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

ERIC M.M. VAN DER DONK, MARTIN SCHUTTEN, ALBERT D.M.E. OSTERHAUS, and ROGER W.J. VAN DER HEIDEN

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_H6 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_L1 gene segment, one from a V_L4, one from a V_L2, and one from a V_L6 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 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. However, it has been demonstrated that several V3 domain-specific antibodies have much broader reactivities than previously suggested. 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. 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. The neutralizing capacity of these antibodies seems to be generally lower, as compared to the V3 domain-specific antibodies. Equimolar mixtures of human MAbs, directed against either of these two sites, may have a synergistic HIV-neutralizing effect. 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), GP13, GP44, and GP68 display...

---

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

---

Department of Virology, Erasmus University, Rotterdam, Dr. Molewaterplein 50, P.O. Box 1738, 3000 DR Rotterdam, the Netherlands.
broadly HIV-1-neutralizing activity, whereas MN215 reacts predominantly with macrophage-tropic and non-syncytium-inducing (NSI) HIV-1 strains. During the asymptomatic stage of the HIV-1 infection predominantly HIV-1 strains of the NSI phenotype are found. 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. 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. 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_{H}-3 gene segment, whereas data suggest a superantigen-like binding of gp120 to (membrane) immunoglobulin V_{H}-3 gene products and the subsequent deletion of V_{H}-3-expressing B cell clones in AIDS patients.

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. Briefly, EBV-transformed B cells producing lgG 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. Five IgG2 MABs from three donors were studied: GP13, GP44, and GP68, recognizing a conformational epitope partly overlapping with the CD4 b.s. of gp120; MN215, recognizing the principal neutralizing domain (V3 domain) of gp120 of the MN isolate; and K14, recognizing an epitope on gp41. 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.

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 10⁷ 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) 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 SstI and HindIII and size selected on a 1% ethidium bromide agarose gel. The purified

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_{H}-1 leader</td>
<td>5' ATAGAGCTCATGGACTGAGTGACCTGGAGG 3'</td>
</tr>
<tr>
<td>V_{H}-3 leader</td>
<td>5' ATAGAGCTCATGGTGCTGAGCTGGAGC 3'</td>
</tr>
<tr>
<td>V_{H}-3 BACK</td>
<td>5' ATAGAGCTCATGGTGCTGAGCTGGAGCTT 3'</td>
</tr>
<tr>
<td>V_{H}-S leader</td>
<td>5' ATAGAGCTCATGGTGCTGAGCTGGAGCTT 3'</td>
</tr>
<tr>
<td>V_{L} leader</td>
<td>5' ATAGAGCTCATGGTGCTGAGCTGGAGCTT 3'</td>
</tr>
<tr>
<td>V_{L} leader</td>
<td>5' ATAGAGCTCATGGTGCTGAGCTGGAGCTT 3'</td>
</tr>
<tr>
<td>V_{L} leader</td>
<td>5' ATAGAGCTCATGGTGCTGAGCTGGAGCTT 3'</td>
</tr>
<tr>
<td>V_{L} leader</td>
<td>5' ATAGAGCTCATGGTGCTGAGCTGGAGCTT 3'</td>
</tr>
<tr>
<td>C_{H}</td>
<td>5' CTCAAGCTTACAGGGAGACCGATGG 3'</td>
</tr>
<tr>
<td>C_{H}</td>
<td>5' CTCAAGCTTACAGGGAGACCGATGG 3'</td>
</tr>
<tr>
<td>C_{H}</td>
<td>5' CTCAAGCTTACAGGGAGACCGATGG 3'</td>
</tr>
</tbody>
</table>

*The SstI restriction site is underlined.

The HindIII restriction site is underlined.
FIG. 1. cDNA sequences of the heavy chain variable regions of HIV-1-specific human monoclonal antibodies. The CDR-I, CDR-II, D, and I_{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 CaCl₂ 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 gene 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 V₄ genes encoding HIV-1 specific human monoclonal antibodies

The complete nucleotide sequences determined for the V₄ region of each antibody are shown in Fig. 1. As a result of the databank searches each of the expressed V₄ 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 V₄ gene contains an open reading frame, has all the features characteristic of a functional V₄ gene, and is most homologous to the previously described V₄₅ germline gene V₄₃₂ (94.2%). 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 V₄ as compared to the analogous framework of V₄₃₂. 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.

The GP44 V₄ gene segment is a member of the V₄₁ gene family and is most homologous to the HIV101 germline gene (94.9%). 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 V₄₃₂ germline sequence. (B) Nucleotide sequence of GP44 compared to the identical DP-77 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 sequence. (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

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Virus neutralizing</th>
<th>(V_H)</th>
<th>(V_L)</th>
<th>(V_H)</th>
<th>(V_L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP13 (IgG_1,\kappa)</td>
<td>CD4 b.s.</td>
<td>Yes</td>
<td>(V_1)</td>
<td>(V_4)</td>
<td>94.2%</td>
<td>94.1%</td>
</tr>
<tr>
<td>GP44 (IgG_1,\lambda)</td>
<td>CD4 b.s.</td>
<td>Yes</td>
<td>(V_H)</td>
<td>(V_2)</td>
<td>94.9%</td>
<td>88.1%</td>
</tr>
<tr>
<td>GP68 (IgG_\kappa)</td>
<td>CD4 b.s.</td>
<td>Yes</td>
<td>(V_H)</td>
<td>(V_3)</td>
<td>87.8%</td>
<td>93.6%</td>
</tr>
<tr>
<td>MN215 (IgG_\lambda)</td>
<td>V3 domain</td>
<td>Yes</td>
<td>(V_H)</td>
<td>(V_6)</td>
<td>96.3%</td>
<td>84.8%</td>
</tr>
<tr>
<td>K14 (IgG_\kappa)</td>
<td>gp41</td>
<td>No</td>
<td>(V_H)</td>
<td>(V_1)</td>
<td>89.8%</td>
<td>89.0%</td>
</tr>
</tbody>
</table>

*--, No significant homology.

similarity with a previously described \(V_H\) 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 \(V_H\) gene family and is most homologous to the DP-10 \(V_H\) germine gene (87.8\%).\(^{28}\)

The MN215 \(V_H\) gene is derived from the largest germine family (\(V_H\)) and is most homologous to the DP-77 germine gene (96.3\%).\(^{29}\) The K14 \(V_H\) gene is also derived from the \(V_H\) family and is 89.8\% identical to the DP-47 germine 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 germine counterpart of K14 \(V_H\).

Owing to the relatively high number of still unidentified members of the \(V_H\) and \(V_H\) families it remains difficult to determine whether our expressed GP44, GP68, MN215, and K14 \(V_H\) genes represent somatically mutated or as yet unidentified germine 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 germine regions 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 \(V_L\) gene segment. There is 94.1\% homology with the previously described \(V_L\) germine gene,\(^{28}\) which is the only \(V_L\) gene found on the human Ig \(\kappa\) 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 \(V_L\) family and is 88.1\% identical to the IGLV21 germine gene.\(^{30}\) However, it is unlikely that the IGLV21 germine gene would be the germine 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,\(^{33}\) also encoding an HIV-1 gp120-specific human MAb. The GP68 \(V_L\) gene is derived from the \(V_L\) gene family and is 93.6\% identical to the HK102 germine gene\(^ {34}\) and 94.7 and 95.4\% identical to the expressed genes 3D6\(^ {35}\) and kA6,\(^ {36}\) 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 \(V_L\) gene family. However, significant homology could be found only with two expressed genes, H95.EBV (93.9\%)\(^ {38}\) and EB4V\(_1\) (94.5\%).\(^ {39}\) The K14 \(V_L\) gene is also derived from the \(V_L\) family and is 89.0\% homologous to the HK137 germine gene.\(^ {40}\) Again, better homology was found with two expressed genes, HQ2 (95.1\%)\(^ {39}\) and A20 (95.2\%),\(^ {41}\) suggesting another germine gene for K14 \(V_L\). There is only 87.6\% homology with the expressed gene No. 86,\(^ {37}\) also encoding an HIV-1 gp41-specific human MAb.

Analysis of \(D, J_\alpha\) and \(J_\kappa\) gene segments

Figure 6 shows the sequences of the expressed \(D\) segments, compared to their closest germine counterpart\(^ {44}\) or to other previously described expressed \(D\) segments.\(^ {42-46}\) Part of the GP13 \(D\) segment may be derived from the \(D_{\alpha}\) germine. 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 germine genes. Identities between sequences are indicated by dashes.
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_{\kappa} 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 V₄ germline sequence. (B) Nucleotide sequence of GP44 compared to the V₂.1 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 EB4V6 and H95.EBV. (E) Nucleotide sequence of K14 compared to the HK137 germline sequence and to the expressed genes A20 and HGO. 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.

1645
D-D fusion, or by an N segment addition. The last three nucleotides (GAT) could not be derived from a J₅ segment and are likely N segment additions. The D segment of K14 may be derived from the D₈₁ 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 Iₙ gene segments are compared with their germline counterparts. Four of the five antibodies express the J₄₅ gene segment and one expresses I₅₆. Nucleotide differences were observed within all four expressed J₄₅ segments, in some cases resulting in amino acid substitutions. Except for MN215, they all miss the first five nucleotides. All four expressed J₄₅ segments displayed the same allelic polymorphism, A to G, as described before; only the GP₆₈ J₄₅ and MN215 J₄₅ segments may display another polymorphism, C to G. Remarkable is the absence of the nucleotide stretch at the 5' end of GP13 I₅₆, normally encoding the five tyrosine residues, characteristic of a I₅₆ segment. Furthermore, two nucleotide differences are observed, neither one causing an amino acid substitution.

K14 expresses the J₁₁ gene segment and GP13 expresses J₁₂ (Fig. 8). Both J₅ segments are unremarkable except for the last three nucleotides (CGT), which are absent. GP68 expresses the J₁₃ 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 J₂ 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 V₁₅₁, two from the V₉₃, and one from the V₉₅ gene family. For the light chains, two are derived from the V₁₉, one from the V₄, one from the V₉₂, and one from the V₇₆ gene family, only for GP13 were we able to identify the germline counterparts of the V genes. The GP13 Vᵢ gene is derived from the V₉₅ gene family, which consists of three members, one of which is a pseudogene. It is most likely that V₁₉₃2 is the Vḥ₅₅ germline counterpart of GP13 Vᵢ₉. Because it has been shown that the smaller human Vᵢ₉ gene families (Vᵢ₄, Vᵢ₅, and Vᵢ₆) display remarkably little polymorphism, the observed nucleotide differences are most likely caused by somatic mutations. The relatively high number of mutations in FR III r:ay indicate a possible role for some of the FR III amino acids in antigen binding, as has been suggested before. The GF:3 Vᵢ gene is derived from the Vᵢ₄ 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 Vᵢ₉ and Vᵢ₁ 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 Vᵢ₂ gene segment shows more homology with two expressed Vᵢ₂ genes (WLR and PV6) than with any Vᵢ₂ 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 framework, suggesting that these three Vᵢ₂ genes may originate from another, as yet unidentified Vᵢ₂ germline gene. A similar observation has been made for the expressed K14 Vᵢ₁ gene and two other expressed Vᵢ₁ genes (A20 and HGGQ) (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 date. 

---

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

**FIG. 8.** (A) Nucleotide sequences of the Iₙ segment of each HIV-1-specific human monoclonal antibody compared to the nucleotide sequence of the closest Iₙ germline gene. (B) Comparison of the deduced amino acid sequences of the Iₙ 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 gp120.3 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 \(_{1\text{g}}\)3-expressing B cells.11,22 Surprisingly, we found two V \(_{1\text{g}}\)3-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 \(_{1\text{g}}\)3 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 V \(_{1\text{g}}\)3-expressing HIV-1 gp41-specific human MAb has been reported.24 A superantigen-like binding of gp120 to membrane immunoglobulin V \(_{1\text{g}}\)3 gene products has been suggested.25 Also serum V \(_{1\text{g}}\)3 IgM from uninfected individuals was shown to bind to gp120. However, binding of gp120 to V \(_{1\text{g}}\)3 IgG from uninfected individuals was significantly lower.26 MN215 and K14 are both V \(_{1\text{g}}\)3 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 \(_{1\text{g}}\)3 gene products MN215 and K14. This may provide an explanation for the presence of V \(_{1\text{g}}\)3-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 present in infection.29 In the near future broadly HIV-neutralizing engineered antibody preparations may play an important role in passive immunization or as therapeutic agents.

ACKNOWLEDGMENTS

The authors thank Marco van de Bildt for skillful technical assistance, Dr. Saskia Ebeling for critical reading of the manuscript, and Conny Kruysse and Miek Eskens for their help in preparing the manuscript. This work was supported by the Dutch Ministry of Defence and by grant 900-506-131 from the Dutch Organization for Scientific Research (NWO).

REFERENCES


46. Raapoorst FM, Timmers E, Kester MHJ, Van Tol MJF, Vosse
SEQUENCING OF HIV-SPECIFIC HUMAN ANTIBODIES


Address reprint requests to:
Albert D. M. E. Osterhaus
Department of Virology
Erasmus University, Rotterdam
Dr. Molewaterplein 50
P.O. Box 1738
3000 DR Rotterdam, the Netherlands