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Molecular Characterization of Variable Heavy and Light Chain Regions of Five HIV Type 1-Specific Human Monoclonal Antibodies*

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ABSTRACT

We have reported the generation and characterization of four HIV-1 neutralizing human monoclonal antibodies. Three antibodies recognize a conformational epitope within the CD4-binding site of HIV-1 gp120 and one recognizes a linear epitope located within the hypervariable V3 domain of gp120. In the present study we report the nucleotide sequences of the cDNAs encoding the variable regions of the heavy and light chains of these antibodies. Molecular characteristics, closest germline genes, and the putative extent of somatic mutation are presented. Two of the four heavy chain variable (V_H) regions are derived from the V_H1 gene family, one from the V_H3 gene family, and one from the V_H5 gene family. In addition, the V_H chain of a previously described human monoclonal antibody, directed against HIV-1 gp41, is derived from the V_H3 gene family. The degree of nucleotide variation between these five antibodies and their closest germline counterparts ranges from 4 to 12%, mainly located in the complementarity-determining regions. Significant nucleotide sequence homology with previously described germline diversity (D) genes could be found for only two of five antibody D segments. Joining (J_H) gene segments utilized are J_H4 or J_H6. Two light chain variable (V_L) regions are derived from a $V_\kappa 1$ gene segment, one from a $V_\kappa 4$, one from a $V_\lambda 2$, and one from a $V_\lambda 6$ gene segment.

INTRODUCTION

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS) is the late-stage disease of infection with human immunodeficiency virus (HIV). Counter-AIDS strategies include the development of active immunization protocols to prevent HIV infection, and passive immunization protocols for postexposure therapy. Passive immunization in particular may be important in preventing transmission of HIV from infected mothers to their offspring[1] and in preventing infection after accidental exposure. These goals may be achieved by the administration of a cocktail of human monoclonal antibodies (MAbs), capable of neutralizing a variety of HIV strains.

HIV-1 infection elicits at least two major types of neutralizing antibodies directed against gp120. One is directed against the hypervariable V3 domain, the other against the conserved CD4-binding site (b.s.). Antibodies directed against the V3 domain are found already in the early phase of infection. Initially it was proposed that V3 domain-specific antibodies would predominantly neutralize the eliciting HIV-1 strain.[2–5] However, it has been demonstrated that several V3 domain-specific antibodies have much broader reactivities than previously suggested.[6–9] Emini *et al.* showed that chimpanzees, passively immunized with an HIV-1 IIIB neutralizing V3 domain-specific antibody, were protected against infection with the homologous HIV strain.[10,11] Antibodies directed against this side are therefore likely candidates for passive immunization. Antibodies directed against the CD4 b.s. are detected later in infection and have a wide range of neutralizing activity against HIV-1 strains, owing to the conserved nature of the CD4 b.s.[12] The neutralizing capacity of these antibodies seems to be generally lower, as compared to the V3 domain-specific antibodies.[13] Equimolar mixtures of human MAbs, directed against either of these two sites, may have a synergistic HIV-neutralizing effect.[14–16] We described the generation and characterization of four HIV-1-neutralizing human MAbs: one V3 domain-specific antibody (MN215) and three antibodies directed against the CD4 b.s. (GP13, GP44, and GP68).[9,17] GP13, GP44, and GP68 display

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*The sequences reported in this article have been submitted to the GenBank database.

broadly HIV-1-neutralizing activity, whereas MN215 reacts predominantly with macrophage-tropic and non-syncytium-inducing (NSI) HIV-1 strains.[9] During the asymptomatic stage of the HIV-1 infection predominantly HIV-1 strains of the NSI phenotype are found.[18] A low efficiency of the primary immune response in eliminating NSI/macrophage-tropic HIV-1 strains or a preferential transmission of these HIV-1 strains has been suggested.[19] This makes MN215 a likely candidate to be used in preparations for early passive immunization therapies. Here we report the molecular characterization of the variable heavy and light chain regions of these four human MAbs, and of a previously described broadly reactive human MAb (K14) directed against an epitope on HIV-1 gp41.[20] Although this is a nonneutralizing MAb, it was included in these studies because of a possible synergistic therapeutic effect in a cocktail of human MAbs.

Molecular characterization of the V genes used by HIV-neutralizing, -nonneutralizing, and -enhancing human MAbs will lead to a better understanding of the interaction between HIV and the antibody repertoire. Furthermore, molecular data are necessary for the *in vitro* construction of broadly reactive, high-affinity HIV-neutralizing human antibodies and in discriminating between neutralizing and enhancing antibodies. These data will provide a valuable contribution to the development of an efficacious anti-HIV vaccine to be used in passive immunization protocols. Therefore, in the present study we give a detailed molecular characterization of the V regions of the five antibodies mentioned above. Remarkably, two of five HIV-specific human MAbs presented here express a V_H3 gene segment, whereas data suggest a superantigen-like binding of gp120 to (membrane) immunoglobulin V_H3 gene products and the subsequent deletion of V_H3-expressing B cell clones in AIDS patients.[21,22]

MATERIALS AND METHODS

HIV-1-specific human monoclonal antibodies

Human MAbs to HIV-1 were isolated from Epstein–Barr virus (EBV)-transformed B cell lines, derived from peripheral blood mononuclear cells from asymptomatic HIV-1-seropositive donors, as described previously.[17,20] Briefly, EBV-transformed B cells producing IgG antibodies specific for HIV-1 were selected by screening for reactivity in enzyme-linked immunosorbent assay (ELISA) with either gp120, gp160, or V3 loop peptides.[9,17] Five IgG_1 MAbs from three donors were studied: GP13, GP44, and GP68, recognizing a conformational epitope partly overlapping with the CD4 b.s. of gp120[17]; MN215, recognizing the principal neutralizing domain (V3 domain) of gp120 of the MN isolate[9]; and K14, recognizing an epitope on gp41.[20] Monoclonal antibodies GP13 and GP68 have been demonstrated to neutralize various HIV-1 laboratory isolates *in vitro*, GP44 neutralizes the SF2 isolate, and MN215 neutralizes the MN and SF2 isolates.[9,17]

Oligonucleotides

The oligonucleotides used in the PCR amplifications were synthesized on an Applied Biosystems (Foster City, CA) DNA synthesizer. Sequences of the oligonucleotides are shown in Table 1.

Single-stranded cDNA synthesis and polymerase chain reaction

Total RNA was extracted from 10^7 EBV-transformed B cells by the RNAzol method (CINNA/Biotex Laboratories, Inc., Houston, TX). Single-stranded cDNA (ss-cDNA) was synthesized by using Moloney leukaemia virus (M-MLV) H^- reverse transcriptase superscript (GIBCO-BRL/Life Technologies, Gaithersburg, MD) and an oligo(dT) primer. Polymerase chain reactions (PCRs)[23] were done essentially via the method recommended by the manufacturer (Perkin-Elmer Cetus, Norwalk, CT). The PCR cycles were as follows: denaturation at 96°C for 1 min, annealing at 60°C for 2 min, and extension at 72°C for 1 min 30 sec, controlled in a DNA thermal cycler (Perkin-Elmer Cetus).

Isolation, cloning, and sequencing of amplified products

Amplified DNA was digested with *Sst*I and *Hind*III and size selected on a 1% ethidium bromide agarose gel. The purified

TABLE 1. SEQUENCES OF OLIGONUCLEOTIDES USED IN POLYMERASE CHAIN REACTION AMPLIFICATIONS

Oligonucleotide	*Sequence*
V_H1 leader	5′ ATAGAGCTCATGGACTGGACCTGGAGG 3′[a]
V_H3 leader	5′ ATAGAGCTCTGGAGTTTGGGCTGAGCTGG 3′[a]
V_H3 BACK	5′ ATAGAGCTCGAGGTGCAGCTGGTGGAGTCT 3′[a]
V_H5 leader	5′ ATAGAGCTCTCGCCCTCCTCCTG 3′[a]
$V_\kappa1$ leader	5′ ATAGAGCTCCTGCTGCTGCTGTGGCTGCCC 3′[a]
$V_\kappa1$ leader	5′ ATAGAGCTCATGGACATGAGGGTCCCC 3′[a]
$V_\kappa4$ leader	5′ ATAGAGCTCATGGTGTTGCAGACCCAG 3′[a]
$V_\lambda2$ leader	5′ ATAGAGCTCTGGACTCCCCTCCTCCTCACT 3′[a]
$V_\lambda6$ leader	5′ ATAGAGCTCCTCACTCACTGTACTGGTTCT 3′[a]
C_γ	5′ CTCAAGCTTCAGGGGAAGACCGATGG 3′[b]
C_κ	5′ CTCAAGCTTAACAGAGGCAGTTCCAGACTT 3′[b]
C_λ	5′ CTCAAGCTTTGTGGCCTTGTTGGCTTG 3′[b]

[a]The *Sst*I restriction site is underlined.
[b]The *Hind*III restriction site is underlined.

A GP13 V_H5

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GAA GTG CAG CTG GTG CAG TCC GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG AGG ATC TCC TGT AGG GGT
 e   v   q   l   v   q   s   g   a   e   v   k   k   p   g   e   s   l   r   i   s   c   r   g

                        CDR-I
TCT GGA TAC AGC TTT CCC AAT TAC TGG GTC AGC TGG GTG CGC CAG ATG CCC GGG AAA GGC CTG GAG TGG ATG
 s   g   y   s   f   p   n   y   w   v   s   w   v   r   q   m   p   g   k   g   l   e   w   m

                            CDR-II
GGA AAG ATT GAT CCT AGT GAC TCT GAG ACC AAC TAC AGC CCG TCC TTC CAA GGC CAC GTC ACC ATC TCG GCT
 g   k   i   d   p   s   d   s   e   t   n   y   s   p   s   f   q   g   h   v   t   i   s   a

GAC AAG TCC CTC AGT ATT GCC TAT CTG CAG TGG AAC AGC CTG AAG GCC TCG GAC ACC GCC ATG TAT TAC TGT
 d   k   s   l   s   i   a   y   l   q   w   n   s   l   k   a   s   d   t   a   m   y   y   c

                                           D
GCG AGA CAT AAG AGG GGC GCG CCG ACG TAT AAA GAT ATC TTG ACT GGT TAT TAT GTC GAT GGT ATG GAC GTC
 a   r   h   k   r   g   a   p   t   y   k   d   i   l   t   g   y   y   v   d   g   m   d   v

            JH6
TGG GGC CAA GGG ACC ACG GTC ACC GTC TCG TCA
 w   g   q   g   t   t   v   t   v   s   s
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B GP44 V_H1

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CAG GTG CAG CTG GTG CAG TCT GGG TCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG GTT TCC TGC AAG GCA
 q   v   q   l   v   q   s   g   s   e   v   k   k   p   g   a   s   v   k   v   s   c   k   a

                              CDR-I
TCT GGA TAC ACC TTC ACC ACC TAC TAT ATA CAC TGG GTG CGT CAG GCC CCT GGA CAA GGG CTT GAG TGG ATG
 s   g   y   t   f   t   t   y   y   i   h   w   v   r   q   a   p   g   q   g   l   e   w   m

                                 CDR-II
GGA ATA ATC AAC CCT AGT GAT GGA AGT AGA AAC TAC GCA CAG AAC TTC CAG GGC AGA GTC ACC ATG ACC AGG
 g   i   i   n   p   s   d   g   s   r   n   y   a   q   n   f   q   g   r   v   t   m   t   r

GAC ACG TCC ACG AGC ACA GTC TAC ATG GAG CTG GGC AGA CTG AGA TCT GAA GAC ACG GCC ATA TAT TAC TGT
 d   t   s   t   s   t   v   y   m   e   l   g   r   l   r   s   e   d   t   a   i   y   y   c

                                 D                                                       JH4
GCG AGA GAT CTC CGC CTA ATG TTA TTT ACT TTG AGG GGG GGT GTG ATG CTT GAC TAC TGG GGC CAG GGA ACC
 a   r   d   l   r   l   m   l   f   t   l   r   g   g   v   m   l   d   y   w   g   q   g   t

CTG GTC ACC GTC CCC TCA
 l   v   t   v   p   s
```

C GP68 V_H1

```
CAG GTG CAG CTG GTC CAG TCT GGG GCT GAA GTG AAG AAG CCT GGG TCC TCG GTG AAG GTC TCC TGC AAG GCT
 q   v   q   l   v   q   s   g   a   e   v   k   k   p   g   s   s   v   k   v   s   c   k   a

                              CDR-I
TCT GGA GGC ACA TTC AGC AGC TCA ACT CTC AAC TGG GTA CGA CAG ACC CCC GGA CAA GGA CTT GAG TGG ATG
 s   g   g   t   f   s   s   s   t   l   n   w   v   r   q   t   p   g   q   g   l   e   w   m

                                 CDR-II
GGC AAA ATC ATC CCT ACC TTG GGT TCA TCA ACC TAT TCA CAG AAC TTC CAG GGG AGA GTC ACC CTT ACC GCG
 g   k   i   i   p   t   l   g   s   s   t   y   s   q   n   f   q   g   r   v   t   l   t   a

GAC GAA TCC ACG AGC ACA GTC TAC ATG GAA CTG AGT GGT CTG ACA TCT GCG GAC ACG GCC GTA TAT TAC TGT
 d   e   s   t   s   t   v   y   m   e   l   s   g   l   t   s   a   d   t   a   v   y   y   c

                                 D                                                   JH4
GCG AAA AGT GGG GCC GGG TGG AGT AAT TTA ATC CGC TCC CCC ATT GAC AAC TGG GGC CAG GGA ACG CTG GTC
 a   k   s   g   a   g   w   s   n   l   i   r   s   p   i   d   n   w   g   q   g   t   l   v

ACC GTC TCC TCA
 t   v   s   s
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D MN215 V_H3

```
      VHIII(BACK')primer
GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC CTG GTC AAG CCT GGG GGG TCC CTG ACA CTC TCC TGT GCA GCC
 e   v   q   l   v   e   s   g   g   g   l   v   k   p   g   g   s   l   t   l   s   c   a   a

                                CDR-I
TCT GGA TTC ACC TTC AGT TCC TAT AGC ATG AAC TGG GTC CGC CAG GCT CCA GGG AAG GGC CTG GAG TGG GTC
 s   g   f   t   f   s   s   y   s   m   n   w   v   r   q   a   p   g   k   g   l   e   w   v

                                    CDR-II
TCA TCC ATT AGT AAT AGT AGT TCT TAC ATC TAT TAC GCA GAC TCA GTG AAG GGC CGA TTC ACC ATC TCC AGA
 s   s   i   s   n   s   s   s   y   i   y   y   a   d   s   v   k   g   r   f   t   i   s   r

GAC AAC GCC AAG AAC TCA CTG TAT CTG CAA ATG AAC AGC CTG AGA GCC GAG GAC ACG GCT GTG TAT TAT TGT
 d   n   a   k   n   s   l   y   l   q   m   n   s   l   r   a   e   d   t   a   v   y   y   c

                           D                                                     JH4
GCG AGT CTT GAG GGG GGG ATG GTG GAA GGA GAT CTT CAC TCC TTT GGC TAC TGG GGC CAG GGA AGG TTG GTC
 a   s   l   e   g   g   m   v   e   g   d   l   h   s   f   g   y   w   g   q   g   r   l   v

ACC GTC TCG TCA
 t   v   s   s
```

E K14 V_H3

```
AAG GTG CAG TTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGT GGG TCC CTG AGA CTC TCC TGT GCA GCC
 k   v   q   l   l   e   s   g   g   g   l   v   q   p   g   g   s   l   r   l   s   c   a   a

                                CDR-I
TCT GGA TTC AGT TTT GGA GCC TAT TAC ATG GCT TGG GTC CGC CAG GCT CCA GGG AAG GGC CTG GAG TGG GTC
 s   g   f   s   f   g   a   y   y   m   a   w   v   r   q   a   p   g   k   g   l   e   w   v

                                    CDR-II
TCA GGT ATT ATT AGT GGC AGT GGT GTT AAC TCA CAC TAC GCA GAC TCT GTG AAG GGC CGG TTC ACC ATC TCC
 s   g   i   i   s   g   s   g   v   n   s   h   y   a   d   s   v   k   g   r   f   t   i   s

AGA GAC AAT TAC AAG AAC ACG CTA TAT CTG CAA ATG AGC AGC CTG AGA GCC GAC GAC ACG GCC ATG TAT TAC
 r   d   n   y   k   n   t   l   y   l   q   m   s   s   l   r   a   d   d   t   a   m   y   y

                        D                                                JH4
TGT ACG AAA CGT GCG GAG GCA GCA TCT GGA CCC TTT GAC TTC TGG GGC CAG GGA ACC CTG GTC ACC GTC TCC
 c   t   k   r   a   e   a   a   s   g   p   f   d   f   w   g   q   g   t   l   v   t   v   s

TCA
 s
```

FIG. 1. cDNA sequences of the heavy chain variable regions of HIV-1-specific human monoclonal antibodies. The CDR-I, CDR-II, D, and J_H segments are denoted. cDNA sequences of the heavy chain of monoclonal antibodies GP13 (A), GP44 (B), GP68 (C), MN215 (D), and K14 (E) are shown.

product was ligated into the *SstI/HindIII* restriction site of a Bluescript phagemid vector and transformed into $CaCl_2$-competent XL1-blue bacteria. Several recombinant clones were selected and sequenced in both orientations using nonradioactive dye-labeled T3 and T7 oligonucleotide primers (Applied Biosystems) on a 370 A automated sequencer (Applied Biosystems).

Owing to limited availability of patient materials we were not able to obtain genomic DNA for the isolation of the respective germline gene counterparts. Therefore the latest update of the total EMBL/GenBank database was searched to identify expressed as well as germline genes displaying the highest nucleotide sequence similarities with the V genes presented in these article. Primary amino acid sequences were deduced and alignments were carried out using the DNAstar program (DNAstar, Inc., Madison, WI).

RESULTS

Analysis of the expressed V_H genes encoding HIV-1-specific human monoclonal antibodies

The complete nucleotide sequences determined for the V_H region of each antibody are shown in Fig. 1. As a result of the databank searches each of the expressed V_H regions could be compared with its closest germline counterpart (Fig. 2). An overview of the five HIV-1-specific human MAbs and their characterization is given in Table 2.

The GP13 V_H gene contains an open reading frame, has all the features characteristic of a functional V_H gene, and is most homologous to the previously described V_H5 germline gene V_H32 (94.2%).[24] There are two nucleotide differences in framework (FR) I, one being silent and one causing an amino acid substitution, and there is one silent mutation in FR II. The most extensive variation is found in the two CDRs (8–22%), which is indicative for an antigen-driven immune response. More than 60% of the total mutations in the complementarity-determining regions (CDRs) result in amino acid substitutions. An unusual number of nucleotide differences ($n = 6$) was observed within framework III of GP13 V_H as compared to the analogous framework of V_H32. Three of the six nucleotide differences in framework III resulted in amino acid substitutions (Fig. 3A). Furthermore, the degree of variation (6%) is higher than the usual mutation rate described for frameworks (i.e., 2%), indicating a possible role for the framework residues in HIV-1 binding.[25]

The GP44 V_H gene segment is a member of the V_H1 gene family and is most homologous to the HV1f10 germline gene (94.9%).[26] Furthermore, there is 88.7% nucleotide sequence

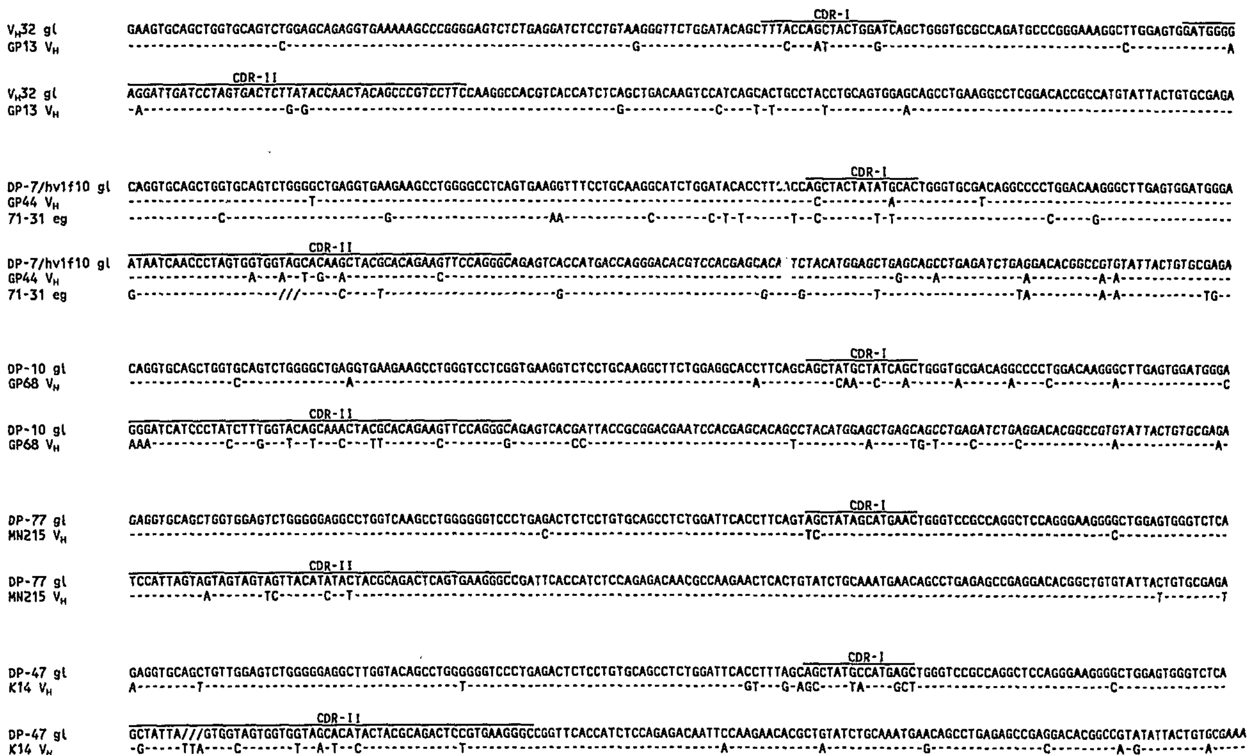

FIG. 2. Nucleotide sequence of each HIV-1-specific heavy chain variable region compared to the nucleotide sequence of the closest germline genes. Identities between sequences are indicated by dashes. (A) Nucleotide sequence of GP13 compared to the V_H32 germline sequence. (B) Nucleotide sequence of GP44 compared to the identical DP-7 germline and hv1f10 germline sequences and to the expressed gene 71-31. (C) Nucleotide sequence of GP68 compared to the DP-10 germline sequence. (D) Nucleotide sequence of MN215 compared to the DP-77 germline seqence. (E) Nucleotide sequence of K14 compared to the DP-47 germline sequence. gl, Germline; eg, expressed gene.

TABLE 2. CHARACTERISTICS OF FIVE HIV-1-SPECIFIC HUMAN MONOCLONAL ANTIBODIES

Antibody	Specificity	Virus neutralizing	V_H	V_L	% homology with closest germline gene	
					V_H	V_L
GP13 (IgG_1,κ)	CD4 b.s.	Yes	V_H5	$V_\kappa 4$	94.2% V_H32	94.1% $V_\kappa 4$
GP44 (IgG_1,λ)	CD4 b.s.	Yes	V_H1	$V_\lambda 2$	94.9% HV1f10	88.1% IGLV21
GP68 (IgG_1,κ)	CD4 b.s.	Yes	V_H1	$V_\kappa 1$	87.8% DP-10	93.6% HK102
MN215 (IgG_1,λ)	V3 domain	Yes	V_H3	$V_\lambda 6$	96.3% DP-77	—[a]
K14 (IgG_1,κ)	gp41	No	V_H3	$V_\kappa 1$	89.8% DP-47	89.0% HK137

[a]—, No significant homology.

similarity with a previously described V_H1 gene, 71-31, expressed by an HIV-1-specific human MAb.[27] However, this MAb is directed against an epitope on HIV-1 p24. The GP68 V_H gene is also derived from the V_H1 gene family and is most homologous to the DP-10 V_H1 germline gene (87.8%).[28]

The MN215 V_H gene is derived from the largest gene family (V_H3) and is most homologous to the DP-77 germline gene (96.3%).[28] The K14 V_H gene is also derived from the V_H3 gene family and is 89.8% identical to the DP-47 germline gene.[28] A remarkable difference between K14 V_H and DP-47 is three additional nucleotides encoding an isoleucine residue in CDR-II of K14. Therefore it is unlikely that DP-47 would be the germline counterpart of K14 V_H.

Owing to the relatively high number of still unidentified members of the V_H1 and V_H3 gene families it remains difficult to determine whether our expressed GP44, GP68, MN215, and K14 V_H genes represent somatically mutated or as yet unidentified germline genes.

Analysis of expressed V_L genes

The complete nucleotide sequences determined for the V_L region of GP13, GP44, GP68, MN215, and K14 are shown in Fig. 4. Each of the expressed V_L regions was compared with the respective, closest germline sequence obtained from the EMBL/GenBank database (Fig. 5).

The GP13 V_L gene contains an open reading frame that has all the features characteristic of a functional $V_\kappa 4$ gene segment. There is 94.1% homology with the previously described $V_\kappa 4$ germline gene,[29] which is the only $V_\kappa 4$ gene found on the human Ig κ locus. There are six nucleotide differences within the frameworks, all causing an amino acid substitution, except for the one in FR III (Fig. 3B). The variation in the three CDRs ranges from 5 to 16%, with most of the nucleotide differences accumulated within CDR-I. Furthermore, 75% of the nucleotide mutations in the CDRs result in amino acid substitutions.

The GP44 V_L gene segment is a member of the $V_\lambda 2$ gene family and is 88.1% identical to the IGLV21 germline gene.[30] However, it is unlikely that the IGLV21 germline gene would be the germline counterpart of the GP44 V_L gene, because there is 94.6 and 94.2% homology with two other expressed V_L genes, WLR[31] and PV6,[32] respectively. Furthermore, there is 92.5% homology with the expressed gene HBW4-1,[32a] also encoding an HIV-1 gp120-specific human MAb. The GP68 V_L gene is derived from the $V_\kappa 1$ gene family and is 93.6% identical to the HK102 germline gene[33] and 94.7 and 95.4% identical to the expressed genes 3D6[34] and kalc6,[35] respectively. Gene 3D6 also encodes an HIV-1-specific human MAb, but this MAb is directed against gp41, a transmembrane glycoprotein of HIV-1. The MN215 V_L gene segment is a member of the $V_\lambda 6$ gene family. However, significant homology could be found only with two expressed genes, H95.EBV (93.9%)[36] and EB4V_λVI (94.5%).[37] The K14 V_L gene is also derived from the $V_\kappa 1$ gene family and is 89.0% homologous to the HK137 germline gene.[38] Again, better homology was found with two expressed genes, HGQ (95.1%)[39] and A20 (95.2%),[40] suggesting another germline gene for K14 V_L. There is only 87.6% homology with the expressed gene No. 86,[32a] also encoding an HIV-1 gp41-specific human MAb.

Analysis of D, J_H, and J_L gene segments

Figure 6 shows the sequences of the expressed D segments, compared to their closest germline counterpart[41] or to other previously described expressed D segments.[42–46] Part of the GP13 D segment may be derived from the D_{XP1} germline. This part could be preceded by another unknown D segment, causing a

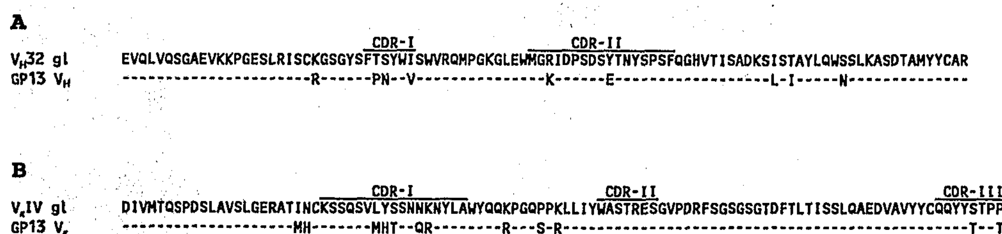

FIG. 3. Deduced amino acid sequences of the heavy (A) and light (B) chain variable regions of GP13 compared to the amino acid sequences of the closest germline genes. Identities between sequences are indicated by dashes.

A GP13 $V_\kappa 4$

```
GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT GTG TCT CTG GGC GAG AGG GCC ACC ATG CAC TGC AAG
 d   i   v   m   t   q   s   p   d   s   l   a   v   s   l   g   e   r   a   t   m   h   c   k
                         CDR-I
TCC AGC CAG AGT GTT ATG CAC ACC TCC AAC CAA AGG AAC TAC TTA GCG TGG TAC CAG CAG AGA CCA GGA CAG
 s   s   q   s   v   m   h   t   s   n   q   r   n   y   l   a   w   y   q   q   r   p   g   q
                                       CDR-II
TCT CCT AGG CTG CTC ATT TAC TGG GCA TCT ACC CGG GAA TCT GGG GTC CCT GAC CGA TTC AGT GGC AGC GGG
 s   p   r   l   l   i   y   w   a   s   t   r   e   s   g   v   p   d   r   f   s   g   s   g

TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG CAG GCT GAA GAT GTG GCG GTT TAT TAC TGT CAG CAA
 s   g   t   d   f   t   l   t   i   s   s   l   q   a   e   d   v   a   v   y   y   c   q   q
   CDR-III                                 Jκ2
TAT TAT ACT ACT CCT ACG TAC ACT TTT GGC CAG GGG ACC AAG CTG GAG ATC AAA
 y   y   t   t   p   t   y   t   f   g   q   g   t   k   l   e   i   k
```

B GP44 $V_\lambda 2$

```
CAG TCT GCC CTG ACT CAG CCT CCC TCC GCG TCC GGG TCT CCT GGA CAG TCA GTC ACC ATC TCC TGC TCT GGA
 q   s   a   l   t   q   p   p   s   a   s   g   s   p   g   q   s   v   t   i   s   c   s   g
                    CDR-I
ACC AGC AGT GAC GTT GGT GCT TAT AAG TAT GTC TCC TGG TTC CAA CAA CAC CCC GGC AAA GCC CCC AAA CTC
 t   s   s   d   v   g   a   y   k   y   v   s   w   f   q   q   h   p   g   k   a   p   k   l
                         CDR-II
ATG ATT TAT GAA GTC AAT GAG CGG CCC TCA GGG GTC CCT GAT CGC TTC TCT GGC TCC AAG TCT GGC AAC ACG
 m   i   y   e   v   n   e   r   p   s   g   v   p   d   r   f   s   g   s   k   s   g   n   t
                                                                                     CDR-III
GCC TCC CTG ACC GTC TCT GGG CTC CAA CCT GAG GAT GAG GCT GAT TAT TAT TGC GCC TCA TAT GCA GGC AGT
 a   s   l   t   v   s   g   l   q   p   e   d   e   a   d   y   y   c   a   s   y   a   g   s
                                 Jλ2
AAC ATC GTG ATA TTC GGC GGA GGG ACA AAG TTG ACC GTC CTA GGT
 n   i   v   i   f   g   g   g   t   k   l   t   v   l   g
```

C GP68 $V_\kappa 1$

```
GAC ATC CAG ATG ACC CAG TCT CCT TCC ACC CTG CCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CGG
 d   i   q   m   t   q   s   p   s   t   l   p   a   s   v   g   d   r   v   t   i   t   c   r
                    CDR-I
GCC AGT CAG AGT ATC AGT GGA TGG CTG GCC TGG TAT CAG CAG AAA CCA GGG AAA GCC CCT AAG CTC CTG ATC
 a   s   q   s   i   s   g   w   l   a   w   y   q   q   k   p   g   k   a   p   k   l   l   i
                    CDR-II
CAT AAG ACG TCT ACT TTA GAA AGT GGG GTC CCC TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAA TTC ACT
 h   k   t   s   t   l   e   s   g   v   p   s   r   f   s   g   s   g   s   g   t   e   f   t
                                                                           CDR-III
CTC ACC ATC AGC AGC CTG CAG CCT GAT GAT TTC GCA ACT TAT TAT TGC CAA CAG TAT AAT AGT TTA ATT ACT
 l   t   i   s   s   l   q   p   d   d   f   a   t   y   y   c   q   q   y   n   s   l   i   t
                    Jκ3
TTC GGC CCT GGG ACC AAA GTG GAT ATC AAA
 f   g   p   g   t   k   v   d   i   k
```

D MN215 $V_\lambda 6$

```
AAT TTT ATG CTG ACT CAG CCC CAC TCT GTG TCG GAG TCT CCG GGG AAG ACG GTA ACC ATC TCC TGC ACC GGC
 n   f   m   l   t   q   p   h   s   v   s   e   s   p   g   k   t   v   t   i   s   c   t   g
                    CDR-I
AGT AGT GGC AGC ATT GCC AGC AAC TAT GTG CAG TGG TAC CAG CAG CGC CCG GGC AGT GCC CCC ACC ACT GTG
 s   s   g   s   i   a   s   n   y   v   q   w   y   q   q   r   p   g   s   a   p   t   t   v
                        CDR-II
ATC TAT GCG GAT AAC CAA AGA CCC TCT GGG GTC CCT GAT CGG TTC TCT GGC TCC ATC GAC AGC TCC TCC AAT
 i   y   a   d   n   q   r   p   s   g   v   p   d   r   f   s   g   s   i   d   s   s   s   n
                                                                                     CDR-III
TCT GCC TCC CTC ACC ATC TCT GGA CTG AAG ACT GAG GAC GAG GGT GAC TAC TAC TGT CAG TCT TAT GAT AGG
 s   a   s   l   t   i   s   g   l   k   t   e   d   e   g   d   y   y   c   q   s   y   d   r
                                 Jλ2
AAC AAT CTG GTA TTC GGC GGA GGG ACC AAG CTG ACC GTC CTA GGT
 n   n   l   v   f   g   g   g   t   k   l   t   v   l   g
```

E K14 $V_\kappa 1$

```
GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC CGG
 d   i   q   m   t   q   s   p   s   s   l   s   a   s   v   g   d   r   v   t   i   t   c   r
                    CDR-I
GCG AGT CAG GGC ATT AGC AAT TAT TTA GCC TGG TAT CAG CAG AAA CCA GGG AAA GGT CCC AAG CTC CTG ATC
 a   s   q   g   i   s   n   y   l   a   w   y   q   q   k   p   g   k   g   p   k   l   l   i
                    CDR-II
TAT GGT GCA TCC ACT TTG CAA TTA GGG GTT CCA TCT CGG TTC AGT GGC AGT GGA TCT GGG ACA GAT TTC ACT
 y   g   a   s   t   l   q   l   g   v   p   s   r   f   s   g   s   g   s   g   t   d   f   t
                                                                           CDR-III
TTC ATC ATC AAC AGC CTG CAG CCT GAA GAT GTC GCA ACA TAT TAC TGT CAA AAG TAT AAC AGT GCC CCT GGG
 f   i   i   n   s   l   q   p   e   d   v   a   t   y   y   c   q   k   y   n   s   a   p   g
                             Jκ1
ACG TGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC AAA
 t   w   t   f   g   q   g   t   k   v   e   i   k
```

FIG. 4. cDNA sequences of the light chain variable regions of HIV-1-specific human monoclonal antibodies. The CDR-I, CDR-II, CDR-III, and J_L segments are denoted. cDNA sequences of the light chain of monoclonal antibodies GP13 (A), GP44 (B), GP68 (C), MN215 (D), and K14 (E) are shown.

FIG. 5. Nucleotide sequence of each HIV-1-specific light chain variable region compared to the nucleotide sequence of the closest germline genes or expressed genes. Identities between sequences are indicated by dashes. (A) Nucleotide sequence of GP13 compared to the $V_\kappa4$ germline sequence. (B) Nucleotide sequence of GP44 compared to the $V_\lambda2.1$ germline sequence and to the expressed genes WLR and PV6. (C) Nucleotide sequence of GP68 compared to the H102 germline sequence and to the expressed genes kalc6 and 3D6. (D) Nucleotide sequence of MN215 compared to the expressed genes EB4Vλ6 and H95.EBV. (E) Nucleotide sequence of K14 compared to the HK137 germline sequence and to the expressed genes A20 and HGQ. gl, Germline; eg, expressed gene.

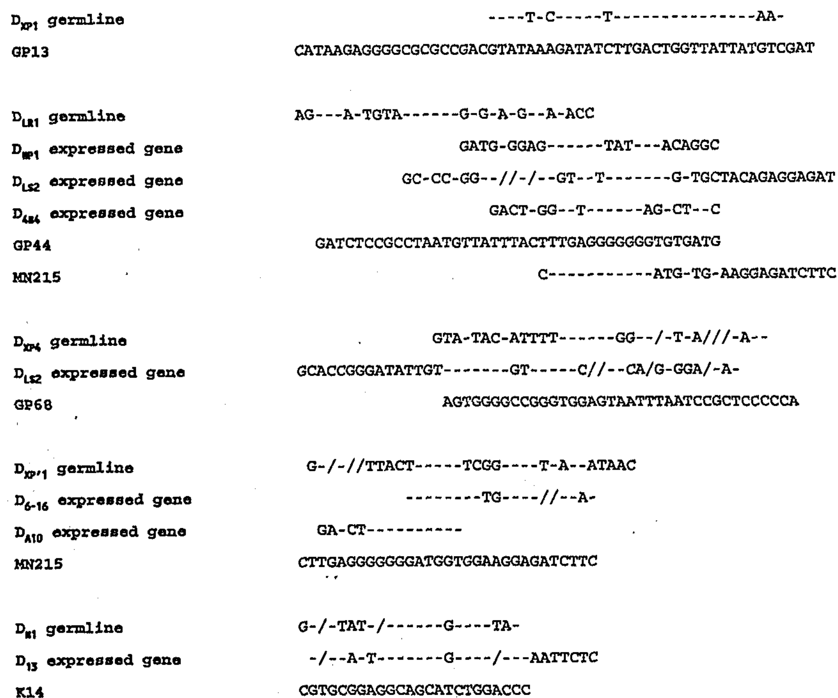

FIG. 6. Possible origins of the D segments of each HIV-1-specific human monoclonal antibody. Identities between sequences are indicated by dashes.

D–D fusion,[47] or by an N segment addition. The last three nucleotides (GAT) could not be derived from a J_H segment and are likely N segment additions. The D segment of K14 may be derived from the D_{N1} germline. For GP44, GP68, and MN215 we found little homology between the expressed D segments and the known germline D segments. Instead, higher homologies with other expressed D segments were observed (Fig. 6).

In Fig. 7 the expressed J_H gene segments are compared with their germline counterparts.[48] Four of the five antibodies express the J_H4 gene segment and one expresses J_H6. Nucleotide differences were observed within all four expressed J_H4 segments, in some cases resulting in amino acid substitutions. Except for MN215, they all miss the first five nucleotides. All four expressed J_H4 segments displayed the same allelic polymorphism, A to G, as described before; only the GP68 J_H4 and MN215 J_H4 segments may display another polymorphism, C to G.[49] Remarkable is the absence of the nucleotide stretch at the 5′ end of GP13 J_H6, normally encoding the five tyrosine residues, characteristic of a J_H6 segment. Furthermore, two nucleotide differences are observed, neither one causing an amino acid substitution.

K14 expresses the $J_{\kappa}1$ gene segment and GP13 expresses $J_{\kappa}2$ (Fig. 8). Both J_{κ} segments are unremarkable except for the last three nucleotides (CGT), which are absent. GP68 expresses the $J_{\kappa}3$ gene segment, which also misses these last three nucleotides. Besides, two nucleotide differences at the 5′ end cause an amino acid substitution. GP44 and MN215 both express the $J_{\lambda}2$ gene segment. For GP44 three nucleotide differences are observed, of which the first one causes an amino acid substitution. For MN215 only the first nucleotide is changed, causing an amino acid substitution.

DISCUSSION

In the present article we have reported the complete nucleotide sequences of the heavy and light chain variable regions of five human MAbs, directed against the envelope glycoproteins of HIV-1. Two heavy chains of these antibodies are derived from the V_H1, two from the V_H3, and one from the V_H5 gene family. For the light chains, two are derived from the $V_{\kappa}1$, one from the $V_{\kappa}4$, one from the $V_{\lambda}2$, and one from the $V_{\lambda}6$ gene family. Only for GP13 were we able to identify the germline counterparts of the V genes. The GP13 V_H gene is derived from the V_H5 gene family, which consists of three members, one of which is a pseudogene.[24] It is most likely that V_H32 is the V_H5 germline counterpart of GP13 V_H. Because it has been shown that the smaller human V_H gene families (V_H4, V_H5, and V_H6) display remarkably little polymorphism,[50] the observed nucleotide differences are most likely caused by somatic mutations. The relatively high number of mutations in FR III may indicate a possible role for some of the FR III amino acids in antigen binding, as has been suggested before.[15] The GP13 V_L gene is derived from the $V_{\kappa}4$ gene family, which consists of only one germline gene. Most of the somatic mutations are concentrated in the CDRs. Therefore, the extensive somatic variation in the V regions of GP13 indicates an antigen-driven (i.e., HIV-1) immune response.

The expressed V_H and V_L genes of the other four human MAbs are derived from V gene families containing an unknown number of still unidentified germline genes. For example, the expressed GP44 $V_{\lambda}2$ gene segment shows more homology with two expressed $V_{\lambda}2$ genes (WLR and PV6) than with any $V_{\lambda}2$ germline gene known at present (Fig. 5). The pattern of nucleotide differences in all three sequences is similar in the CDRs, and even more in the frameworks, suggesting that these three $V_{\lambda}2$ genes may originate from another, as yet unidentified $V_{\lambda}2$ germline gene. A similar observation has been made for the expressed K14 $V_{\kappa}1$ gene and two other expressed $V_{\kappa}1$ genes (A20 and HGQ) (Fig. 5).

Extensive computer analysis was performed on the V region nucleotides as well as the deduced primary amino acid sequences of the human MAbs presented in this article. Comparisons were also made with the sequences of all HIV-1-specific human antibody V regions known to data[27,32a,34,52] and

```
JH4 germline   ACTACTTTGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCA
JH4 GP44            C-----------------G------------------C-----
JH4 GP68            A-----A-----------G-----G------------------
JH4 MN215      ---C-----G-------------G----GGT-------------G---
JH4 K14             -------T----------G------------------------

JH6 germline
ATTACTACTACTACTACGGTATGGACGTCTGGGGGCAAGGGACCACGGTCACCGTCTCCTCA
                 -----------------C-----------------------G---
                 JH6 GP13
```

```
JH4 germline   YFDYWGQGTLVTVSS
JH4 GP44        L-----------P-
JH4 GP68        I-N-----------
JH4 MN215      S-G-----R------
JH4 K14         --F-----------

JH6 germline   YYYYYGMDVWGQGTTVTVSS
JH6 GP13            ---------------
```

FIG. 7. (A) Nucleotide sequences of the J_H segment of each HIV-1-specific human monoclonal antibody compared to the nucleotide sequence of the closest J_H germline gene.[48] (B) Comparison of the deduced amino acid sequences of the J_H segments as described in (A). Identities between sequences are indicated by dashes.

```
Jκ1 germline   TGGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAACGT
Jκ1 K14        ------------------------------------

Jκ2 germline   TACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGT
Jκ2 GP13       ------------------------------------

Jκ3 germline   TTCACTTTCGGCCCTGGGACCAAAGTGGATATCAAACGT
Jκ3 GP68       A-T---------------------------------

Jλ2 germline   GTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAGGT
Jλ2 GP44       ---A----------------A---T--------------
Jλ2 MN215      C--------------------------------------
```

```
Jκ1 germline   WTFGQGTKVEIKR
Jκ1 K14        ------------

Jκ2 germline   YTFGQGTKLEIKR
Jκ2 GP13       ------------

Jκ3 germline   FTFGPGTKVDIKR
Jκ1 GP68       I-----------

Jλ2 germline   VVFGGGTKLTVLG
Jλ2 GP44       -I-----------
Jλ2 MN215      L------------
```

FIG. 8. (A) Nucleotide sequences of the J_L segment of each HIV-1-specific human monoclonal antibody compared to the nucleotide sequence of the closest J_L germline gene.[48] (B) Comparison of the deduced amino acid sequences of the J_L segments as described in (A). Identities between sequences are indicated by dashes.

with the sequences of the V regions of human Fab fragments from combinatorial libraries, recognizing either the CD4 b.s. or the V3 domain of HIV-1 gp120.[53] Also, the corresponding D segments were compared. However, information on antigen–antibody binding could not be obtained by these analyses. Instead of using linear amino acid sequences, three-dimensional modeling of antibody V regions may provide useful structural information.

To date only little information is available on the immunoglobulin variable region gene repertoire used by HIV-1-neutralizing human MAbs (see Refs. 27, 32a, 52, and this article). It was reported that in AIDS patients there is a clonal deficit of V_H3-expressing B cells.[21,22] Surprisingly, we found two V_H3-expressing HIV-1-specific human MAbs, MN215 and K14, obtained from two different HIV-1-seropositive donors. It is unlikely, that this could however, be due to the reported expansion of the V_H3 B cell pool in early clinical stages of HIV infection,[22] because MN215 and K14 were obtained from the donors 3–4 years after seroconversion. So far only one other V_H3-expressing HIV-1 gp41-specific human MAb has been reported.[34] A superantigen-like binding of gp120 to membrane immunoglobulin V_H3 gene products has been suggested.[22] Also serum V_H3 IgM from uninfected individuals was shown to bind to gp120. However, binding of gp120 to V_H3 IgG from uninfected individuals was significantly lower.[22] MN215 and K14 are both V_H3 IgG antibodies, and therefore we suggest a high affinity in binding of MN215 and K14 to their respective specific antigenic sites on HIV-1. This in contrast with a lower binding affinity of gp120 as a superantigen for the immunoglobulin V_H3 gene products MN215 and K14. This may provide an explanation for the presence of V_H3-expressing B cells in later stages of HIV infection. Additional V region nucleotide sequences of HIV-neutralizing human MAbs are necessary to obtain significant structural information which will eventually lead to a better understanding of HIV neutralization by antibody–antigen interactions. Furthermore, potential clinical benefits may be associated with understanding the extent of the V gene repertoire directed against specific HIV antigens presenton infection.[32a] In the near future broadly HIV-neutralizing engineered antibody preparations may play an important role in passive immunization or as therapeutic agents.

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