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Research Article

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Characterization of the cDNA of a Broadly Reactive Neutralizing Human Anti-gp120 Monoclonal Antibody

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Abstract

The F105 mAb, identified in an HIV-1-infected individual, binds to a discontinuous epitope on the HIV-1 gp120 envelope glycoprotein, blocks the binding of gp120 to the CD4 viral receptor, and neutralizes a broad range of HIV-1 isolates. This study reports the primary nucleotide and deduced amino acid sequences of the rearranged heavy and light chains of the mAb F105. This IgG₁k mAb uses a V_H gene member of the V_H4 gene family (V71-4) and is productively rearranged with a D-D fusion product of the dlr4 and da4 germline D_H genes and the J_{H5} gene. This rearranged heavy chain gene expresses the V_H4-HV2a idiotope, which is seen in human monoclonal IgM cold agglutinins. The F105 V_L appears to be derived from the *Humvk325* germline gene and is rearranged with a J_{k2} gene. For both chains, the mutational pattern in the rearranged V_H and V_L genes is indicative of an antigen-driven process. These studies show that production of a broadly neutralizing anti-HIV-1 antibody that recognizes determinants within the CD4 recognition site of the envelope glycoprotein is achieved by rearrangement of the V71-4 and *Humvk325* germline variable region genes along with selected individual point mutations in the rearranged genes. (*J. Clin. Invest.* 1992. 90:1467-1478.) Key words: acquired immunodeficiency syndrome • immunoglobulins • gene rearrangement • autoantibody • restricted idiotypic

Introduction

HIV-1 productively infects human CD4⁺ cells, including CD4⁺ T cells and cells of the monocyte/macrophage lineage (1-5). The first step in infection by HIV-1 is the specific binding of gp120, the envelope glycoprotein of HIV-1, to its cellular receptor, the CD4 molecule (6, 7). Infection with HIV-1 leads, in most cases, to a progressive decline in the number and functions of CD4⁺ T cells with the eventual appearance of clinical manifestations of cellular immunodeficiency, such as opportunistic infections and malignancies, i.e., AIDS (8).

Serum antibodies reacting with the HIV-1 gp120 can neutralize viral infection by binding to several sites on the molecule (9, 10). The earliest neutralizing human antibody re-

sponse is directed to epitopes in the third hypervariable region of gp120, the principle neutralizing domain, which is contained within a loop formed by disulfide bonding (11-13). These antibodies are frequently strain specific (14). Envelope glycoprotein variation both within the linear epitope and outside the epitope can allow escape of viruses from neutralization by these antibodies (15, 16). Later in the course of HIV-1 infection, more broadly neutralizing antibodies appear (17). A large fraction of these broadly neutralizing antibodies, which are present in low concentrations in patients' sera, are directed to conformationally sensitive epitopes on gp120 (18, 19). A subset of the broadly reactive antibodies, found in the serum of patients, interferes with the binding of gp120 and CD4 (10, 20-22). These antibodies appear to be reactive with a discontinuous epitope on gp120 that encompasses the CD4 binding region (23, 24). This region of gp120 is well conserved although not invariant.

Several human mAbs derived from HIV-infected individuals, which block CD4 binding, recognize conformation-dependent epitopes on gp120, and neutralize a broad range of HIV-1 isolates, have recently been described (25-27). One of these human mAbs is F105 (25). This IgG₁k antibody reacts with a conformationally defined epitope on HIV-1/gp120, which appears to be within or topographically near the CD4-binding site (24, 25). This mAb recognizes an epitope on the surfaces of HIV-1-infected malignant T cell and monocyte cell lines and on intact virions. In addition, F105 neutralizes a number of diverse HIV-1 isolates, including IIIB, MN, RF, and SF₂ strains (25). This antibody is potentially useful in immunoprophylaxis after exposure, in preventing the vertical transmission of HIV, and in monitoring the humoral immune response in HIV-1-infected individuals and vaccinated volunteers.

One of the aims of elucidating nucleotide sequences of antibodies specific for a single antigen is to determine the extent of diversity or restriction in the use of germ line genes (28). Recently, the primary nucleotide and deduced amino acid sequences of several human anti-gp120 V3 loop and anti-gp41 heavy chains have been reported (29, 30). To understand the molecular nature of the broadly neutralizing human antibodies that are directed at or near the CD4-binding domain of gp120, the rearranged heavy and light chains of the F105 mAb were cloned. These studies provide the first molecular characterization of the broadly neutralizing anti-gp120 antibodies and form the basis for future molecular studies aimed at investigating the humoral immune response to the CD4-binding domain of gp120.

Methods

cDNA synthesis and polymerase chain reaction amplification of F105 immunoglobulin genes. The F105 hybridoma was derived by fusion of

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EBV transformants with the HMA2.11TG/0 cell line, a nonsecreting human-mouse myeloma analogue (25). Both cell lines were used for cDNA synthesis and polymerase chain reaction (PCR)¹ amplification experiments. First strand cDNA was synthesized in a 25- μ l reaction from 5 μ g of total RNA by using oligo(dT) priming and the Moloney murine-leukemia virus reverse transcriptase according to published protocols (31). 5–10% of the first strand cDNA was used to perform the PCR reactions. The temperatures used for the PCR are melt 94°C, 1 min; primer anneal 52°C, 2 min; primer extension 72°C, 2 min. 1-min ramp times were used except a 2-min ramp time was used between annealing and extension. Ethidium bromide-stained 2% agarose gels were used to separate the PCR fragments. The appropriate band was excised, gene cleaned (Bio 101, Inc., La Jolla, CA), Klenow repaired, restriction enzyme digested and cloned into pSL1180 (Pharmacia LKB Biotech. Inc., Piscataway, NJ) using SURE bacteria (Stratagene Inc., La Jolla, CA) as hosts. At least three separate transformants were sequenced using both forward and reverse sequencing primers. DNA sequence analysis was performed by the method of Sanger et al. (32). The sequencing primers were designed to be complementary to the polylinker sequences of pSL1180.

The heavy chain primer pair consists of a V_H primer and a J_H or C_{H1} primer, each containing convenient restriction sites for cloning. The Kabat database on immunoglobulins was used to analyze the amino acid and codon distribution found in the six distinct human V_H families (33). Based on this analysis, the 35-bp universal 5' V_H primer was designed TTTGCGGCCGCTCAGGTGCA(G/A)CTGCTCGAGTC-(T/C)GG, which is degenerate for two different nucleotides at two positions and will anneal to the 5' end of FR1 sequences. A 5' NotI site (left-underlined) has been introduced for cloning the amplified DNA and is located 5' to the first codon of the V_H gene. An internal XhoI site has been introduced as well (right-underlined). Similarly, a 35-bp J_H region oligonucleotide has been designed for reverse priming at the 3' end of the heavy chain variable gene, TTAGCGCGCTGAGGTGACCGTGACC(A/G)(G/T)GGT. Based on the nucleotide sequence of the six human J_H region minigenes, this primer contains two degenerate positions with two nucleotides at each position. A BssHII site (left-underlined) 3' to and immediately adjacent to the codon determining the last amino acid of the J region allows convenient cloning at the 3' end of the V_H gene. An internal BstEII site (right-underlined) has been introduced as well. To obtain unbiased sequence data in the J_H region, a reverse IgG₁ C_{H1} primer was designed, which terminates just 3' of the first cysteine codon in the hinge exon, TTAGCGCGCACAAAGATTGGGCTC (33). A BssHII site was similarly introduced for cloning. The resulting gene product (H fragment) was intended to correspond to a Fd of IgG₁ isotype and conserves the H-L disulfide bond (34). A primer corresponding to amino acids 183 to 189 of IgG₁ CH1 region was used for DNA sequencing, TGCTGAGGGAGTAGAGT.

A similar strategy has been used to design PCR primers for the human kappa chain variable genes. There are four families of human V_{κ} genes. The 5' V_{κ} primer TTTGCGGCCGCTGAGCTC(G/C)(T/A)G(A/C)TGACCCAGTCTCCA, which will anneal to the 5' end of the FR1 sequences, is degenerate at three positions (two nucleotides each) and contains a Not I (left-underlined) site at a position similar to that of the V_H region. An internal Sac I site is also present (right-underlined). The reverse 39-bp J_{κ} primer CGAGGATCC-TTATTAACGCGTGATCTCCA(C/G)(C/T)TTGGTCCC, designed from the sequence of five human J_{κ} minigenes, is degenerate at three positions (two nucleotides at each position) and contains an internal Mlu I site (right-underlined) immediately flanking the J_{κ} coding region and is then followed by two stop codons and a BamHI cloning site (left-underlined). To obtain unbiased sequence data in the J_{κ} region, two reverse kappa constant region primers were designed. The first primer CGAGGATCCTTATTAACGCGTGGTGCAGCC-

ACAGT will anneal to the most 5' end of the kappa constant region and has a similar flanking sequence arrangement as the J_{κ} primer. A second primer TGGGGATCCTTATTAACACTCTCCCCTGTTGA-A was designed to anneal to the most 3' kappa constant region nucleotides and this region is followed by two stop codons and a BamHI cloning site.

After the primary nucleotide sequence was determined for both the F105 heavy and kappa chain genes and the germ line genes were identified, PCR primers were designed based on the leader sequences of the V_H 71-4 (35) and *Humvk325* (36) germ line genes. The V_H 71-4 leader primer TTTACCATGGAACATCTGTGGTTC and the *Humvk325* leader primer GGAACCATGGAAACCCAGCGCAG both contain a 5' NcoI site (underlined). These leader primers were used in conjunction with the respective C region primers for PCR amplification experiments.

ELISA. F105, or IgM ODO (a gift from Dr. Gregg Silverman, University of California, San Diego, CA) were resuspended in coating buffer (100 mM sodium carbonate, pH 8.5) at 150 ng/ml and incubated overnight (100 μ l/well) at 4°C on ELISA plates (Immulon No. 1; Dynatech Labs, Inc., Chantilly, VA). After washing with Tris-buffered saline containing 0.2% Tween 20 (TBS-T) and blocking with TBS-T containing 1% BSA, the plates were incubated at varying concentrations with various rabbit anti-peptide antisera that recognize V_H III or IV framework or V_H IV CDR2 determinants (37). A rabbit antiserum raised against an unrelated peptide served as a negative control (rabbit anti-formyl Met-Leu-Phe/BSA). In some cases CDR2 peptides were preincubated at 1 μ g/ml with anti-CDR2 peptide antisera for 1 h at room temperature before addition to the plate. After overnight incubation the plates were washed extensively and bound rabbit antibody was detected with affinity column-purified, alkaline phosphatase-coupled goat anti-rabbit IgG that had been absorbed with human serum (Sigma Chemical Co., St. Louis, MO) and *p*-nitrophenylphosphate as alkaline phosphatase substrate (BioRad Laboratories, Richmond, CA) per manufacturer's instructions. Absorption at 405 nm was recorded using an ELISA reader (Titertek Multiskan Plus; Labsystems, Inc., Finland).

Cold agglutinin and rheumatoid factor assays. For cold agglutinin activity, 200 μ l of F105 (1 mg/ml) was diluted 10-fold in 6% BSA and then tested against reagent human red cells with the following phenotypes: A₁, I, low H; B, I, mid-range H; O, I, strong H; and O, i, strong H. Antibody was tested against each cell at 22°C, 18°C, and 4°C. Reactions were tested by both direct hemagglutination and also using mouse monoclonal anti-human IgG (Coombs sera).

For rheumatoid factor activity, various dilutions of F105 were mixed with human IgG-coated latex particles and assayed for agglutination.

Results

F105 heavy chain V_H gene is derived from germ line V_H IV 71-4. The F105 hybridoma cell line was used as the source of mRNA. First strand cDNA was prepared by the method of Gubler and Hoffman (31) and 5–10% of this reaction mixture was used in the PCR experiments. The degenerate V_H and J_H primers were initially used to amplify by PCR an ~ 370-nucleotide fragment. This is the expected molecular weight of the rearranged heavy chain gene. This amplified DNA was inserted into the pSL1180 plasmid. Subsequent to the identification of the germ line V_H gene, the cDNA was reamplified using primers that recognize the leader and IgG₁ constant regions to obtain unbiased DNA sequence information of the FR1 and J_H regions.

DNA sequence analysis was performed by the method of Sanger et al. (32). The results revealed that the rearranged F105 V_H gene is derived from a member of the V_H IV gene family as evidenced by > 88% similarity with all of the

1. Abbreviations used in this paper: CDR, complementarity determining region; FR, framework; PCR, polymerase chain reaction; TBS-T, Tris-buffered saline containing 0.2% Tween 20.

pared with V_H 71-4 and these changes are summarized in Table II. Nine of the changes are in the framework residues and are distributed in all three framework regions (FRs). These changes include four transitions and five transversions. The remaining three changes are all transitions and occur in the

	-19								-10							
V _H 71-4:	ATG	AAA	CAT	CTG	TGG	TTC	TTC	CTT	CTC	CTG	GTG	GCA	GCT	CCC	AGA	T/GG
F105V _H :	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
VH411:
VH415:
VH416:
	1								10							
V _H 71-4:	GTC	CTG	TCC	CAG	GTG	CAG	CTG	CAG	GAG	TCG	GGC	CCA	GGA	CTG	GTG	AAG
F105V _H :
VH411:
VH415:
VH416:
	20															
V _H 71-4:	CCT	TCG	GAG	ACC	CTG	TCC	CTC	ACC	TGC	ACT	GTC	TCT	GGT	GGC	TCC	GTC
F105V _H :A	A..
VH411:	A..
VH415:	A..
VH416:C	A..
	30															
	CDR1															
V _H 71-4:	AGT	***	***	AGT	***	TAC	TAC	TGG	AGC	TGG	ATC	CGG	CAG	CCC	CCA	GGG
F105V _H :	C..	T..
VH411:
VH415:
VH416:
	50															
	CDR2															
V _H 71-4:	AAG	GGA	CTG	GAG	TGG	ATT	GGG	TAT	ATC	TAT	TAC	AGT	GGG	AGC	ACC	AAC
F105V _H :	C..AC
VH411:
VH415:	G..
VH416:
	60															
	70															
V _H 71-4:	TAC	AAC	CCC	TCC	CTC	AAG	AGT	CGA	GTC	ACC	ATA	TCA	GTA	GAC	ACG	TCC
F105V _H :G.G
VH411:
VH415:
VH416:
	80															
V _H 71-4:	AAG	AAC	CAG	TTC	TCC	CTG	AAG	CTG	AGC	TCT	GTG	ACC	GCT	GCG	GAC	ACG
F105V _H :C.	...	A..
VH411:
VH415:
VH416:
	90															
V _H 71-4:	GCC	GTG	TAT	TAC	TGT	GCG	AGA									
F105V _H :	C..									
VH411:									

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Table II. Summary of Differences between F105 and Germline Genes V_H 71-4 and Hum vk325

Position	Location	Nucleotide		Change	Amino acid		Change
		71-4	F105		71-4	F105	
A. Summary of differences between F105 V _H and germ line V _H 71-4							
26	FR1	T	A	TV	Gly	Gly	Silent
29	FR1	G	A	TS	Val	Ile	Conservative
32	CDR1	T	C	TS	Tyr	His	Nonconservative
40	FR2	C	T	TS	Pro	Ser	Nonconservative
46	FR2	G	C	TV	Glu	Gln	Nonconservative
49	FR2	G	A	TS	Gly	Gly	Silent
52	CDR2	T	C	TS	Tyr	Tyr	Silent
60	CDR2	A	G	TS	Asn	Ser	Semiconservative
72	FR3	C	G	TV	Asp	Glu	Conservative
82A	FR3	G	C	TV	Ser	Thr	Conservative
82C	FR3	G	A	TS	Val	Met	Conservative
94	FR3	A	C	TV	Arg	Arg	Silent
B. Summary of differences between F105 V _k and germ line Humvk325							
Position	Location	Nucleotide		Change	Amino acid		Change
		vk325	F105		vk325	F105	
15	FR1	C	G	TV	Pro	Ala	Nonconservative
31	CDR1	C	G	TV	Ser	Arg	Nonconservative
78	FR3	C	G	TV	Leu	Val	Conservative
90	CDR3	G	A	TS	Glu	Glu	Silent
92	CDR3	G	A	TS	Gly	Asp	Nonconservative
93	CDR3	A	C	TV	Ser	Ser	Silent
94	CDR3	G	A	TS	Ser	Asn	Nonconservative
95A	CDR3	C	G	TV	Pro	Val	Nonconservative
95B	CDR3	C	T	TS	Pro	Val	Nonconservative

TS, transition; TV, transversion.

CDR1 and CDR2 regions. Table I also compares the nucleotide sequence of F105 V_H with three additional nonrearranged V_H IV germ line genes (V_H 4.11, 4.15, and 4.16) that are most closely related to V_H 71-4 (38). All three of these germ line genes and F105 share the guanosine to adenosine; valine to isoleucine change in position 29 supporting the possibility that this change may represent an allelic polymorphism. F105 however has many more nucleotide changes (12) compared with these genes (one, 4.11; or two, 4.15 and 4.16), suggesting that additional somatic mutations might have arisen in F105 after recombination.

At the amino acid level, four of the F105 V_H nucleotide changes result in silent mutations that are distributed equally in each of the three FR regions (positions 26,FR1; 49,FR2; 94,FR3) and there is one silent mutation in CDR2 (position 52). The remaining eight nucleotide changes result in amino acid changes that occur in the three FRs more frequently than in the CDRs (Table II). Two nonconservative changes occur in the FR2 region and three conservative changes occur in the FR3 region. One additional FR1 region framework residue at position 29, which results in a conservative change valine to isoleucine, is located immediately adjacent to the complementarity determining region (CDR) defined by Kabat et al. (33). This region may also contribute to the topography of the anti-

body-combining site (39). The presence of isoleucine at position 29 in four of seven germ line V_H IV genes suggests that isoleucine may represent an allelic difference and not a somatic mutation (35, 38). In the CDR regions, two changes occur with a non-conservative replacement of tyrosine to histidine in CDR1, position 32 and a semiconservative change of asparagine to serine in CDR2, position 60. The ratio of substitution to silent mutations is 2:1. The substitution mutations occur in a FR/CDR ratio of 3:1.

Comparative analysis of F105 V_H and other V_H 71-4-derived rearranged V_H genes. Table III, A compares the nucleotide sequence of F105 V_H to two mAbs that, by nucleotide sequence analysis, appear to use a rearranged V_H 71-4 gene. One IgM lambda mAb (Ab26) was derived from CD5⁺ B cells of a healthy donor and represents a naturally occurring polyreactive antibody that binds to many antigens (40). Ab26 has greatest sequence similarity with V_H 71-4 (92.3%) compared with the next closest germ line gene V_H 71-2 (89.5%). Also shown in Table III is antibody 268-D, an IgG₁ mAb that is specific for the principle neutralizing domain of gp120 of HIV-1 strain MN and is 88.5% identical to V_H 71-4 (29). As can be seen in Table III, all three of these V_H 71-4-related heavy chains have undergone extensive somatic mutation with F105 showing the least number of nucleotide changes. For mAbs Ab26

Table III. Comparison of F105 V_H- and V_H 71-4-related Antibody GenesA. Nucleotide Sequence Comparison of F105 V_H to Somatic Rearranged V_H71-4 Genes

	-19									-10						
V _H 71-4:	ATG	AAA	CAT	CTG	TGG	TTC	TTC	CTT	CTC	CTG	GTG	GCA	GCT	CCC	AGA	T/GG
F105V _H :	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Ab26:
268-D:	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
				1									10			
V _H 71-4:	GTC	CTG	TCC	CAG	GTG	CAG	CTG	CAG	GAG	TCG	GGC	CCA	GGA	CTG	GTG	AAG
F105V _H :
Ab26:
268-D:	---	---	---	T..T.
							20									
V _H 71-4:	CCT	TCG	GAG	ACC	CTG	TCC	CTC	ACC	TGC	ACT	GTG	TCT	GGT	GGC	TCC	GTC
F105V _H :A	A..
Ab26:A	C..T	A..
268-D:	C..	A..
							30									
V _H 71-4:	AGT	***	***	AGT	***	TAC	TAC	TGG	AGC	TGG	ATC	CGG	CAG	CCC	CCA	GGG
F105V _H :	C..	T..
Ab26:	GGT	G..TCA
268-D:	..A	***	***	..A	***	GC..CAA
V _H 71-4:	AAG	GGA	CTG	GAG	TGG	ATT	GGG	TAT	ATC	TAT	TAC	AGT	GGG	AGC	ACC	AAC
F105V _H :	C..AC
Ab26:CC	..A
268-D:G	..AAC	C..	..A	...	G..	...	C..T	..C	..A	GT..
V _H 71-4:	TAC	AAC	CCC	TCC	CTC	AAG	AGT	CGA	GTC	ACC	ATA	TCA	GTA	GAC	ACG	TCC
F105V _H :GG
Ab26:T	G..GT
268-D:TC	..G	C..	A..	A..
V _H 71-4:	AAG	AAC	CAT	TCC	TCC	CTG	AAG	CTG	AGC	TCT	GTG	ACC	GCT	GCG	GAC	ACG
F105V _H :C	...	A..
Ab26:
268-D:	..G	..GAC	..GCAG	..T	T..
V _H 71-4:	GCC	GTG	TAT	TAC	TGT	GCG	AGA									
F105V _H :	C..									
Ab26:C									
268-D:AT									

B. Amino Acid Sequence Comparison of F105 V_H to Somatic Rearranged V_H71-4 Genes

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Table III (Continued).

	80														90												
V _H 71-4	V	D	T	S	K	N	Q	F	S	L	K	L	S	S	V	T	A	A	D	T	A	V	Y	Y	C	A	R
F105Vh	.	E	T	.	M
Ab26	N	N	.	N	S	.
268-D	I	.	.	.	R	K	.	L	.	.	S	.	K	F	S

Dashes after F105 represent primer annealing sequences. Dashes after 268-D represent sequences that are not reported (29).

and 268-D, there are 19 and 42 nucleotide changes, respectively. In all of these examples, nucleotide changes are scattered in both the CDR and FR regions. All three antibodies have the guanosine to adenosine change in amino acid position 29 as does F105. Table III, B shows the corresponding amino acid changes in these three mAbs. For antibodies Ab26 and 268-D there are 11 and 23 amino acid changes, respectively, compared with F105, which has 8 changes. The only change shared by three antibodies is valine to isoleucine in position 29.

Analysis of the F105 D_H gene encoding CDR3. The D_H portion in V_H-D_H-J_H structure corresponds to the CDR3 of H chain (41). Over 20 human germ line genes have been identified and it is estimated that the total number of D_H genes is ~ 30 (42). As shown in Table IV, A, the F105 D_H region appears to be composed of a D-D fusion product (43-46). A seven-nucleotide sequence in the center of the F105 D_H gene is identical to the germ line D_H gene *dlr4*. This region of identity is immediately flanked on its 5' end by an additional two of three nucleotides that are identical when compared with *dlr4*. The additional 5' nucleotides found in F105 D_H that are rich in deoxyguanosines and deoxycytosines probably represent ran-

dom "N segment" nucleotide additions since these are the favored nucleotides of the enzyme terminal deoxynucleotidyl-transferase (47). Examination of the 3' end of the F105 D_H region shows that 11 of 13 nucleotides are identical to the da4/da1 germ line gene. The 3' end of this nucleotide sequence may extend into the 5' end of the J_H region, the precise boundary of which is difficult to define accurately because of the identity with the da4/da1 germ line D_H gene. Since both of these D_H genes are in the same transcriptional orientation, rearrangement probably occurred by deletion rather than by inversion (43).

A GeneBank search for other rearranged human heavy chain genes that have nucleotide sequence homologies with the F105 D_H gene revealed two rearranged genes (L17 and L37). These have a relatively short D_H segment (~ 24 nucleotides) compared with F105 but contain near their 5' end the same 12 nucleotide "core" that is identical to 12 nucleotides at the 3' end of the F105 D_H gene (Table IV, B) (48). The finding that three unrelated antibodies have this same da4/da1-related segment suggests that this sequence is related to a germ line gene and that the two base changes, both guanosine to adenosine

Table IV. F105 Heavy Chain D Region Sequence Comparisons

A. Comparison of F105 D_H gene with human germ line D_H region genes

dlr4	AGGATATTGTACTAGTACCAGCTG	CTATGCC
F105	GgccccGTgCCAGCTGtcttCTAcGgtgactac	
F105	ggccccgtgccagctgtcttCTACgGTgACTAC	
da4		TGACTACAGTAAGTAC
da1		TGACTACAGTAAGTAC

Nucleotide sequences of three human germ line D_H genes *dlr4*, *da4*, and *da1* (42). Underlined nucleotides show identity between F105 D region and a known germ line D_H gene.

B. Comparison of the F105 and L17/L37 D_H/J_H boundaries

Kabat Position	95	96	97	98	99	100	A	B	C	D	E		
F105 Amino acid	Gly	Pro	Val	Pro	Ala	Val	Phe	Tyr	Gly	Asp	Tyr		
F105 Nucleotide	GGC	CCC	GTG	CCA	GCT	GTC	TTC	<u>TAC</u>	<u>GGT</u>	<u>GAC</u>	<u>TAC</u>		
L17/L37 Nucleotide						ggg	agT	<u>ACG</u>	<u>GTG</u>	<u>ACT</u>	<u>ACg</u>	gga	gat
L17/L37 Amino acid						Gly	Ser	Thr	Val	Thr	Thr	Gly	Asp
Kabat Position						96	97	98	99	100	A	B	C

L17/L37 are rearranged D_H region gene that are derived from tonsillar lymphocytes that express the V_H I related G6 cross-reactive idiotype (48). Underlined nucleotides show identity between F105 D region and the L17/L37 D region genes.

Table V. Comparison of F105 J_H to Germ Line J_{H5}

	CDR3 FR4
J _{H5}	Asn-Trp-Phe-Asp-Ser-Trp-Gly-Gln-Gly-Thr-Leu-Val-Thr-Val-Ser-Ser
J _{H5}	AAC-TGG-TTC-GAC-TCC-TGG-GGC-CAA-GGA-ACC-CTG-GTC-ACC-GTC-TCC-TCA
F105	T**C*A-C** - *** - C** - *** - *** - **G - *** - *** - *** - *** - *** - ***
F105	Tyr-Arg-Leu-*** - Pro-*** - *** - Gln-*** - *** - *** - *** - *** - *** - ***
Pos.	100E100F101-102-103-104-105-106-107-108-109-110-111-112-113-114

Four somatic mutations that are clustered at the 5' end of F105 J_H in positions 100E, 100F, 101, and 103 result in substitution mutations whereas the fifth somatic mutation in position 106 is silent.

changes, may represent allelic differences in the da4/da1 germ line genes rather than somatic mutations. Table IV, B also demonstrates that F105 and L17/L37 use different reading frames for the 12 identical nucleotides, which results in different amino acids for this portion of CDR3 (48). This observation is in agreement with several studies that show that all of the three coding frames appear to be used equally in the human (42, 45). However, preferential use of one reading frame has been demonstrated with anti-DNA antibodies (49) and with certain germ line D_H genes (45).

Analysis of the F105 J_H region gene. Comparison of the F105 J_H region with the six human J_H germ line genes reveals greatest sequence similarity with J_{H5} (Table V). The F105 J_H region differs by six nucleotides from that of J_{H5}. Five of these differences are clustered at the 5' end of the J_H gene. These five differences result in four amino acid changes. Nonconservative changes occur at positions 100E, 100F, 101, and 103. The sixth nucleotide change, occurring in amino acid position 106, which lies outside of CDR3, is silent. It is possible that the amino acid corresponding to position 100E in J_{H5} is deleted in F105 J_H.

F105 heavy chain shares the CDR2 idiotope HV2a. Silverman and Carson (37) used peptide antisera raised against consensus peptides from the first framework (FR1) and second CDR region (CDR2) of human V_H IV heavy chains to make heavy chain class assignments for cold agglutinin and rheumatoid factor antibodies (37, 50). The CDR2 consensus peptide HV2a for these antibodies could not distinguish among three different V_H IV germ line genes (V71-2, V71-4, and V79) (37). The results demonstrated that both cold agglutinin and a subgroup of rheumatoid factor heavy chains both reacted with FR1 antibodies but only cold agglutinin antibodies reacted with anti-V_H4-HV2a antisera. Fig. 1 illustrates ELISA experiments with F105 and the cold agglutinin IgM ODO. The anti-V_H4-FR1 antisera reacts strongly with both F105 and IgM ODO whereas anti-V_H3-FR1 antisera shows only background activity compared with the unrelated rabbit anti-formyl-Met-Leu-Phe antisera. F105 also reacts with anti-VH4-HV2a, albeit weakly, and this binding is inhibited by preincubation of anti-VH4-HV2a with VH4-HV2a peptide and to a lesser extent with the closely related VH4-2c peptide. This result indicates that the F105 V_H is structurally more closely related to the cold agglutinin heavy chains than to the rheumatoid factor heavy chains.

F105 light chain V_κ gene is derived from germ line Humvk325 germ line gene. For the analysis of the F105 kappa chain, the source of mRNA, method of first strand cDNA synthesis, and conditions for amplification by PCR were identical

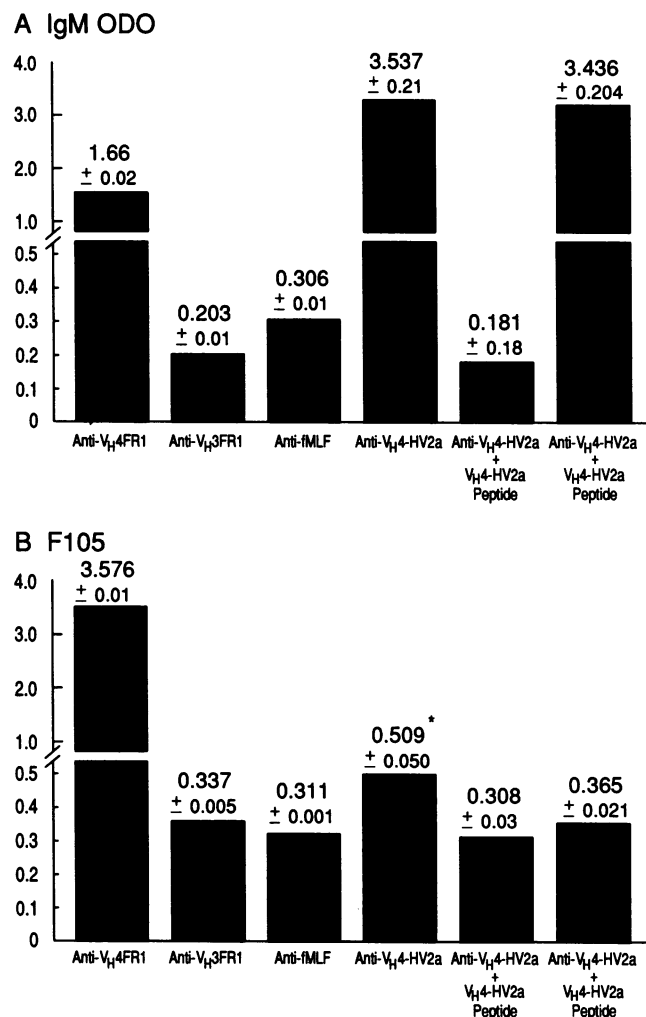


Figure 1. ELISA analysis of F105 and IgM ODO. 150 ng/well of F105 or IgM ODO were incubated overnight on ELISA plates. After blocking with TBS-T with 1% BSA, the plates were incubated with various rabbit anti-peptide antisera (1:1500 final dilution) that recognize V_H III or IV framework or V_H IV CDR2 determinants (37). A rabbit antisera raised against an unrelated peptide served as a negative control (rabbit anti-formyl Met-Leu-Phe/BSA). In some cases CDR2 peptides were preincubated at 1 µg/ml with anti-CDR2 peptide antisera for 1 h at room temperature before addition to the plate. Bound rabbit antibody was detected with affinity column-purified, alkaline phosphatase-coupled goat anti-rabbit IgG that had been absorbed with human serum. Absorption at 405 nm was recorded. Data represent mean of triplicate determinations ± SD. * *P* < 0.01 compared with anti-V_H3FR1 or anti-fMLF using Student's two-tailed *t* test. (A) IgM ODO-coated plates; (B) F105-coated plates.

to those used for the heavy chain. The degenerate V_{κ} and J_{κ} primers were initially used to amplify by PCR an ~ 350 -nucleotide fragment. This is the expected molecular weight of the rearranged kappa light chain gene. This amplified DNA was inserted into the pSL1180 plasmid. Subsequent to the identification of the germ line V_{κ} gene, the cDNA was reamplified using primers that recognize the leader and kappa chain constant regions to obtain unbiased DNA sequence information of the FR1 and J_{κ} regions.

DNA sequence analysis revealed that the rearranged F105 V_{κ} gene is derived from a member of the V_{κ} III subgroup gene family (33). The V_{κ} III subgroup can be further subdivided into sub-subgroups, one of which is the V_{κ} IIIb sub-subgroup. F105 shares the greatest sequence similarity with the V_{κ} IIIb germ line gene *Humvk325* (97.7%, 343/351 nucleotides) (Table VI, A). There are a total of nine nucleotide changes in F105 V_{κ} compared with *Humvk325* (Table II). Two of the changes, which are both transversions, are located in the framework regions FR1 and FR3. Seven additional changes, including four transitions and three transversions, are located in CDR1 (one) and CDR3 (six).

At the amino acid level, only two of the nucleotide changes result in a silent mutations. Of the remaining seven nucleotide changes, two changes occur in framework regions and five occur in the CDR regions (Table II and Table VI, B). The two framework changes include a nonconservative change at position 15 in FR1 and a conservative change at position 78 in FR3. In the CDR regions, one change occurs with a nonconservative substitution at position 31 in CDR1. There are three nonconservative amino acid changes in CDR3 in positions 92, 94, and 95. The change at position 95 (proline to valine) is the result of two individual point mutations that occur in one codon. The ratio of substitution to silent mutations is 7:2 and these changes occur in a FR/CDR ratio of 2:7. This pattern of changes is in marked contrast to the pattern of changes observed for the F105 V_H sequence where the ratio of substitution to silent mutations is 2:1 and the substitution mutations occur in a FR/CDR ratio of 3:1 (Table II).

Analysis of the F105 J_{κ} region gene. The F105 J_{κ} region is most similar to the germ line $J_{\kappa 2}$ gene (Table VI, A). The codon at position 96 is formed by the recombination of V_{κ} and J_{κ} and has a single nucleotide change that results in the nonconservative replacement of tyrosine for cysteine. A silent thymidine to adenosine change also occurs at position 108, otherwise the F105 J_{κ} gene is in $J_{\kappa 2}$ germ line configuration.

Comparative analysis of the F105 rearranged kappa chain with other rearranged *Humvk325*-related kappa chains. Rheumatoid factor antibodies are typically IgMk antibodies and are directed against IgG antibodies. Many IgMk rheumatoid factors use the *Humvk325* gene in the germ line unmutated configuration (51, 52) and several also use the $J_{\kappa 2}$ gene in unmutated form (51) as in F105. Table VI, B compares the amino acid and nucleotide sequence of the rearranged F105 kappa chain to the kappa chain of the closely related IgMk rheumatoid factor *neu*. The *neu* kappa chain is similar to F105 with two identical point mutations in positions 31 and 96. The latter change occurs as a result of an uncommon V/J joining event.

Discussion

This study reports the primary nucleotide and deduced amino acid sequence analysis of the human antibody F105 that is

directed toward the conformationally sensitive CD4-binding domain of gp120. The results show that the F105 antibody is created by rearrangement of the V_H 71-4 and *Humvk325* germ line variable region genes along with selected individual point mutations in the rearranged genes.

The mutational pattern in the rearranged V_H and V_L genes is indicative of an antigen-driven process. For F105 V_H the ratio of substitution to silent mutations is 2:1. These substitution mutations occur in a FR/CDR ratio of 3:1. This type of change involving a high number of amino acid substitutions in the FR regions has been attributed to stochastic rather than selective forces since antigen-driven selective forces would be expected to impose higher selection pressure on the CDR regions. It is possible that selection has occurred but has acted to retain CDR 1 and 2 in near germ line configuration, whereas more divergence is allowed in the FR regions where these residues may play a role in antigen binding (53). It is also possible that these FR mutations may play a role in network regulation, i.e., are selected by necessity of idiotypic regulation in the anti-gp120 response (54). However, changes in the V_H gene segment must be considered in the context of the overall selection forces that act on the heavy chain. The power of antigen selection can make D-D fusions, a relatively rare event in B cell development, which occur at a lower frequency than normal joining, biologically significant (43). In addition, heavy chain selection may be biased to changes in CDR3 since four of five amino acid changes occur in the J_{H5} gene and these changes are concentrated in the 5' end of the gene, which contributes to the formation of CDR3 (Table V).

For the F105 kappa chain, the ratio of substitution to silent mutations is 7:2 and these mutations occur in a FR/CDR ratio of 2:7. This is in marked contrast to the mutational pattern seen with F105 V_H (Table III) and strongly supports the notion that these somatic changes are the result of an antigen-driven process. Most notable are the higher number of mutations in CDR3 than are generally found when compared with cloned *Humvk325*-derived light chain genes from patients with chronic lymphocytic leukemia (55), small cell non-Hodgkin's lymphoma (56), monoclonal rheumatoid factors (51), and Sjögren's syndrome (57). These somatic mutations could reflect the chronic B cell activation that occurs both in the regional lymph nodes and peripheral blood of patients with AIDS (58).

The F105 V_H gene is derived from a member of the V_H IV gene family, V_H 71-4. This is a relatively small family with ~ 10 members. The expression of V_H IV genes in humans has been described only recently; indeed, the heavy chain families, V_H IV, V, and VI were identified only in the last five years (35). Expressed V_H IV genes have been found at an unexpectedly high frequency in human autoantibodies (28, 40, 53, 59–61). Only a few of these antibodies have been shown to use the V_H 71-4 germ line gene (Table V) (59, 62). The most studied of the V_H IV-derived heavy chain genes include the cold agglutinin and rheumatoid factor antibodies. Several laboratories have determined that cold agglutinins use the V_H IV 2-1 germ line gene segment (53, 62–64) whereas a subgroup of autoantibodies of the IgM anti-IgG type (rheumatoid factors) use members of a V_H IV gene family that is serologically distinct from the cold agglutinin V_H IV 2-1-derived genes (50). The molecular characterization of these V_H IV rheumatoid factor heavy chains has not been reported. Our studies demonstrate that the VH4-HV2a idiotope is expressed, albeit weakly, on the

A. Nucleotide sequence comparison of F105 V_{kanna} with germ line gene *Humvk325*

B. Amino acid sequence comparison of F105 V_{kappa} with germ line *Humvk325* and a related rearranged kappa chain

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F105 heavy chain. The weak binding may be the result of the asparagine to serine substitution in position 60. Accordingly, by this type of antiidiotypic analysis the F105 V_H is more closely related structurally to the V_H IV heavy chains of anti-I cold agglutinin antibodies than to the V_H IV heavy chains of the rheumatoid factor antibodies. However, in spite of this structural similarity, F105 displays neither cold agglutinin nor rheumatoid factor activity (data not shown).

Restricted heavy and light chain V gene use occurs in several autoimmune (57, 63, 65) and infectious diseases (66–68). Restricted V gene use in response to gp120 is highly suggested by several recent observations with antiidiotypic antibodies (69, 70), however, only limited anti-gp120 sequence data are available at present for comparison with F105. Andris et al. (29) recently reported the heavy chain nucleotide sequences of two human anti-gp120 V3 loop and two anti-gp41 mAbs that were produced by EBV transformation of peripheral blood cells of HIV-infected patients. Their analysis showed that one of the anti-V3 loop antibodies (268-D) (Table III) used the V_H 71-4 germ line gene whereas the other anti-V3 loop antibody (257-D) used a V_H V germ line gene V_H251. Both of the anti-gp41 antibodies (120-16 and 98-6) used V_H IV germ line genes, V_H 71-2 and V_H 2-1, respectively. The predominance of V_H IV genes in these examples is evident. Additional sequence data will be required to identify any restricted use of V region genes.

The V region of the F105 light chain appears to be derived from the *Humkv325* germ line gene and is a member of the VκIIIb sub-subgroup. The phenotypic marker of the human germ line gene *Humkv325* is the 17.109 cross-reactive idiotype (36, 71, 72) as defined by a murine mAb that was raised against human rheumatoid factor (73). The 17.109 cross-reactive idiotype is expressed by ~ 2% of circulating IgM from normal adults (74). In addition to the aforementioned occurrence of *Humkv325* light chains in monoclonal rheumatoid factors (51), in B cell malignancies, including chronic lymphocytic leukemia (55) and small cell non-Hodgkin's lymphoma (56), and in lymphocytes from biopsy specimens of patients with primary Sjögren's syndrome (57), *Humkv325* light chains have also been detected in non-rheumatoid factor paraproteins and in antibodies with anti-LDL, anti-intermediate filament, and anti-DNA histone activity (51, 52, 72).

Whether HIV-1 infection and/or gp120 itself can directly or preferentially stimulate an immune response that uses *Humkv325*-derived V_κ genes is uncertain. Other viruses, most notably members of the herpes virus family have been implicated in such stimulation. The 17.109 cross-reactive idiotype is expressed by *in vitro* and *in vivo* EBV-infected B cells and, in the latter, IgM associated 17.109 cross-reactive idiotype was elevated in acute infection and later declined whereas IgG-associated 17.109 cross-reactive idiotype rose (75). The human anticytomegalovirus antibody EV1-15 has also been shown to use a V_κIIIb light chain that is probably derived from *Humkv325* (76). In the peripheral blood of HIV-infected patients with AIDS, resting B cells appear to be lacking, and there are both decreased numbers of partially activated cells and increased numbers of fully differentiated B cells (58). The polyclonal B cell activation seen in these AIDS patients may be due to concomitant EBV or cytomegalovirus infections, which are often seen in these patients, or may be due to a direct effect of HIV (58). Whether this polyclonal B cell activation that is seen in HIV-infected patients is manifested by a increased frequency of 17.109 cross-reactive idiotype expression cannot be

answered by the present study but represents an important avenue for future investigations.

The adult repertoire of antibody specificities is acquired in a developmentally programmed fashion (77). Schroeder et al. (77) and Kipps et al. (78) have demonstrated that rearranged V_H 71-4 genes (V58P2 and V37P1) and the 17.109 cross-reactive idiotype are expressed in fetal tissue, respectively. Since the restricted use of V_H genes may limit immunocompetence in fetal and neonatal life (77), it will be of importance to determine if the anti-gp120 antibodies produced by these neonatal and pediatric HIV-infected patients, in particular those antibodies that recognize determinants within the CD4 recognition site of gp120, use these same V region germ line genes with or without somatic mutations.

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