JOURNAL OF VIROLOGY, Mar. 1994, p. 1581–1585
0022-538X/94/$04.00+0

Vol. 68, No. 3

A New Subtype of Human Immunodeficiency Virus Type 1 (MVP-5180) from Cameroon

LUTZ G. GÜRTLER,[1]* PETER H. HAUSER,[2] JOSEF EBERLE,[1] ALBRECHT VON BRUNN,[1] STEFAN KNAPP,[2] LEOPOLD ZEKENG,[3] JEAN M. TSAGUE,[3] AND LAZARE KAPTUE[3]

Max von Pettenkofer Institute, University of Munich, D-80336 Munich,[1] and Behring Werke, D-35001 Marburg,[2] Germany, and Labo et Transfusion Sanguine, Centre Hospitalier Universitaire, Yaoundé, Cameroon[3]

Received 16 August 1993/Accepted 7 December 1993

A new subtype (MVP-5180) of human immunodeficiency virus type 1 (HIV-1) was isolated from a Cameroonian AIDS patient. MVP-5180 was grown in several human T-cell lines and the monocytic U937 line. MVP-5180 DNA could not be amplified by nested primer PCR with conventional *env* primers and could be only very faintly amplified with *gag* and *pol* primers. Most German, Ivoirian, and Malawian anti-HIV-1 sera reacted faintly or moderately with Env proteins in an MVP-5180 immunoblot, whereas some Cameroonian sera reacted strongly. Of HIV-1-infected Cameroonians, 8% were identified by serological methods as infected with MVP-5180; 7% were positive when MVP-5180-specific PCR *env* primers were used. DNA sequence analysis of MVP-5180 showed that its genetic organization was that of HIV-1, with 65% similarity to HIV-1 and 56% similarity to HIV-2 consensus sequences. The *env* gene of MVP-5180 had similarities to HIV-1 and HIV-2 of 53 and of 49%, respectively. V3 loop analysis identified a crown of Gly-Pro-Met-Arg by using cloned DNA and Gly-Pro-Leu-Arg by using PCR-amplified DNA, neither of which configuration has been described for other HIV strains. In an analysis of relationships, MVP-5180 occupied a position distant to all other HIV-1 strains, including the chimpanzee simian immunodeficiency virus type 1 SIVcpz and the Uganda virus U455, and closer to the HIV-1/HIV-2 divergence node. MVP-5180, together with another Cameroonian isolate, ANT-70, constitutes a group subtype O of the most divergent HIV-1 isolates yet identified. Characterization of MVP-5180 is important for understanding the natural history of the primate immunodeficiency viruses and for the development of vaccines and diagnostics.

Nucleic acid sequencing has distinguished between five human immunodeficiency virus type 1 (HIV-1) and two human immunodeficiency virus type 2 (HIV-2) subtypes (13); the subtype MVP-5180 reported here is additional to the five HIV-1 subtypes and has been grouped together with ANT-70 as subtype O (13). The most divergent HIVs have been identified in Africa, for HIV-1 particularly in Central Africa and for HIV-2 in West Africa. A simian immunodeficiency virus (SIVcpz) was isolated from a chimpanzee captured in Gabon (16), a country adjacent to Cameroon, and the sequence of another chimpanzee virus from Zaire, CPZant (SIV-1ant), even more divergent from the HIV-1 subtype described in this report, will be published soon (15a). In 1990, a long terminal repeat (LTR) nucleic acid sequence of a Cameroonian HIV-1 isolate, ANT-70, differing considerably from sequences of other HIVs, was described (4). Its LTR sequence is close to that of MVP-5180, and its complete sequence will be published shortly (20). Complete nucleic acid sequences are available for both viruses (accession numbers L20587 for ANT-70 and L20571 for MVP-5180) (13).

Even more divergent HIVs may exist. Such divergent HIVs are likely to be transmitted by the usual routes (sexual and blood contact and mother-to-infant transmission), leading to wider distribution. They will have to be taken into account in vaccine development and diagnostic test sensitivity and specificity.

* Corresponding author. Mailing address: Max von Pettenkofer Institute of Hygiene and Medical Microbiology, University of Munich, Pettenkofer Strasse 9A, D-80336 Munich, Germany. Phone: 49-89-5160 5274/5273. Fax: 49-89-538 0584.

MATERIALS AND METHODS

Isolation procedure. MVP-5180 was isolated by conventional methods (3) from the heparinized blood of a Cameroonian patient in 1991, after phytohemagglutinin stimulation of Ficoll-separated lymphocytes in the presence of 10 U of interleukin-2 and 2 μg of Polybrene per ml. This 34-year-old, female patient who had never been outside the country died with AIDS about 1 year later. During cocultivation of the isolate with acceptor lymphocytes from HIV-uninfected donors, syncytia formed. MVP-5180 was adapted easily to growth in such permanent human T-cell lines as HUT-78, MT-2, Jurkat, and C8166. After 3 weeks, MVP-5180 grew productively in the monocytic U937 cell line. T-cell entry of MVP-5180 was CD4 receptor dependent, since monoclonal anti-CD4 inhibited cell infection (17).

For comparison, other HIV isolates obtained by the same isolation procedure were used. MVP-11971 is an HIV-2 isolate, available through the NIH AIDS Research and Reference Reagent Program, number 1107; MVP-899 is an HIV-1 isolated in Germany in 1987; and MVP-9841 is an Ivoirian HIV-1 isolate from 1991.

Viral growth was monitored in tissue culture supernatants by determining reverse transcriptase activity and p24 antigen content. A nonradioactive procedure using biotin- and digoxigenin-labeled oligonucleotides was used for the reverse transcriptase determinations (7). A commercial p24 antigen assay (Abbott, Wiesbaden, Germany) was used as specified by the manufacturer.

Nucleic acid sequence analysis. DNA was extracted from MVP-5180-infected HUT-78 cells. The 3′ part of the *env* gene was amplified by PCR (11), using either the 5′ primer sk68 (15) (AGCAG CAGGA AGCAC TATGG, positions 1538 to 1557) (13) and the 3′ primer Benvb (GAGTT TTCCA GAGCA

ACCCC, positions 1774 to 1755) or the 5′ primer B163env (CAGAA TCAGC AACGC CTAAA CC, positions 1729 to 1741) and the 3′ primer Benvend (GCCCT GTCTT ATTCT TCTAG G, positions 2515 to 2495). Amplified DNA was subjected to agarose gel electrophoresis in borate buffer, eluted from the gel, and purified by phenol-chloroform extraction and ethanol precipitation (18). DNA was then subjected to sequence analysis, using *Taq* dideoxy terminator cycle sequencing (19) (Applied Biosystems, Foster City, Calif.). DNA was labeled with [^{32}P]CTP with a random-primed labeling kit (Boehringer, Mannheim, Germany) to identify MVP-5180 DNA-containing clones as described below.

The MVP-5180 genome was cloned by constructing a genomic library from MVP-5180-infected HUT-78 cells. Clones were established by digesting cellular DNA with *Sau*3A. DNA fragments in the size range of 10 to 21 kbp were separated by agarose gel electrophoresis, extracted from the gel, and precipitated with ethanol. 5′ ends were dephosphorylated with alkaline phosphatase. The DNA fragments obtained were ligated into *Bam*HI-digested lambda DASH vector (Stratagene, La Jolla, Calif.). Ligated DNA was packaged in vitro into T phage heads (18). Infected *Escherichia coli* plaques were screened by hybridization on nitrocellulose, using radioactively labeled probes from the PCR-amplified *env* gene, followed by X-ray autoradiography (18). Clones were obtained by *Eco*RI digestion of recombinant phage DNA and incorporated in the Bluescript KS− vector (Stratagene). Clones were sequenced as described above with the *Taq* dideoxy terminator cycle sequencing kit.

Once the nucleic acid sequence of the cloned DNA had been established, the V3 loop DNA was amplified further by PCR, using the 5′ primer AGAAT CAGCA AAGAA TATCA TAG (positions 820 to 843) and the 3′ primer TTGTT GTAGG GCATT TTCCC A (positions 1005 to 985), and sequenced as described above.

Nucleic acid and amino acid sequences were analyzed and compared with those of other isolates, using the University of Wisconsin Genetics Computer Group program (5).

Epidemiology of MVP-5180 in Cameroon. Two methods were used to examine the distribution of MVP-5180 in Cameroonian HIV-1-infected subjects. The first method detected anti-HIV in plasma by competitive immunoblotting. Competitive immunoblotting examines the simultaneous development of reaction patterns in two immunoblot strips coated with equal amounts of viral proteins either of HIV-1 isolate MVP-899 or of isolate MVP-5180 in a single chamber to one set of reagents. Immunoblot strips were prepared as described previously (10). The second method detected HIV DNA in mononuclear blood cells by PCR with nested primers. To amplify specific regions of the *env* gene, the 5′ primer 51enva (TGCTA TTCTT GGGGG TGCT, positions 1517 to 1535) and 3′ primer 51envb (TGGTC CCATT GCTGC CATG, positions 1864 to 1845) were used for the first set of cycles, and the 5′ primer 51envc (CCCAC AGTGT ACTGA AGGG, positions 1589 to 1607) and 3′ primer 51envd (CTGTC AT CAT TATAT CCTCC, positions 1832 to 1813) were used for the second cycle. Normal (subtype A and B) HIV-1 specimens were identified by using primers enva (TGTTC CTTGG GTTCT TG, positions 1520 to 1537) and envb (GAGTT TTCCA GAGGA ACCCC, positions 1773 to 1754) for the first set of 30 cycles and primers sk68 and sk69 (15) for the second set. For the PCR procedure, DNA was liberated from the Ficoll-separated lymphocytes by proteinase K-detergent digestion (11). The PCR cycles were carried out at 55, 72, and 92°C (11). Amplied products were separated and identified by agarose gel electrophoresis (18).

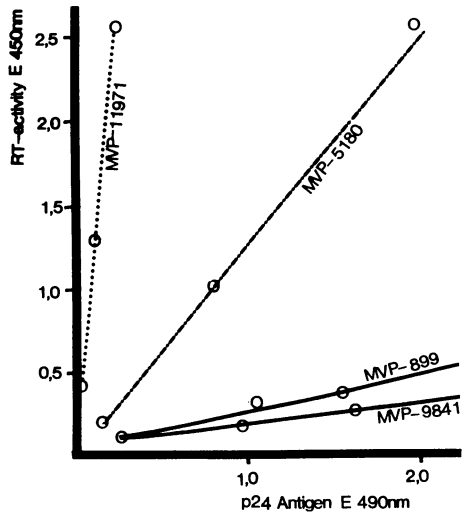

FIG. 1. Comparison of the reactions of the reverse transcriptase activities of four HIVs with a monoclonal anti-p24. MVP-899 is a German and MVP-9841 is an Ivoirian HIV-1 isolate; MVP-11971 is an HIV-2 isolate from Senegal/Germany. The Cameroon isolate MVP-5180 reacted less with anti-p24 than the HIV-1 isolates did but more than the HIV-2 isolate did. Reverse transcriptase activity was measured with a nonradioactive enzyme-linked immunosorbent assay (ELISA) method (7); p24 antigen was measured with a commercial ELISA kit; extinction values are given on the axis. Reactions were determined with serial dilutions of virus suspensions. Each line of reaction is based on at least three determinations. Repetition of this comparison gave similar results.

RESULTS

Biological characterization of MVP-5180. Three weeks after the lymphocyte cultures were established, giant cells formed, and p24 antigen and reverse transcriptase activity were detected in the culture supernatants. By cocultivation, MVP-5180 was adapted easily to growth in permanent T-cell lines such as HUT-78, MT-2, Jurkat, and C8166. Pronounced syncytium formation was observed in all cell lines, and titers of up to 10^5 50% tissue culture infectious doses ($TCID_{50}$) per ml were obtained by retitration on cells of the respective cell lines.

Most HIV isolates do not replicate in the monocytic U937 cell line (2), but MVP-5180 began replication 3 weeks after inoculation U937 cell cultures with 10^2 $TCID_{50}$ of supernatant from the HUT-78 cells. In U937 cells, MVP-5180 grew to 10^3 $TCID_{50}$/ml when retitrated on HUT-78 cells. Neither MVP-899 nor MVP-11971 replicated in U937 cells in five consecutive assays.

As expected, the activity of the reverse transcriptase of MVP-5180 was Mg^{2+}-dependent (data not shown). When the activity of the reverse transcriptase was plotted against the extinction obtained in the p24 antigen assay, it was evident that the reactivity of the monoclonal anti-p24 was less than with other HIV-1 proteins such as MVP-899 and MVP-9841 but was higher than the reactivity of the HIV-2 isolate MVP-11971 (Fig. 1).

Nucleic acid sequence. The failure to amplify considerable amounts of DNA by PCR using conventional primers for HIV-1 and HIV-2 indicated diversity in the conserved parts of the genome. Screening of the phage library led to the selection of one clone out of 2×10^6 plaques for subcloning. Infectivity of this clone was not tested. Extracted phage DNA was digested with *Eco*RI, and subclones pSP1 to pSP4, containing

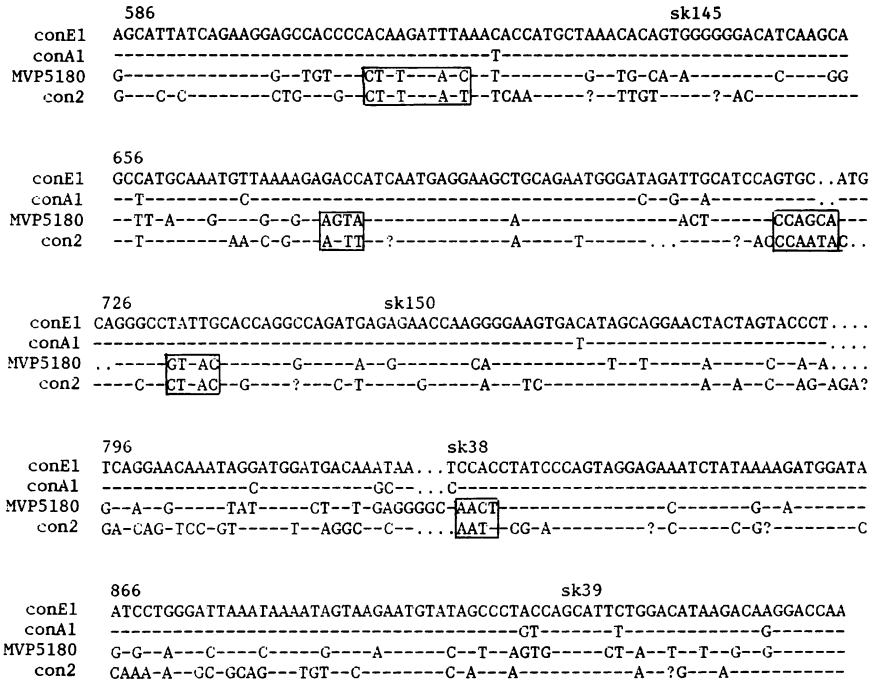

FIG. 2. Comparison of the nucleic acid sequences of part of the *gag* gene of MVP-5180 with the HIV-1 European/American consensus sequence (conE1), the HIV-1 African consensus sequence (conA1), and the HIV-2 consensus sequence (con2). The consensus sequences are taken from reference 13. This part of the *gag* gene is used mostly for choosing the primer binding sites in PCR. Numbers correspond to nucleotide numbers of the Los Alamos data bank in 1991. Dashes represent homologous nucleotides, points indicate gaps, and question marks represent variable positions. The regions of similarity of the MVP-5180 sequence with that of HIV-2 are boxed and located in positions 541 to 552, 607 to 610, 649 to 654, 664 to 668, and 758 to 761. As expected for an HIV-1 subtype, the major part of the MVP-5180 nucleotide sequence is close to the HIV-1 sequence.

MVP-5180 DNA from nucleotides 1 to 1785, 1786 to 5833, 5834 to 7415, and 7660 to 9793, respectively, were obtained. The missing fragment from nucleotides 7416 to 7659 was obtained by PCR using primer 157 (CCATA ATATT CAGCA GAACT AG), primer 226 (GCTGA TTCTG TATAA GGG), and phage DNA as the template.

The sequence obtained by cloning the MVP-5180 DNA in the Bluescript vector showed a genomic arrangement identical to that of HIV-1: LTR, *gag*, *pol*, *vif*, *vpr*, *vpu*, *tat*, *env*, *nef*, and LTR. The full sequence has been deposited in the HIV Sequence Database and in GenBank (accession number L20571). In contrast to most other HIV-1 isolates, the 5′ end of the *env* gene carried two start ATG codons, like the HIV-1 isolate CDC4 (13). Figure 2 shows part of the *gag* gene of MVP-5180 and the corresponding sequences of the HIV-1 and HIV-2 consensus sequences (13). This region of *gag* is used frequently for commercial PCR kits, and the difference in this region may explain the difficulties in amplifying MVP-5180 DNA with these kits.

Similarities with HIV-1 and HIV-2 nucleotide consensus sequences (13) are summarized in Table 1. MVP-5180 is more similar to HIV-1 than to HIV-2 and, in the LTR region, especially to ANT-70 (4, 13). Direct sequencing of the *gag* gene by PCR from DNA of MVP-5180-infected HUT-78 cells by using the 5′ primer TGGCG CCCGA ACAGG GACTT GAA (positions 635 to 658 of the LTR gene) and the 3′ primer CCAAA GAGTG ATTGA GGGAG (positions 185 to 165 of the *pol* gene) revealed 2% of exchanged nucleotides, indicating that the cloned DNA was representative of MVP-5180 DNA.

When the V3 loop, selected as a hypervariable region of the genome, was amplified by PCR using the primers described above, an inclusion of six nucleotides in the 3′ part of the loop and a mutation in the crown of the loop, converting the methionine codon (ATG) to a leucine codon (CTG), were found (Fig. 3).

Comparison of the MVP-5180 amino acid sequence with that of the Gabon chimpanzee virus (16) showed similarities of 70, 78, and 53% in the *gag*, *pol*, and *env* genes, respectively; similarities of 70, 76, and 51% to the Uganda HIV-1 (U455)

TABLE 1. Comparison of the nucleotide sequence of MVP-5180 with the consensus sequences from HIV-1 and HIV-2 (13) and the sequence published for ANT-70 (4)

MVP-5180 gene	No. of exchanged nucleotides	No. of total nucleotides	% Similarity (approx)
LTR	207	630	HIV-1, 67
	308		HIV-2, 51
	115	630	ANT-70, 82
gag	448	1,501	HIV-1, 70
	570		HIV-2, 62
pol	763	3,010	HIV-1, 74
	1,011		HIV-2, 66
vif	183	578	HIV-1, 68
	338		HIV-2, 42
env	1,196	2,534	HIV-1, 53
	1,289		HIV-2, 49
nef	285	621	HIV-1, 54
	342		HIV-2, 45
	59	354	ANT-70, 83
Total	3,082	8,874	HIV-1, 65
	3,858		HIV-2, 56

```
LAI:          CTRPNNNTRKSIRIQRGPGRAF.VTIGKIGNM.......RQAHC

5180 clone:   -I-EGIAEVQD-YT..--M-WRSM-L.-RS-N..TSPRS-V-Y-

5180 PCR:     -I-EGIAEVQDLHT..--L-WRSM-L.-KSSNSHTQPRSKV-Y-

ROD:          -K--G-KIV-Q-MLMS..-HV-HSHYQP-.-KR...P..---W-
```

FIG. 3. Amino acid sequences of the V3 loop of gp120 of HIV-1 isolate LAI (13) and HIV-2 isolate ROD (13) compared with that of MVP-5180. Homologous amino acids are represented as dashes; gaps are represented as points. The one-letter code has been used: A, Ala; R, Arg; N, Asn; D, Asp; C, Cys; Q, Gln; E, Glu; G, Gly; H, His; I, Ile; L, Leu; K, Lys; M, Met; F, Phe; P, Pro; S, Ser; T, Thr; W, Trp; Y, Tyr; V, Val. The sequence GPGR marks the crown of the loop. From MVP-5180, the two sequences obtained by the cloned and the PCR-amplified DNA sequencing are given. PCR-amplified DNA showed an exchange of M to L in the crown and two additional amino acids, S and H, in the C-terminal part of the loop. Cloned and PCR-amplified DNA was from the same culture, taken approximately 8 weeks after adaption of the MVP-5180 grown on peripheral blood mononuclear cells to HUT-78 cells.

(14) and of 54, 57, and 34% to the HIV-2 isolate D205 (6) were found. The phylogenetic tree from the *gag* part of the amino acid sequence data analysis in Fig. 4 illustrates the remote position of MVP-5180. Phylogenetic analysis of other genes (data not shown) and analysis of the nucleotides of the *pol* gene (12a) produced a similar tree. In summary, the data from the nucleic acid and amino acid sequences indicate that MVP-5180 occupies an extreme position within the other HIV-1 strains, and it has been grouped together with ANT-70 in a new subtype, O (13).

Epidemiology in Cameroon. Analysis of the immunoblot bands of MVP-5180 components indicated that the molecular weights of some proteins were different from those of other HIV-1 isolates. The inner core protein consisted of two protein species with molecular weights of 24,000 and 25,000, and the reverse transcriptase-RNase H complex had molecular weights of about 48,000 and 60,000, compared with 51,000 and 66,000 for HIV-1.

By using competitive immunoblotting, an MVP-5180 prevalence in HIV-1-infected Cameroonians from the Centre Hospitalier Universitaire in Yaoundé of 8% (21 of 261) was found. Using PCR in lymphocyte DNA from a further set of specimens from the same hospital, a prevalence of 7% (5 of 74) was obtained. Thus, even in Cameroon, strains immunologically similar and structurally very close to MVP-5180 are uncommon.

DISCUSSION

It became clear that HIV was a heterogeneric virus when patients with the characteristic clinical picture of AIDS were found to be negative for HIV-1 but infected with HIV-2 (1). Since then, several HIV variants have been described, one of which (ANT-70) (4, 20) was also isolated from Cameroon. The ANT-70 isolate is also characterized by a lower reverse transcriptase molecular weight and low reactivity of its p24 with anti-p24 (4). From the sequence data available, ANT-70 and MVP-5180 belong to the same subtype variant (HIV-1, subtype O) of the group of West Central African HIV-1 isolates, and this subtype lies closer to the point where both types of primate immunodeficiency viruses diverge than the chimpanzee virus SIVcpz does (Fig. 4). Despite the differences in their nucleic acid sequences, both ANT-70 and MVP-5180 are currently the most divergent from the other subtypes of HIV-1 yet described, and they must be taken into account when the natural

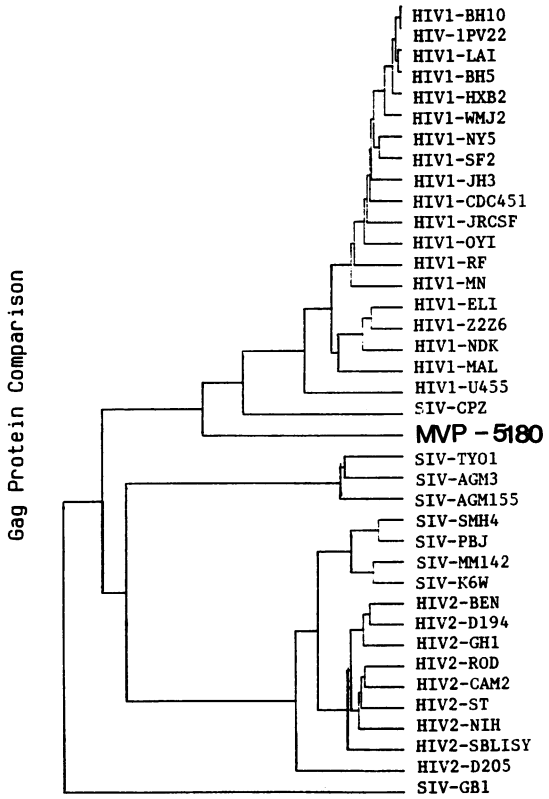

FIG. 4. Relationship of different HIV-1 and HIV-2 isolates established by the University of Wisconsin Genetics Computer Group program (5), using the UPGMA algorithm based on the amino acid sequences of the Gag polyprotein. The extreme position of MVP-5180 within the HIV-1 strains can be seen. Within the HIV-1 pedigree, MVP-5180 has an even more divergent extreme position than the chimpanzee virus SIVcpz. Together with ANT-70 (20), MVP-5180 constitutes a new HIV-1 sequence subtype, assigned to a sequence subtype designated as subtype O (13).

origin of HIV is considered. The heterogeneity of this HIV subtype population is unknown, but the low overall prevalence of all HIV-1 subtypes (3 to 7%) found in the general population of Cameroon (9) means that the MVP-5180-related virus infections are few in number and have been detected as yet only in the southern part of the country.

It is highly likely that this variant HIV causes AIDS. The patient from whom MVP-5180 was isolated died with a typical clinical picture of AIDS; other HIV-1 subtypes were not detected in this patient by PCR, but isolation only of this subtype virus cannot exclude the possibility that other HIVs were involved.

Compared with other HIVs, the configuration of the V3 loop within the sequence of MVP-5180 is the most striking divergence. The crown is composed of either Gly-Pro-Met-Arg (clone) or Gly-Pro-Leu-Arg (PCR sequencing), which is very similar to the crown Gly-Pro-Met-Ala of ANT-70 (13, 20). Also uncommon in the loop is the formation of the N-terminal part by acidic amino acids, together (as usual) with hydrophobic amino acids. Finally, compared with the LAI isolate (13), the V3 loop had three or five additional amino acids (Fig. 3). The V3 loop is one of the dominant neutralization domains of HIV-1 (8), and as its structure in MVP-5180 compared with isolates like LAI, MN, or SF-2 (13) is less than 50% similar,

vaccines intended to induce neutralizing antibodies must be adapted to the configuration of this loop in order to be effective also in Central Africa.

Viruses of subtype O are likely to be prevalent also in countries adjacent to Cameroon. Divergent strains have been observed by Murphy et al. (12) in the Central African Republic. Since movements of infected individuals may lead to distribution of viruses of this subtype to other parts of the world, knowledge of the sequence can avoid misinterpretation of immunoblot patterns and explain failures in PCR amplification.

ACKNOWLEDGMENTS

We acknowledge with gratitude the initiation of this work by the late Friedrich Deinhardt. We thank B. Seed, Massachusetts General Hospital, Boston, and H. E. Pauly, U. Grundmann, and G. Zettelmeissl, Behring-Werke, Marburg, Germany, for their kind support of the project; and B. Schmidt-Ehry and T. Rehle, Gesellschaft für technische Zusammenarbeit, Eschborn, Germany, for valuable support and interest.

REFERENCES

1. **Clavel, F., D. Guétard, F. Brun-Vézinet, S. Chamaret, M. A. Rey, M. O. Santos-Feirrera, A. G. Laurent, C. Dauguet, C. Katlana, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier.** 1986. Isolation of a new retrovirus from West African patients with AIDS. Science **233:**343–346.
2. **Cloyd, M. W., and B. E. Moore.** 1990. Spectrum of biological properties of human immunodeficiency virus (HIV-1) isolates. Virology **174:**103–116.
3. **Coombs, R. W., A. C. Collier, and L. Corey.** 1991. Plasma viremia as an endpoint in evaluating the effectiveness of drugs against human immunodeficiency virus type-1 (HIV) infection: natural history of plasma viremia and monitoring antiretroviral therapy, p. 9–19. *In* J. M. Andrieu (ed.), Viral quantitation in HIV infection. John Libbey Eurotext, Montrouge.
4. **De Leys, R., B. Vanderborght, M. vanden Haesevelde, I. Heyndrickx, A. van Geel, C. Wauters, R. Bernaerts, E. Saman, P. Nijs, B. Willems, H. Taelman, G. van der Groen, P. Piot, T. Tersmette, J. G. Huisman, and H. van Heuverswyn.** 1990. Isolation and partial characterization of an unusual human immunodeficiency retrovirus from two persons of West-Central African origin. J. Virol. **64:**1207–1216.
5. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
6. **Dietrich, U., M. Adamski, R. Kreutz, A. Seipp, H. Kühnel, and H. Rübsamen-Waigmann.** 1989. A highly divergent HIV-2-related isolate. Nature (London) **342:**948–950.
7. **Eberle, J., and R. Seibl.** 1992. A new method for measuring reverse transcriptase activity by ELISA. J. Virol. Methods **40:**347–356.
8. **Freed, E. O., D. J. Myers, and R. Risser.** 1991. Identification of the principal neutralizing determinant of human immunodeficiency virus type 1 as a fusion domain. J. Virol. **65:**190–194.
9. **Garcia-Calleja, J. M., L. Zekeng, J. P. Louis, J. L. Mvondo, A. Trebucq, D. Sokal, D. Yanga, A. Ndoumou, D. Andela, R. Salla, J. Gardon, S. Abbenyi, and L. Kaptue.** 1992. HIV infection in Cameroon: 30 months' surveillance in Yaoundé. AIDS **6:**881–882.
10. **Gürtler, L. G., J. Eberle, B. Lorbeer, and F. Deinhardt.** 1987. Sensitivity and specificity of commercial ELISA kits for screening anti-LAV/HTLV-III. J. Virol. Methods **15:**11–23.
11. **Kellog, D. E., and S. Kwok.** 1990. Detection of human immunodeficiency virus, p. 337–347. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols—guide to methods and applications. Academic Press, San Diego, Calif.
12. **Murphy, E., B. Korber, M. C. Georgers-Courbot, B. You, A. Pinter, D. Cook, M. P. Kieny, A. Georges, C. Mathiot, F. Barré-Sinoussi, and M. Girard.** 1993. Diversity of the V3 region sequences of human immunodeficiency viruses type 1 from Central African Republic. AIDS Res. Hum. Retroviruses **9:**997–1006.

12a. **Myers, G.** Personal communication.

13. **Myers, G., B. Korber, S. Wain-Hobson, R. F. Smith, and G. N. Pavlakis.** 1993. Human retroviruses and AIDS 1993. A compilation and analysis of nucleic acid and amino acid sequences. Los Alamos National Laboratory, Los Alamos, N. Mex.
14. **Oram, J. D., R. G. Downing, M. Roff, J. C. S. Clegg, D. Derwada, and J. W. Carswell.** 1990. Nucleotide sequence of a Ugandian HIV-1 provirus reveals genetic diversity from other HIV-1 isolates. AIDS Res. Hum. Retroviruses **6:**1073–1078.
15. **Ou, C. Y., S. Kwok, S. W. Mitchell, D. H. Mack, J. J. Sninsky, J. W. Krebs, P. Feorino, D. Warfield, and G. Schochetman.** 1988. DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science **239:**295–297.

15a. **Peeters, M.** Personal communication.

16. **Peeters, M., C. Honoré, T. Huet, L. Bedjabaga, S. Ossari, P. Bussi, R. W. Cooper, and E. Delaporte.** 1989. Isolation and partial characterization of an HIV-related virus occurring naturally in chimpanzees in Gabon. AIDS **3:**625–630.
17. **Rieber, E. P., C. Federle, C. Reiter, S. Krauss, L. Gürtler, J. Eberle, F. Deinhardt, and G. Riethmüller.** 1992. The monoclonal CD4 antibody M-T413 inhibits cellular infection with human immunodeficiency virus after viral attachment to the cell membrane: an approach to postexposure prophylaxis. Proc. Natl. Acad. Sci. USA **89:**10792–10796.
18. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
20. **Vanden Haesevelde, M., J.-L. Decourt, R. J. De Leys, B. Vanderborght, G. van der Groen, H. van Heuverswijn, and E. Saman.** 1994. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. J. Virol. **68:**1586–1596.