HIV-red-1 probe, like Jeffreys' "minisatellites" [4], is thus an effective marker for human genotyping: for the identification of individuals ail analysis of paternity in forensic medicine, for upping the genome, establishing the zygosity of twins, and for human population and medical-genetic research.

The authors are grateful to A. Ya. Spunde for synthesizing the oligonucleotides and to M. E. Vartanyan for support with the work.

LITERATURE CITED

1. J. I. Bell, M. J. Selby, and W. J. Ratter, Nature, **295,** 31 (1982).
2. D. J. Capon, E. Y. Chen, A-D. Levinson, et al., Nature, **302,** 33 (1983).
3. A. P. Feinberg and B. Vogelstein, Analyt. Biochem., **137,** 266 (1984).

4. D. R. Higgs, S. E. V. Goodbourn, J. S. Wainsoat, et al., Nucl. Acids Res. **9,** 4213 (1981).
5. A. I. Jeffreys, V. Wilson, and S. L. Thein, Nature, **314,** 67 (1985).
6. A. I. Jeffreys, V. Wilson, and S. L. Thein, Nature, **316,** 77 (1985).
7. T. Naniatis, E. F. Fritsch, and J. Sambrook, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor (1982).
8. R. Patarca and W. A. Haseltin, J. Theor. Biol., **125,** 213 (1987).
9. E. I. Rogaev, Nucl. Acids Res., **17,** 1246 (1989).
10. F. Wong-Staal and R. C. Gallo, Nature, **317,** 395 (1985).