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Genetic Comparison of HIV-1 Isolates from Africa, Europe, and North America

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INTRODUCTION

THE DEFINITION OF THE GENETIC, biological, and immunological properties of human immunodeficiency virus type 1 (HIV-1) constitutes an important element in vaccine development. Current projections indicate that the African continent may bear an increasing share of the burden of the worldwide HIV epidemic in the coming decades. However, many questions remain unanswered regarding the extent of variation of HIV-1 in Africa, the prevalence of different variants, and their geographic distribution. It previously was shown by genetic analysis[1] that HIV-1 isolates from Africa are both highly diverse and generally distinct from those prevalent in North America and Europe. Previous genetic characterization has included isolates from Gabon,[2] Zaire,[3–6] and Uganda.[7] The objective of this work was to evaluate further variability among HIV-1 isolates from Africa. A genetic typing system based on the polymerase chain reaction (PCR) was developed and used to characterize isolates from diverse locales in Africa and from North America and Europe. The results indicate that the diversity of HIV-1 in Africa may be considerably greater than anticipated and that distantly related variants sometimes are intermixed in a single location.

METHODS

Amplification of DNA by PCR is influenced by the homology of oligonucleotide primers with the template. We have developed a procedure, termed anchored PCR, whereby nucleotide sequence alterations in the template can be directly related to the quantity of amplified product.[8] A panel of 24 primers in the *gag* gene (Fig. 1a) was chosen from the compilation of HIV-1 DNA sequences in the Los Alamos database.[1] DNA from virus cultures was amplified in separate reactions with each of the 24 primer pairs. Amplified DNA was detected by Southern blot hybridization with a ^{32}P-labeled probe common to all of the amplified segments. Radioactivity was quantitated directly with a Betagen blot analyzer. Criteria for evaluation of results were established previously[8] by quantitation of product from paired and mispaired template/primer combinations. Decreased PCR product yields of 20-fold or greater relative to control samples indicated template/primer mispairing.

Some isolates were studied by DNA sequencing. DNA from virus cultures was amplified, cloned in a plasmid, and sequenced using fluorescent dye primers or terminators (Applied Biosystems) and Taq polymerase (Cetus). Data were collected using an Applied Biosystems 370A DNA sequencer. Multiple clones were sequenced per isolate and data were assembled using DNAStar software and Macintosh computers.

RESULTS

In a pilot study, a panel of African isolates was evaluated by PCR and by DNA sequencing (Fig. 1b). The isolates were found to differ greatly with respect to the number of mispaired PCR reactions. Some of the samples showed no mispaired reactions, a pattern that generally was found in North American isolates.[8] An isolate from the Ivory Coast showed five mispaired PCR relations. Four isolates from Zambia shared a particularly distinctive pattern, with 6 of the primers consistently unable to amplify these samples and additional mispaired primers common to some of the samples.

The degree of correspondence of the PCR patterns to phylogenetic relationships of isolates was determined by DNA sequencing of the p17 coding region of GAG. A tree reflecting the genetic relationships (Fig. 1c) indicated that two isolates with a North American-like PCR pattern were genetically similar to

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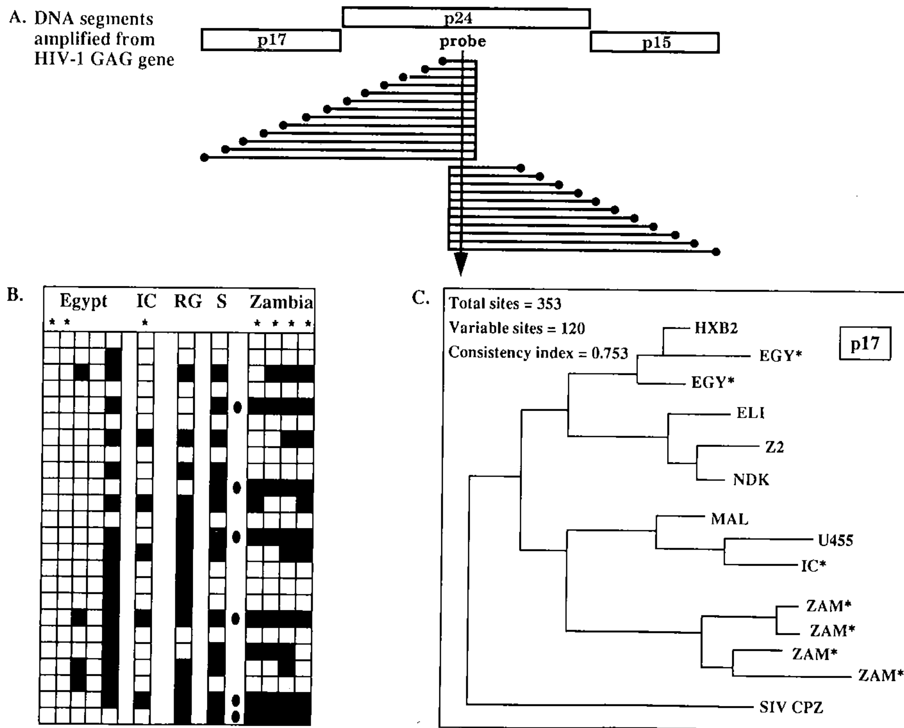

FIG. 1. Anchored PCR analysis of HIV-1 isolates. (a) Location of the 24 primer combinations along the HIV-1 *gag* gene. Each combination is represented by a line showing the position and length of the different fragments. The location of the probe is given by a vertical arrow. (b) Graphic representation results. Open boxes represent reactions with a product yield of > 5% of a positive control, filled boxes represent reactions considered as mispaired (product yield of < 5%). Horizontal axis: virus isolates, vertical axis: primer combinations. (c) Phylogenetic tree of different HIV isolates based on the DNA sequence of the coding region for p17; corresponding PCR patterns are indicated by (*).

HIV_{HXB2}. The Ivory Coast isolate, with an intermediate PCR pattern, was in the HIV_{MAL} branch of the P17 tree. The four Zambian isolates were similar to one another but distinct from previously described HIV-1 isolates, clustering in a separate branch. This showed that the PCR profiles reflected the genetic relationships of HIV-1 isolates.

In a larger study, 39 HIV-1 isolates from Europe and North America and 64 isolates from 10 widely separate sites in Africa

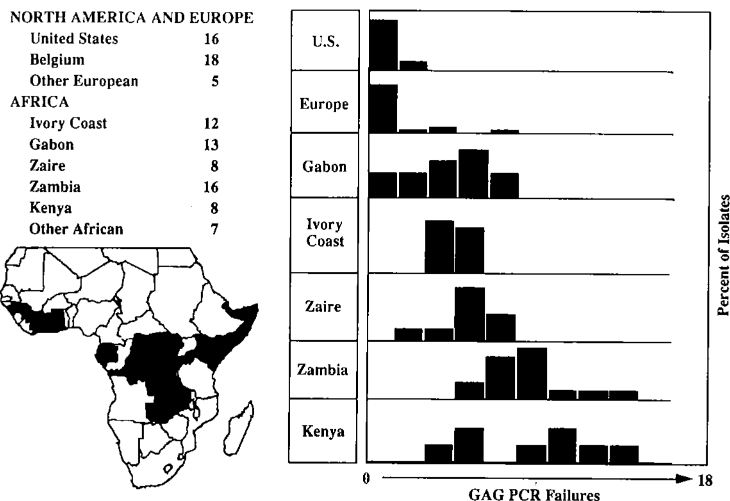

FIG. 2. Anchored PCR analysis of North American, European, and African isolates. (a) List of isolates and their geographic origin. (b) Mispaired PCR relations for isolates of different geographical origin, horizontal axis: number of mispaired PCR reactions; vertical axis: percent of isolates with a specific number of mispaired PCR reactions.

were analyzed using anchored PCR (Fig. 2). North American and European isolates showed very few mispaired PCR reactions. Totally different PCR profiles, with extensive primer mispairing, were observed for the African isolates. West and West Central African isolates appeared closest to the European/North American samples, while those from East Africa were the most divergent from them. Central African isolates showed an intermediate pattern.

Geographic locations differed with respect to the diversity of PCR pattern among the HIV-1 isolates. The North American isolates (n = 14) were the most homogeneous with respect to PCR profile. The 25 European samples were also homogeneous, but three of them showed some additional mispaired reactions not typical of the majority of North American and European isolates. A broad diversity of pattern was seen with the isolates from Gabon (n = 13), some overlapping the North American/European group while others were clearly distinct from it. The Ivory Coast isolates (n = 12) were similar by this analysis but were clearly separated from the North American and European group. The groups of isolates from Zaire (n = 8) and Zambia (n = 12) appeared more distant from the North American/European samples. By our analysis the eight isolates from Kenya fell into two groups, with one group showing the most extensive PCR primer mispairing encountered in this study. The remaining African isolates were from several different locations and were heterogeneous, but additional samples from each location will be needed to complete the study. Many of the isolates described here are being characterized by DNA sequencing of major structural genes, and the preliminary results (data not shown) largely confirm the PCR-defined genetic relationships.

DISCUSSION

Primer sequences for PCR typing of HIV-1 isolates were selected at a time when the database of genetic information about the virus was limited to isolates from North America, Europe, and Zaire. Thus the relatively few instances of primer mispairing with isolates from these locales was expected. The numerous instances of PCR primer mispairing among the African isolates studied here is noteworthy. It suggests that the range of variation exhibited by HIV-1 in Africa may be very large. The only African sites that conformed to any extent to the North American/European pattern were Gabon and Zaire, and even there only a few of the isolates fell into this category. Many locations in Africa with significant incidence and prevalence of HIV-1 infection have not been studied, and additional genetic diversity of the HIV-1 virus may still be undocumented. The complex mixtures of variants found in some locales also is of note, since the impact of virus diversity on therapy or intervention could be greatly increased in these sites.

Genetic information about diverse African isolates will provide a valuable resource for many avenues of research leading to an anti-HIV-1 vaccine. We see application for both PCR typing and DNA sequencing in this regard. An expanded DNA sequence database will document phylogenetic relationships and guide selection of prototypic variants for vaccine development. More comprehensive sequence information will permit construction of new PCR primers for improved discrimination of HIV-1 variants. PCR typing could be applied to the very large sample sizes that may be needed to adequately document HIV-1 variation in Africa. This technique can be accommodated to a clinical laboratory setting, and may be valuable in connection with potential vaccine field trials to document incidence and prevalence of local variants. Lastly, it may be of importance to understand the "boundary conditions" for HIV-1 genetic variation. Without this, the goal of a globally effective vaccine may be delayed by the emergence of virus variants distant from current vaccine prototypes.

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