A Comparison of HIV-1 Group M and Group O Functional and Immunogenic Domains in the Gag p24 Protein and the C2V3 Region of the Envelope Protein

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The major focus of the sequencing effort of group O viruses to date has been on the p24 Gag and C2V3 gp120 env regions. A total of 42 p24 sequences and 45 C2V3 sequences derived from group O isolates have either been published or provided to the Los Alamos database prior to publication. The primary goals of this review are: i) to provide a brief summary of the accumulating knowledge concerning HIV-1 group O serology and epidemiology; ii) to outline differences and similarities group M and O HIV-1 viruses in the the heavily sequenced 24 Gag and C2V3 gp120 regions, with an emphasis on well-defined functional domains; and iii) to provide an alignment of the region of gp41 that harbors an immunodominant domain important for diagnostics. Only published sequences will be shown in alignments (Table 1), but complete sets of unpublished and published sequences currently in the database were used to generate the consensus sequences for group O sequences shown in the accompanying alignment figures.

Introduction

Group O characterization: After the discovery of the virus ANT70 by a group in Belgium [de Leys (1990)], and the further characterization of this isolate, as well as the MVP5180 and VAU isolates (VAU was isolated from a French woman with no ties to Africa) in 1994 [vanden Haesevelde (1994), Gurtler (1994), Charneau (1994)], it became clear that a new group of viruses, distinct from the HIV-1 Major (M) group, was present in the human population. This group was named the HIV-1 group O for "Outlier" group. The group O, group M, and chimpanzee viruses are genetically quite distant from one another, but these three groups share a distinct lineage relative to other primate lentiviruses (Figure 1). It is notable that four years separated the publications concerning the first and second of these outlier viruses.

Since the isolation of these first highly unusual HIV-1 strains, a second Caucasian patient was found in France [Cohen (1995)], and a quite large number of viruses of this group have been isolated, especially from Cameroonian patients [Loussert-Ajaka (1995)]. The main method for identifying group O viral infections is obtaining a paradoxical pattern of positive and negative serological reactivity using a set of HIV-1 enzyme-linked immunosorbent assay detection kits [Simon (1994)]. A variety of Western blot reactivity patterns can occur with group O serum. Phenotypic differences between group O and M have also been detected. HIV-1 group O isolates exhibited a high level of resistance to non-nucleoside inhibitors ([Descamps (1995)], and Descamps et al., manuscript in preparation), and group M, but not group O viruses, require the incorporation of cyclophilin A for the production of infectious virions [Braaten (1996)].

Sequence analysis of env and gag regions of the first ten group O isolates revealed a peculiar feature of this group relative to group M isolates – limited phylogenetic clustering patterns with only a few sequences grouping into clades in both the p24 and C2V3 regions [Loussert-Ajaka (1995)]. This is in marked contrast to the clear phylogenetic subtypes that emerge in phylogenetic analysis of the group M sequences. The original ten group O isolates are a subset of the 40-plus sequences currently stored in the Los Alamos database, and the observation of limited subtype structure among O group sequences [Loussert-Ajaka (1995)] is consistent with the additional data now available. The nucleotide

Table 1. List of public HIV-1 O group sequences included in this paper. The first two characters preceding the period indicate the country of residence of the individual who was the source of the isolate, followed by the sequence name.

Sequence	Accesion	Reference	Note
O_DE.HAM112		Hampl et al.	The protein sequences were entered by hand at the Los Alamos Database, from the paper by Hampl et al. The source was a 27 year old Cameroonian who had moved to Germany, and was tested for HIV infection because of eczematous skin lesions. This was the first group O infection to be found in Germany.
O_CM.ANT70	L20587	De Leys et al and vanden Haesevelde et al.	This was the first O subtype isolate discovered. The complete viral genome has been sequenced. The virus was derived from a symptomatic Cameroonian, CDC stage III.
O_FR.VAU	X80020	Charneau et al.	This sequence was from an isolate from a French woman who died of AIDS in 1992, who had no known ties to West Africa. DNA was extracted from VAU infected PBMCs, PCR amplified, cloned, and gp160 env was sequenced. The viral isolate was highly cytopathic.
O_CM.MVP5180	L20571	Gurtler et al.	The complete viral genome has been sequenced from the MVP5180 isolate derived from a Cameroonian woman, sampled in 1991; the donor died of AIDS in 1992. The viral isolate MVP5180 was grown in several human T-cell lines and the monocytic U937 line.
O_FR.BCF 01, 02, 03, 06, 07, 08, 11	C2V3 region U24562-U24568, p24 U24706-U24712	Loussert-Ajaka et al.	These seven sequences are from Cameroonian patients who were living in France. PBMC proviral DNA was PCR amplified and 3-6 clones from each patient were sequenced. The consensus of the 3-6 clones was presented. 07, 08 and 11 were CDC stage II; 01 was CDC IV; and 02, 03 and 06 were CDC IVC1.
O_CM.CA9	X78476 U53175	Janssens et al, Braaten et al.	The env sequence was kindly released for publication in the 1995 Human Retroviruses and AIDS database by Dr. Wouter Janssens. The Pol region was published by Janssens et al, 1994, and the p24 region by Braaten et al., 1996.
O_GA.VI686	X78476 X96526	Janssens et al. Delaporte et al.	The VI686 viral sequence spanning the pol region was published by Janssens et al., and the complete Env gp160 was published in a diversity study of HIV isolates from Gabon by Delaporte et al.
O_CM.YBF 22,26,28,32,35, and 37		(F. Simon, P. Mauclere, and S. Saragosti, submitted)	These sequences are derived from a set of individuals from Yaounde and have been submitted for publication. 22 and 26 were CDC stage II, the other four had AIDS. They were isolated in 1996.

Table 1 cont.

Sequence	Accesion	Reference	Note
O_FR.DUR or RUD	X84327		This group O viral sequence was derived from a French woman who had a Cameroonian sexual contact. It was made available in GenBank prior to publication by J. Cohen.
O_CM.MVP2171		(Lutz Gurtler, personal communication)	The complete genome of this sequence has been finished, but it is not yet available at the database. The immunodominant region of gp41 was available, and is included here.

GenBank accession numbers of additional sequences shown in the alignments.

Env sequences

A_U455 M62320
A_KENYA L22943
B_D31 U43096
B_LAI K02013
C_SM145A L22946
C_DJ373A L23065
D_Z2Z6 M22639
D_ELI K03454
E_TN235 L03698
E_CM240X L14572
F_BZ163A L22085
F_93BR020.17 U27401
G_LBV217 U09664
G_92RU131.9 U30312
H_CA13 U09667
H_VI557 L11793
CPZGAB X52154
CPZANT U42720

p24 sequences

CONSENSUS-M
A_KE_K88 L11773
A_ZR_VI57 L11794
B_DE_D31 U43096
B_FR_LAI K02013
C_SO_SM145 L11803
C_ZR_VI313 L71787
D_ZR_ELI K03454
D_ZR_Z2Z6 M22639
F_BR_BZ162 L11751
F_ZR_VI174 L11782
G_ZR_VI191 L11783
H_GA_VI525 L11792
H_ZR_VI557 U09666
CPZGAB X52154
CPZANT U42720

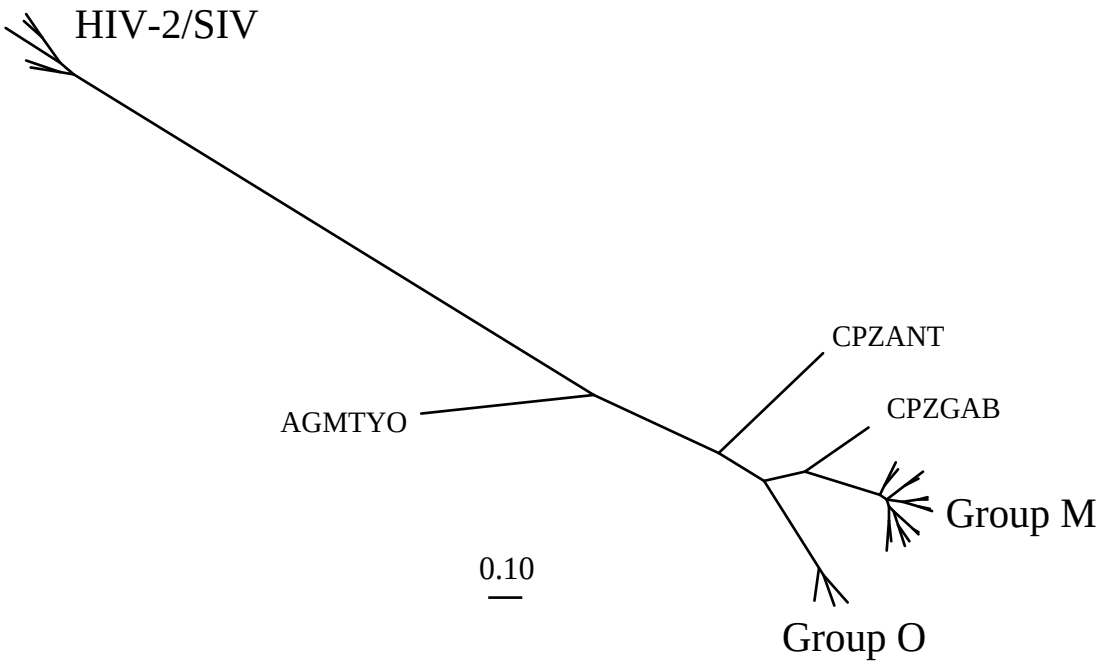


Figure 1. A maximum likelihood tree illustrating the phylogenetic relationship between HIV-1 Group M, HIV-1 Group O, and chimpanzee viral sequences relative to other primate lentiviral sequences. This tree was created using PHYLIP DNAML 4.0 (J. Felsenstein, `http://evolution.genetics.washington.edu/phylip`), with a series of optimization tests performed to select the parameters of three rate categories (1 3 and 9 with frequencies of 0.25, 0.50 and 0.25, respectively) and a transition/transversion ratio of 1.3. The sequences used were the group M isolates subtypes A–G from the alignments in Figure 1 and 2; the full length envelope group O sequences (MVP50, ANT70, VAU); AGMTYO; HIV-2/SIV sequences BEN, D205, ROD, and SIVMNE and SIVSTM; and the chimpanzee viral sequences CPZGAB and CPZANT70. There were 1866 positions spanning gp160 included in the alignment after gapstripping. The taxa included in this comparison were aligned using a Hidden Markov Model (HMMER version 1.8 `http://genome.wustl.edu/eddy/HMMER/main.html`) [Myers & Farmer(1996), Eddy (1995)]. The clade including the HIV-1 and chimpanzee viral sequences is also seen in 100/100 PHYLIP Neighbor Joining bootstrap replicates using a p24 region alignment. The chimpanzee sequences are sometimes linked in phylogenetic reconstructions, and other times are separated as is seen is this tree, with CPZGAB branching with the Group M isolates. The associations of the CPZ sequences depend upon the region of HIV-1 used to construct the tree (data not shown), and may reflect an early recombination event.

sequences of the envelope gene fragments spanning the C2V3 region have been shown to be particularly phylogenetically informative [Leitner (1996)] and to give accurate phylogenetic reconstructions of the M group clades using a variety of phylogenetic tree reconstruction methods [Korber (1994), Leitner(1996)]. Despite this, very little clear phylogenetic structure can be resolved among the group O sequences, in either the region encoding C2V3 of envelope or p24 (analysis was conducted on all sequences available, data not shown). This lack of clearly defined phylogenetic branching patterns in group O could be due to one or a combination of the following three possibilities.

i) An artifact of sample size; accumulation of sequences from new isolates should help us to evaluate this hypothesis.

ii) The length of the sequences in the regions studied not being adequate to reveal phylogenetic associations in group O, although the subtypes are clearly distinguishable using these regions for group M.

iii) A distinct evolutionary history of the two groups, such that the epidemiological history and biology of group M virus yields geographically and phylogenetically distinct subtypes, whereas the group O isolates had a different natural history and simply radiated out from an original ancestral virus [Loussert-Ajaka (1995)]. Interestingly, the first case of AIDS documented in Europe was a Norwegian sailor infected with a group O virus [Jonassen (1997)]. He developed clinical manifestations of HIV infection in 1966, his wife in 1967, and daughter in 1969. All of them died in the 1970s [Froland (1988)].

Geographical distribution: Group O isolates can be identified genetically or serologically. Group O isolates that have been genetically characterized originated in Norway [Jonassen (1997)], Belgium [de Leys (1990)], France [Loussert-Ajaka (1995)], Germany [Hampl (1995)], Spain [Soriano (1996)], the United States [Rayfield (1996)], and, of course, Cameroon [Mauclere (1997)]. Group O infections have been serologically characterized using sera originating in Cameroon and Gabon [Nkengasong (1994)], Equatorial Guinea [Hunt (1996)], Benin [Heyndrickx (1996a)], and Kenya [Songok (1996)]. In Cameroon, 7 group O infections were found among 332 sera tested (7/332), Gabon (2/213), Niger (5/1459), Nigeria (2/183), Senegal (1/1283), Chad (2/619), and Togo (1/670) [Peeters (1997)].

Cameroon has the highest known prevalence of group O infections. Epidemiology studies conducted in Cameroon indicate a prevalence of HIV-1 group O infection of 2% [Zekeng (1994)], and more recently, Mauclere et al. found prevalence of group O infections ranging from 1% in the northern part of the country, to 6.3% in the capitol; overall, 82 group O infections were found among 2458 HIV-1 samples [Mauclere (1997)]. Among the 19 cases found in France, 17 were from patients originally from Cameroon, and of the two French Caucasian patients, one acknowledged having a Cameroonian sexual partner.

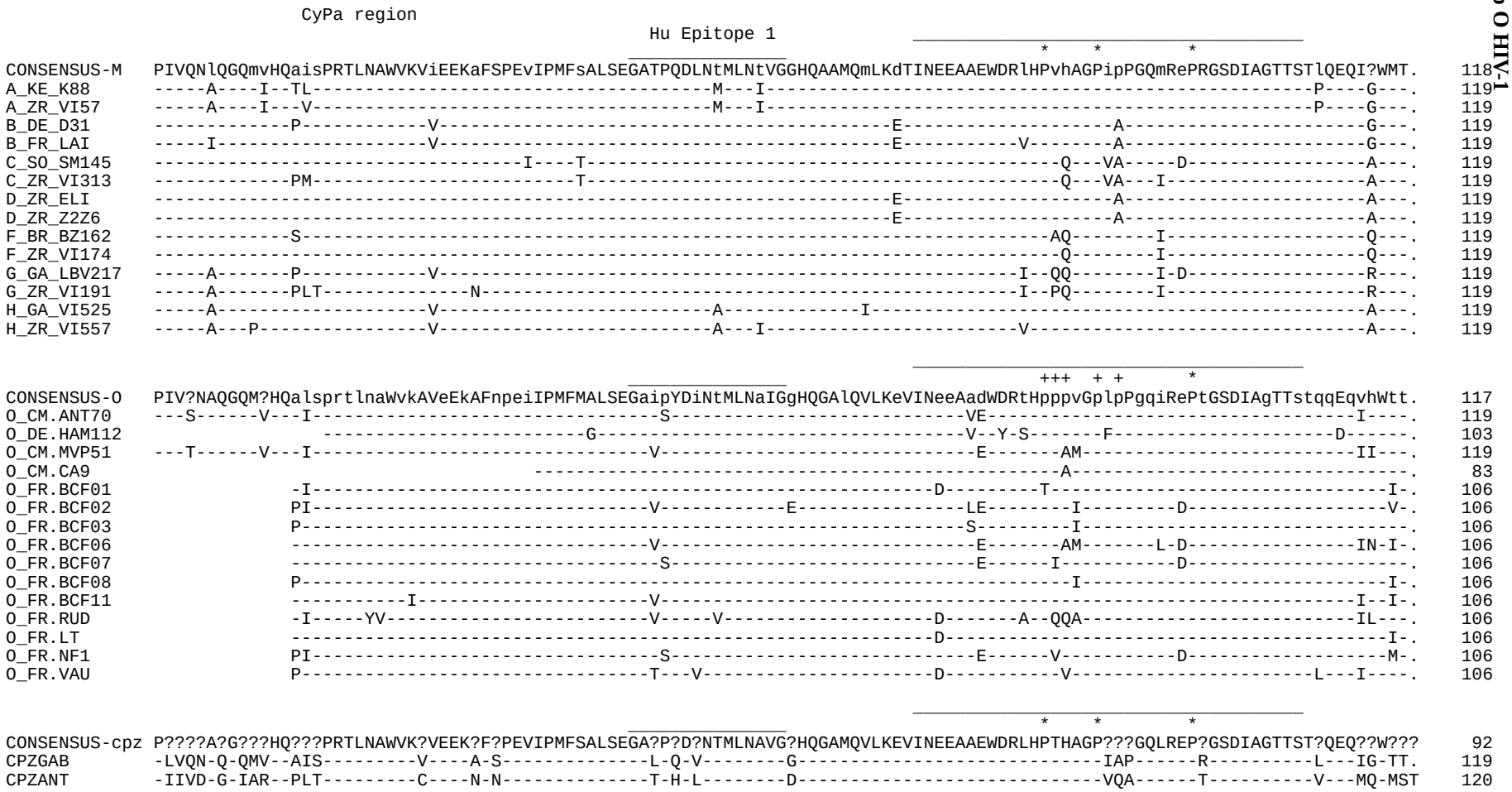
The algorithm developed to characterize group O viruses [Gurtler(1996), Mauclere (1997)] has proven to be very effective. The development of new tools (for example, new peptides used in combination, LIA (Innogenetics)) has allowed the easy serological characterization of group O isolates and the screening of large numbers of samples. Alternatively, group O infections can be detected by generic methods, including PCR using group O and group M specific primers and restriction analysis of a POL fragment [Janssens (1995), Heyndrickx (1996b)]

The gag capsid (p24) protein

Background: The gag precursor poly-protein is initially incorporated into the budding viral particle, and is cleaved into the structural proteins of the mature virion by viral protease. The Gag precursor self-assembles into virion like proteins even in the absence of other viral proteins; it binds and packages viral RNA, and interacts with other viral proteins and envelope lipid to assemble the viral particles (for a review of Gag proteins, see [Gorelick & Henderson(1994)]). The capsid antigen (CA) is the p24 protein of HIV-1, and forms the viral core.

The major homology region: The major homology region (MHR) is a part of the capsid protein which is well conserved among lentiviruses of primates, cats, and ungulates [Otteken (1996), Matsuo (1992), Grund (1994), Gorelick & Henderson(1994)]. This region plays a role in viral particle formation and is critical for viral replication. Four highly conserved positions among all retroviruses (human, simian, caprine, bovine, equine, and feline) [Mammano (1994)] are indicated by asterisks in the alignment shown in Figure 2, on the following two pages, and in the consensus MHR alignment (Figure 3). The first three of these positions are critical for HIV-1 particle production, and mutations in forth residue result in aberrant particle size and inappropriate viral cores [Mammano (1994)].

The MHR is conserved among group O viral sequences, as expected, although slightly greater variation is apparent in the group O viruses than the M group viruses. The conserved arginine (R) is present in most retroviral sequences, but it is substituted with a serine (S) in two of the group O viral sequences (data not shown).


CyPa region
Hu Epitope 1
CONSENSUS-M PIVQNlQGQmvHQaisPRTLNAWVKViEEKaFSPEvIPMFsALSEGATPQDLNtMLNtVGGHQAAMQmLKdTINEEAAEWDRlHPvhAGPipPGQmRePRGSDIAGTTSTlQEQI?WMT. 118
A_KE_K88 -----A----I--TL----------M---I----------P----G---. 119
A_ZR_VI57 -----A----I---V----------M---I----------P----G---. 119
B_DE_D31 -------------P-----------V----------E----------A----------G---. 119
B_FR_LAI -----I----------V----------E----------V--------A----------G---. 119
C_SO_SM145 ----------I----T----------Q---VA-----D----------A---. 119
C_ZR_VI313 ------------PM----------T----------Q---VA---I----------A---. 119
D_ZR_ELI ----------E----------A----------A---. 119
D_ZR_Z2Z6 ----------E----------A----------A---. 119
F_BR_BZ162 ------------S----------AQ--------I----------Q---. 119
F_ZR_VI174 ----------Q--------I----------Q---. 119
G_GA_LBV217 -----A-------P-----------V----------I--QQ--------I-D----------R---. 119
G_ZR_VI191 -----A-------PLT-------------N----------I--PQ--------I----------R---. 119
H_GA_VI525 -----A----------V----------A-------------I----------A---. 119
H_ZR_VI557 -----A---P----------V----------A---I----------V----------A---. 119
CONSENSUS-O PIV?NAQGQM?HQalsprtlnaWvkAVeEkAFnpeiIPMFMALSEGaipYDiNtMLNaIGgHQGAlQVLKeVINeeAadWDRtHpppvGplpPgqiRePtGSDIAgTTstqqEqvhWtt. 117
O_CM.ANT70 ---S------V---I----------S----------VE----------I----. 119
O_DE.HAM112 ----------G----------V--Y-S-------F----------D------. 103
O_CM.MVP51 ---T------V---I----------V----------E-------AM----------II---. 119
O_CM.CA9 ----------A----------. 83
O_FR.BCF01 -I----------D---------T----------I-. 106
O_FR.BCF02 PI----------V-----------E----------LE--------I---------D----------V-. 106
O_FR.BCF03 P----------S---------I----------. 106
O_FR.BCF06 ----------V----------E-------AM-------L-D----------IN-I-. 106
O_FR.BCF07 ----------S----------E------I-----------D----------. 106
O_FR.BCF08 P----------I----------I-. 106
O_FR.BCF11 -----------I----------V----------I--I-. 106
O_FR.RUD -I-----YV----------V-----V----------D-------A--QQA----------IL---. 106
O_FR.LT ----------D----------I-. 106
O_FR.NF1 PI----------S----------E------V-----------D----------M-. 106
O_FR.VAU P----------T---V----------D-----------V----------L---I----. 106
CONSENSUS-cpz P????A?G???HQ???PRTLNAWVK?VEEK?F?PEVIPMFSALSEGA?P?D?NTMLNAVG?HQGAMQVLKEVINEEAAEWDRLHPTHAGP???GQLREP?GSDIAGTTST?QEQ??W??? 92
CPZGAB -LVQN-Q-QMV--AIS---------V----A-S----------L-Q-V--------G----------IAP------R----------L---IG-TT. 119
CPZANT -IIVD-G-IAR--PLT---------C----N-N----------T-H-L--------D----------VQA------T----------V---MQ-MST 120

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```
                                                     Hu Epitope 2
            ________________
                                                            MHR
                          _________________________
                                                    *   *    *  *
CONSENSUS-M    ..sNPpiPVG?IYKRWIIlGLNKIVRMYSPvSILDirQGPKEPFRDYVDRFfKtLRAEQAtQeVKnWMTdTLLVQNANPDCKtILkALGpgAtlEEMMTACQGVGGPgHKArVL    229
A_KE_K88       ..--------D------------------------VK----------------------------G---E------------S--R---T------------------------    231
A_ZR_VI57      ..--------D-------------------------K----------------V------S-D------E------------S--R---T-----------------S------    231
B_DE_D31       ..N-------E-------------------T--------------------Y-----------------E--------------------A-----------------------    231
B_FR_LAI       ..N-------E-------------------T--------------------Y--------S--------E--------------------A-----------------------    231
C_SO_SM145     ..--------D-------------------------K-------------------------D----------------------R------S--------------A------    231
C_ZR_VI313     ..N-------D-------------------------K-------------------------D----------------------R------S---------------------    231
D_ZR_ELI       ..--------E-------V--------------------------------Y--------S-D------E--------------------Q----------------S------    231
D_ZR_Z2Z6      ..--------E----------------------------------------Y--------S----G---E--------------------Q----------------S------    231
F_BR_BZ162     ..----V---E------------------------------------------------------G------------------------------------------------    231
F_ZR_VI174     ..N---V---E------------------------------------------------------G------------------------------------------------    231
G_GA_LBV217    ..--------E---------------------------------------------------D--------------------------------------------S------    231
G_ZR_VI191     ..--------D------------------------------------------------------G-------------------R----------------------------    231
H_GA_VI525     ..G--A----D-------------------------K-------------------------D-------------------N------T--SI-------------S------    231
H_ZR_VI557     ..--------D------------------------------------------A-----------G-------------------R---Q--SI-----------------K--    231

                                                     _______________
                                                   ________________________
CONSENSUS-O    .r?nnpiPvGDIYrkWIVlgLnkmVkmyspvsiLDIkQGPKePFrDYvDrfyKTLRAEQAtQEVKNWMTETLLVQNaNPDCKQILKsLGPgAtLEEMMVACQGVGGPTHKa??L    226
                                                    *   *    *  *
O_CM.ANT70     .-P-Q----------------------------------------------------------------------------------------------------------RV-    232
O_DE.HAM112    .-A-Q-V---------------------HS    132
O_CM.MVP51     .-GA-S------------------------------R---------------------------------------S---------A---E--------------------KI-    232
O_CM.CA9       .-A-H------------------L------------K-------K------------    139
O_FR.BCF01     .-P-------------------------    133
O_FR.BCF02     .-NP---------W----F----L----    133
O_FR.BCF03     .-P-Q-----------------------    133
O_FR.BCF06     .-P---V-------------------L-    133
O_FR.BCF07     .-P-------------------------    133
O_FR.BCF08     .-A-QS----------------------    133
O_FR.BCF11     .-GG-S----------------------    133
O_FR.RUD       .-AG------------------------    133
O_FR.LT        .-A-------------------------    133
O_FR.NF1       .-A-------------------------    133
O_FR.VAU       .-A-Q-------------I---------    133
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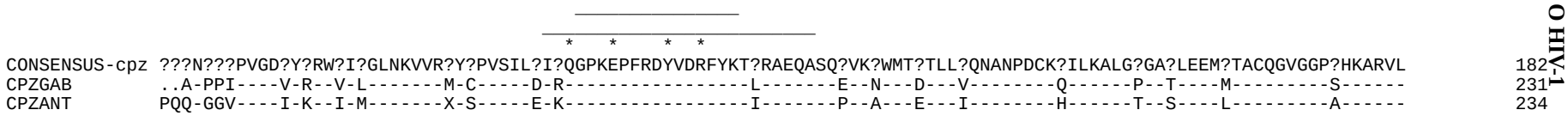

Figure 2. Alignment of the p24 protein. The group M consensus is based on the sequences shown, so each M subtype is equally represented, by two sequences. Upper case letters signify invariant amino acids, and lower case indicate the most common amino acid in a position with some variation. The group O consensus was constructed from all 42 sequences available, but only those published or released are shown. A dash indicates identity with the consensus, a period indicates a insertion made to maintain the alignment. Hu Epitope 1 and 2 are immunodominant linear B cell epitopes that have been identified in group M [Janvier (1996)], and are highly conserved between groups M and O. The MHR is the major homology region and the CyPa region is the cyclophilin A binding domain. Asterisks in the MHR mark highly conserved, functionally important residues, and in the CyPa region mark prolines critical for group M viral replication. The plus signs over the O group consensus indicate prolines that are common among O group sequences, but not perfectly conserved.

```
                    *   *    *  *
M group consensus:  irQGPKEPFRDYVDRFfKtLRAEQA
group O consensus:  -k----e--r--v-rfy--------
     cpz consensus: -?--------------Y--?-----
```

Figure 3. A comparison of the consensus sequences across the MHR of gag p24. Highly conserved amino acids essential for group viral particle formation are marked with asterisks.

There is an immunodominant region within the MHR that can stimulate both human and murine antibodies (see [Korber (1996)] for a summary). One of the immunodominant linear epitopes defined for HIV-1 M group viruses is located within this region, defined as: GPKEPFRDYVDRFYK, (called Hu Epitope 2 in Figure 2) [Janvier (1996)]. Only a single amino acid differs in this epitope between the group O consensus the M group consensuses. The tyrosine (Y) in the second to last position of the epitope matches the consensus of group O sequences, and is found in all B and D clade sequences, as well as among the Thai A subtypes. But in all other clades in the M group, this position is most commonly occupied by a phenylalanine (F). It is not clear whether this substitution would influence the antigenic specificity of the peptide, though it is very likely that the antigenic peptide studied by Janvier et al. would react with sera from individuals infected with group O strains of HIV-1, because the group M subtype B derived peptide perfectly matches the group O consensus. 9/20 sera from HIV-1 infected individuals reacted with this peptide [Janvier (1996)]. Among HIV-2 and SIV sequences, the immunogenic region is conserved except for two substitutions: the central RD in positions six and seven become QS (see the Gag protein alignments in this compendium). These substitutions may not be critical for binding, as murine monoclonal antibodies that bind to this region have been shown to be cross-reactive with HIV-1, HIV-2, and SIVmac and SIVagm strains [Matsuo (1992), Robert-Hebmann (1992), Niedrig (1989)]. Therefore, this immunodominant domain could potentially be useful for serological identification of all primate lentiviral infections, by sequence analogy, including those of group O.

Cyclophilin A binding region: Cyclophilin A (CyPa) is a human protein that binds to a proline rich region in the HIV-1 p55 Gag precursor protein, and is incorporated into group M HIV-1 viral particles, (). Group M HIV-1 viral particles that lack cyclophilin A are not infectious, in contrast to other retroviruses [Luban (1994), Thali (1994), Franke (1994)]. Cyclophilins are a family of proteins that catalyze protein folding and are protective against heat shock [Fisher & Schmid(1990), Gething & Sambrook(1992), Sykes (1993)]. In contrast to HIV M group virus and the SIVcpz GAB strain, and the group O viruses do not have to bind and incorporate CyPa to produce infectious virions; this is one of the first major biological differences between group M and group O to be documented [Braaten (1996)].

Three of the prolines in the highly conserved CyPa binding region of p24 are required for the production of infectious virions in M group strains. These prolines are marked with an asterisk in Figures 2 and 4. The proline at position 222 (AG**P**) was required for CyPa binding and incorporation into virions, and was required for replication. The proline at position 225 (**P**GQ) was not essential for replication, and so is not marked. Mutations in prolines 217 (lH**P**) and 231 (**P**RG) decreased particle yield and the mutant virus did not replicate [Franke (1994)]. Proline 222 was highly, but not perfectly, conserved among group O viruses, in spite of the lack of a requirement for cyclophilin A binding. Proline 231 was perfectly preserved among group M, group O, and SIVcpz. Proline 217 is perfectly preserved among group M and SIVcpz viruses, but varies somewhat in group O viruses. Two additional prolines are present in group O viruses proximal to position 217 (Figure 2).

There are many murine monoclonal antibodies that bind to the CyPa binding domain [Korber (1996)]. One particular epitope that has been defined by peptide reactivity (ETINEEAAEWDRVHP) was cross-reactive with sera from HIV-1, HIV-2 and SIV infections [Niedrig (1988)]. While there are frequent substitutions among group O isolates in this region, most are conservative aspartic acid (D) to glutamic acid (E) substitutions, and there are some O strains that are identical to the M consensus sequence in this region. Thus it is likely that group O sera would react with this peptide.

```
                                *    *        *
M group consensus:    INEEAAEWDRlHPvhAGPipPGQmRePRGSDIAGTTS
group O consensus:    --ee-ad---t-pppv-plp-gqi-e-t-----g--s
     cpz consensus:   ----------L--TH---???--L-E-?---------
```

Figure 4. A comparison of the consensus sequences across the Cyclophilin A binding region in gag p24. Highly conserved prolines essential for group M viral replication are marked with asterisks.

Additional immunogenic domains in p24: A peptide covering positions 178-192 (HIV-1 IIIB) was recognized by sera from 8/20 HIV-1 positive people (Hu Epitope 1 in Figure 2) [Janvier (1996)]. The linear immunodominant antigenic regions (Hu Epitope 1 and 2) in p24 group M viruses are conserved in group O as well, particularly human epitope 2, suggesting that they may provide broadly cross-reactive antigenic peptides recognized by sera of individuals with O and M group infections.

Through studies involving M group strains, HIV-1 epitopes have been defined for both helper and cytotoxic T cells across the p24 protein. The p24 T-cell epitopes that have been defined in M group tend to cluster, some near the C-terminal region, and some crossing the CyPa binding site and the MHR [Korber (1996)]. Because of the conservation of p24, it is highly probably that some cross-reactivity between M and group O would be observed. For example, there is an HLA-A25 restricted CTL epitope, (ETINEEAAEW) that has been defined using CTL clones from long term survivors [van Baalen (1996)]. This epitope is conserved in viruses of the B and D subtypes, but the form DTINEEAAEW is commonly in other clades, and this variant was not recognized by CTL specific for the index peptide. While group O sequences show some variation in this epitope, some are very similar to the reactive M group epitope, and may be cross-reactive: (*e.g.* MVP51: EVINEEAAEW).

The C2V3 Region

Variation and immunogenicity of the V3 loop: The variation in the V3 loop has been the focus of extensive international research efforts, originally because it was noted in the late 1980s that antibodies directed against the tip of V3 loop could potently neutralize laboratory strains of HIV-1 in a type-specific manner [Javaherian (1989), Palker (1988), Rusche (1988)]. More recently, anti-V3 monoclonal antibodies have been shown in general to be potent neutralizing agents when directed against HIV-1 laboratory-adapted strains, but far less so when directed against primary isolates. This presumably is due to distinct conformations of envelope, such that the V3 loop of primary isolates is inaccessible (see [Moore & Ho(1995), Poignard (1996)] for review). Most anti-V3 monoclonal antibodies are type-specific, and monoclonals directed against one strain can be completely unreactive even with closely related viral strains, due to the hypervariable nature and high rate of amino acid substitution in the immunogenic tip of the V3 loop [Korber (1996)]. Other neutralizing epitopes, both continuous and discontinuous, have been identified in envelope [Poignard (1996), Moore & Ho(1995)]. Many neutralizing antibodies (but not all, *e. g.* [Trkola (1995)]) share the property of reduced neutralization of primary isolates relative to laboratory adapted strains [Moore & Ho(1995)].

The turn at the tip of the loop is the focus of most anti-V3 neutralizing antibodies, and is most often found to be GPGR or GPGQ among M group viruses [Foley (1996)]. These two motifs are not found among group O strains. With the exception of some common amino acids flanking the cysteines at the base of the V3 loop, group O and group M V3 loops are very different from each other. Helper and cytotoxic T cell epitopes, as well as antibody binding sites, have been identified within the V3 loop of M group viruses. Because group O and M viruses are highly variable in the C2V3 region within a group, and because the extent of the substitutions found between group O and M is so great, it is highly unlikely that there would be antigenic O and M cross-reactivity for either B or T cell epitopes in the V3 loop.

Phenotypic determinants in the V3 loop: The V3 loop has been shown to have a critical functional role in determining the phenotype of the virus [Hwang (1991), Chesebro (1992), de Jong (1992)]. Positively charged amino acids in certain positions in the V3 loop are correlated with a syncytium-inducing (SI), T-cell-tropic viral phenotype among group M subtype B viruses [Fouchier (1992)] (the two most critical positions are indicated in Figure 5). The observations concerning these two positions, and a correlation between net positive charge on the loop and rapid high and slow low

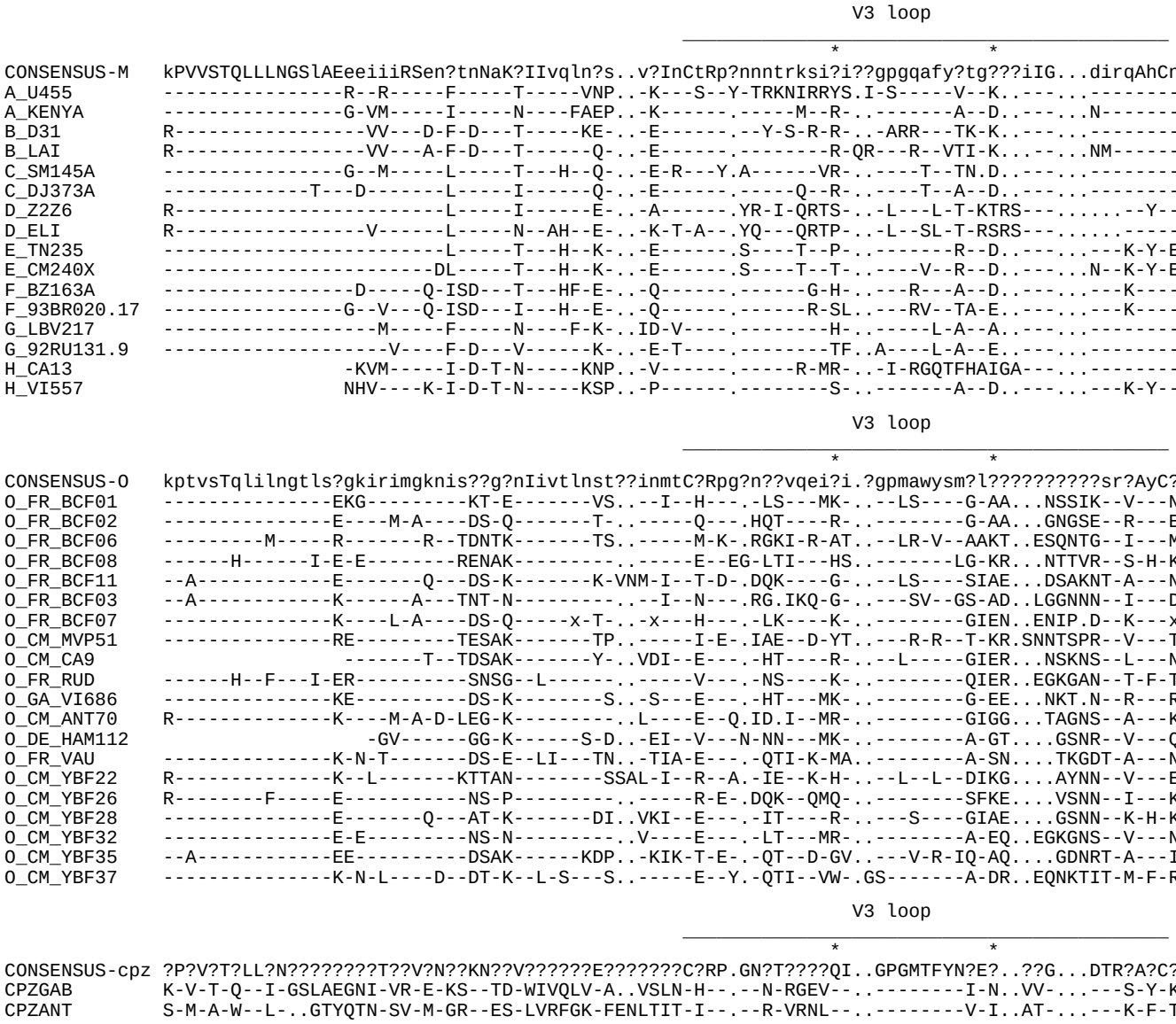

Figure 5. Alignment of the C2V3 region of gp120. The boundaries of the V3 loop are shown, and the positions that are associated with viral phenotype (SI phenotype and positive charge) are marked with asterisks. 45 group O sequences were used to generate the consensus, but only those published or released are shown.

phenotypes, were extended beyond subtype B to other group M subtypes [de Wolf (1994), Rubsamen-Waigmann (1994)]. Recently, a correlation between positive charge and phenotype was shown for the group O viruses [de Jong (1996)]. Because of the length variation between different strains and the diversity found in this region, it is difficult to be certain the alignment of V3 loop shown in Figure 5 is biologically appropriate. Despite this, in a best estimate the two critical sites in the V3 loop associated with group M SI/NSI phenotype switching display a similar pattern of variation among group O strains and group M sequences. Both are highly variable positions, and the first position alternates between neutral and positively charged amino acids, and the second position alternates between negative, neutral and positively charged amino acids.

Recently it has been shown that chemokine second receptor usage for HIV entry into CD4-positive immune cells is associated with the phenotype of the virus, and not with the genetically defined HIV-1 group or subtype [Zhang (1996), Choe (1996)]. CXCR4 (fusin) usage is associated with viruses of with T-cell tropic, SI phenotypes; CCR5 usage is associated with a macrophage tropic, non-SI (NSI) phenotype; and some SI viruses are able to use both CXCR4 and CCR5 [Zhang (1996)]. The group O viruses, similar to the M group, have both patterns of second receptor usage [Zhang (1996)]. The two group O isolates for which second receptor usage has been determined are CA9, with an NSI phenotype and second receptor usage restricted to CCR5; and MVP5180, with an SI phenotype and the ability to use both CCR5 and CXCR4. The pattern of positive charge substitutions in the V3 loop, at least in these two group O isolates, is consistent with what has been observed in the M group. The SI isolate MVP5180 has greater positive charge overall, and in particular, in the second of the two sites associated with the SI phenotype in the M group is positively charged in O_CM_MVP51 and negatively charged in O_CM_CA9 (Figure 6). The C-C chemokines RANTES, MIP-1α, and MIP-1β produced by CD8+ cells, can inhibit HIV-1 infection of primary and macrophage-tropic group M HIV-1 isolates, whereas T-cell tropic isolates tend to be resistant to the C-C chemokine suppressive effects [Cocchi (1995)]. The V3 loop was shown to be a critical region for determining the susceptibility to chemokine-mediated supression using chimeric gp120 proteins [Cocchi (1996)]. It will be interesting to see if SI group O viruses, like MVP5180, will have a similar pattern of resistance.

```
                          *              *
                              + +    ++
O_CM_MVP51    CIREGIAEVQDIYTGPMRWRSMTLKRSNNTSPRSRVAYC
  O_CM_CA9    CERPGNHTVQEIRIGPLAWYSMGIERNSKNSSR--LAYC
```

Figure 6) The V3 loop sequences of SI strain MVP51 and NSI strain CA9. The positions that have been associated with viral phenotype are marked with asterisks. The positively charged amino acids arginine (R) and lysine (K) near the tip of the loop V3 found in SI isolate MVP51 are marked with a plus (+). The dashes (-) indicated insertions made to maintain the alignment.

Immunodominant domain in gp41

The immunodominant region in gp41 is conserved between different group M strains, and is reactive with sera from individuals infected with a variety of group M subtypes [Engelbrecht (1994), Petrov (1990), Shafferman (1989), Gnann (1987)]. Although only a limited number of sequences are avalailable in the database across this region, because of its importance for diagnostics, a comparison of the and group M and O consensus sequences in this region is shown in Figure 7.

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```
CONSENSUS-M  EaqQHlLqLTVWGIKQLqaRvLAvErYLkDQqlLGiWGCSGKlICtT?VPwNssWS.Nks?eeIW?NmTwmeWerEisnYt??iy?l?eeSQnQQekneqd
A_U455       -------K--------------------Q-----------------T--------.---Q-D--N----LQ--K---S--GI--Q-I-----------L-
A_KENYA      -------K--------------------R-------------I----N--------.---QS---E----LQ-DK------DI--N-L-------------
B_D31        ---------------------------R-----------------A----A---.---MDM--N-----------D---SL--T-I------------E
B_LAI        --------------------I--------------------------A----A---.---L-Q--N-------D---N---SL-HS-I------------E
C_SM145A     -----M-----------R-----I----E-----------------A--------.-RTQ----E-L---Q-DK------DT--R-L-V---------K-
C_DJ373A     -VH--M-----------T----I----------------------A--------.---Q----D-----Q-D---N---ET--R-L-V--T---Q--K-
D_Z2Z6       --------------------I-------------------------T--------.-R-LND--Q-----------D---GL--R-I----T-------E
D_ELI        --------------------I--------------------H----N--------.-R-LN---Q-----------D---GL--S-I----T------KE
E_TN235      ------------------------------KF--L------I----A--$--T--.-R-Y----N----I----------NQ--EILT------DR--K-
E_CM240X     ------------------------------KF--L------I----A-----T--.-R-F----N---RI----------NQ--EILT------DR--K-
F_BZ163A     ----------------------------Q------L-----------N--------.---Q----E-------QK-----SNEV-R-I-K----------G
F_93BR020.17 ---------------------------------L-----------N--------.---L----G--------K-V---SKE--R-I-D--------K-E
G_LBV217     ----------------------------Q-----------------N----T---.---FN---D----I------N---HQ--S-L----I--------
G_92RU131.9  ------------------------K-----------------P-N----T---.---FN---D----I------N---YQ--N-L----------D---

CONSENSUS-O  QAQQqLLRlSVWGIRQLRARLlALETliQNQQlLnLWGCkgklvCYTSVkWN?tW?G?ds...IWd?LTWQeWDqqi?NiSs?IydeIQ?AQvQQE?Nekk
O_FR.RUD     ----H---------------------M----------R--AI-----Q--E--G-N--...---R----Q-----A-V--F---K--E--E--
O_CM.MVP51   ----H----------------Q----------R----------I--------TS-S-RYNDDS---N----Q---H-N-V--I------A--D---K-V-A
O_CM.ANT70   ----------x---------------L------S-----------------R--I-NE-...---T-------R--S----T--E---K------Q----
O_CM.2901    ----H----------------Q-----M---------------I--------E--G-NL-...---S----Q----VA-V--L---K--E--E---K--RA
O_FR.VAU     ----H---P-----------------F-------------NR-I--------K--G-DNES..---E----Q-----N-V--F--EK--E--E---K---E
```

Figure 7. Alignment of amino acids from gp41 spanning the immunodominant domain of gp41. This alignment starts at 50 amino acids from the start of gp41 and continues for the next 100 amino acids. The M consensus is based on the set of sequences shown. The group O consensus is based on the 9 sequences currently available at the Los Alamos database covering this region; the five that have been published or made public are shown in the alignment.

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