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Molecular Characterization of Biologically Diverse Envelope Variants of Human Immunodeficiency Virus Type 1 Derived from an Individual

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The envelope genes of six viruses derived from a single sampling from an individual chronically infected with human immunodeficiency virus type 1 (RJS-4) have been analyzed. Here we present the nucleotide and predicted amino acid sequences of these variants and show a correlation between biological properties and disturbance of the envelope reading frame.

Genetic variation among human immunodeficiency virus (HIV) isolates is well established, and viruses analyzed either directly from infected individuals or after propagation in vitro comprise groups of related but genetically and biologically distinct viral subtypes or quasispecies (8, 20, 24, 25). These viruses have differing capacities to replicate in target cells of lymphoid and monocytoid origin or in brain-derived tissues (4, 8, 9, 14, 30), and in some cases replicative preference can be localized to alterations in the envelope protein (5, 15, 17, 26, 27). Here we report a molecular analysis of six infectious provirus constructs containing variant *env* genes which have been shown to possess differing biological phenotypes (8).

The HIV-1 isolate RJS-4 was obtained by short-term coculture (<1 week) of peripheral blood mononuclear cells from a chronically infected individual with Jurkat cells (clone D4), and this was used to prepare a recombinant λ phage library as described previously (11, 24). To provide a panel of related viruses (designated JS4) in which the effects of envelope variation could be measured independently of the effects created by differences in other areas of the genome, *Sal*I-*Bam*HI fragments encompassing the *env* gene were substituted into the corresponding site of the biologically active molecular clone pHXB2gpt as described elsewhere (8) (Fig. 1). The infectious potentials of the clones were assessed by transfection of Cos-1 cells and incubation of these transfected cells or supernatants derived therefrom with target T-cell lines, fresh peripheral blood T cells, or normal monocytes in culture. The results from this study for immune precipitation of *gag*- and *env*-related products, measurement of reverse transcriptase (RT) activity, and viruslike particles produced by JS4-transfected Cos-1 cells are summarized (Table 1).

The complete nucleotide sequences of the *Sal*I-*Bam*HI fragments of pHXB2gpt and the six JS clones were determined by using 1 μg of NaOH-denatured plasmid DNA, dideoxynucleotides, RT, and 5′-^{32}P-labelled oligonucleotides as described previously (6) and submitted to GenBank (accession numbers: JS4-6, M37491; JS4-15, M37573; JS4-16, M37577; JS4-22, M37574; JS4-24, M37575; JS4-26, M37576). The overall sequences of the six JS4 variants are very similar. All contain a series of 11 triplet base insertions or deletions compared with pHXB2gpt (Fig. 1). At the nucleotide level, each of the variants is more similar to its JS4 relatives than to pHXB2gpt. For example JS4-6 shows 90.3, 97.8, and 99.1% homology with pHXB2gpt, JS4-26, and JS4-22, respectively, over the 2.9 kb of DNA sequenced.

Figure 1 summarizes the key features of the sequences, all JS4 clones contain a mutated *Bam*HI site compared with pHXB2gpt. Instead of a *Bam*HI site at nucleotide position 8474 (as in pHXB2gpt), RJS-4 viral genomes have a *Bam*HI site 157 bases downstream, such that JS4 hybrid genomes contain an almost perfect 157-base tandem repeat. This leads to a frameshift which results in the JS4 clones encoding 58 amino acids at the carboxy terminus of gp41 (compared with 53 in pHXB2gpt), contributed from the gene encoding the carboxy terminus of the pHXB2gpt *rev* gene (Fig. 1 to 3). The frameshift also results in the carboxy terminus of *rev* being extended by 20 amino acids in all JS4 clones (Fig. 3). While these modifications do not account for the biological differences seen between JS4 viruses (since all contain the same changes), they do complicate our analyses and may lead to slight attenuation of replicative and cytopathic properties compared with pHXB2gpt (Table 1).

The JS4-6 envelope amino acid sequence, contributed by the RJS-4 genome, was aligned with published HIV-1 envelope sequences, and its closest relative, with 86.5% homology, was found to be isolate SF_{162} (3). In comparison with the consensus sequence published by Myers et al. (22), JS4-6 contains two amino acid substitutions (225 A→V, 662 K→T; Fig. 2) at previously invariant positions. The relevance of these substitutions to the biological properties of the JS4 clones is unknown. Only four of the clones (JS4-6, -22, -24, and -26) contain an intact open reading frame for *env*, generating proteins sharing between 96.0 and 98.6% homology. Clone 16 contains a premature stop at amino acid position 111, due to the mutation of a tryptophan codon (TGG) to TAG (Fig. 2). Clone 15 has an altered reading frame after amino acid residue 303, due to the insertion of an additional adenosine residue in a run of seven. This alteration would be predicted to generate a protein of only 330 amino acids. These truncations probably explain the lack of immune precipitation of envelope proteins in Cos-1 cells transfected with JS clones 15 and 16 (Table 1). However, since both variants are capable of generating HIV-like particles with RT activity, we conclude that generation of an intact transmembrane envelope glycoprotein is not required

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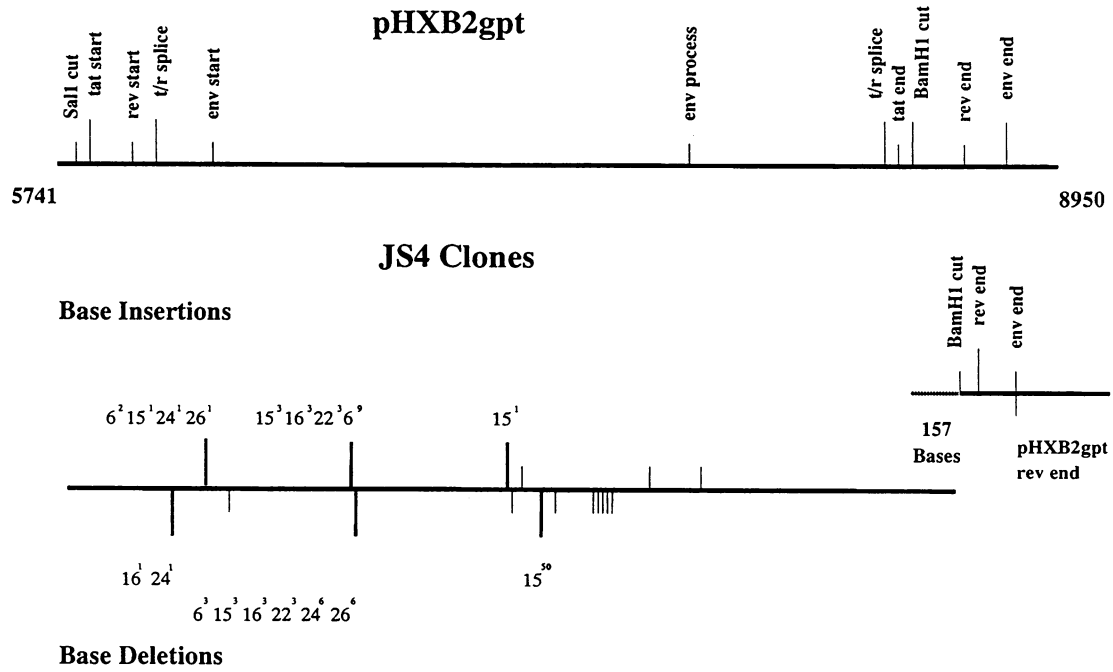

FIG. 1. Construction and molecular characterization of JS4 *SalI-Bam*HI hybrid genomes. The eleven features shown for pHXB2gpt occur, from left to right, at residues 5785, 5830, 5969, 6045-6046, 6224, 7756, 8376-8377, 8421, 8474, 8650, and 8792, respectively, numbered as by Myers et al. (22). Major features of the JS4 constructs relative to pHXB2gpt are shown. Short bars indicate triplet base insertions and deletions common to all JS4 prototypes. Other insertions and deletions (long bars) show the clone in which the superscript value indicates the number of nucleotides involved. All JS4 clones contain *tat* termination signals at a position equivalent to residue 8466 in pHXB2gpt (encoded in RJS genomes), whereas the frameshift of 157 bases introduced because of the altered location of the *Bam*HI site in RJS genomes compared with pHXB2gpt results in *rev* and *env* reading frames being terminated at residues 8535 and 8650, respectively, derived from pHXB2gpt.

for particle formation. In support of this it has been shown that expression of the simian immunodeficiency virus *gag* gene in isolation can result in viruslike particle formation (7). In addition, virus derived from transfection of Cos-1 cells with JS4-15 and JS4-16 was shown to be infectious for primary monocyte-macrophage cultures as judged by immune precipitation of *gag* proteins (Table 1). This suggests that glycoprotein-deficient virus particles may be sequestered in macrophages, gaining entry via a non-CD4-dependent pathway. Support for this suggestion is derived from recent publications showing that transmission of HIV from monocytes to epithelia can occur by three routes: phagocytosis, direct membrane fusion, and receptor-mediated endocytosis (2), HIV produced in macrophages is relatively deficient of envelope glycoprotein compared with that produced in T cells (19) and a study of an HIV-1 clustered

TABLE 1. Biological properties of the hybrid JS-4 viruses[a]

Clone	Result of transfection of Cos-1 cells				Result of virus infection of cells[b]		
	Virus particles detected by electron microscopy	RT activity (%)[c]	Immunoprecipitation of HIV-1 proteins		Infection of T cells	Infection of cord blood T-cell lines	Monocytes and macrophages in culture
			Gag	*Env*			
pHXB2-gpt	+	65	+	+	+++	+++	−
JS4-6	+	69	+	+	+	+	+
JS4-15	+	94	+	−	−	−	+
JS4-16	+	87	+	−	−	−	+
JS4-22	+	85	+	+	++	+	+
JS4-24	+	52	ND[d]	ND	+/−	+/−	−
JS4-26	+	100	+	−	−	−	−
SP65-gpt	−	0	−	−	−	−	−

[a] The data shown summarize the results of previous work (8).

[b] Virus infection was judged by detection of HIV-1 *gag* p24 expression by target cells, 2 weeks (T-cell lines and lymphocyte cultures) and 5 weeks (monocyte-macrophage cultures maintained in lipopolysaccharide, colony-simulating factor-1, and interleukin 3 after addition of an innoculum of concentrated virus containing supernatant. The supernatants were obtained from Cos-1 cells after transfection with JS4 clones.

[c] Expressed as a percentage of the maximum level detected, determined by three to seven independent experiments. Thus, RT activity for clone JS4-26 is shown as 100% and RT activity of SP65gpt (a clone devoid of HIV-1 sequences) is shown as zero.

[d] ND, not done.

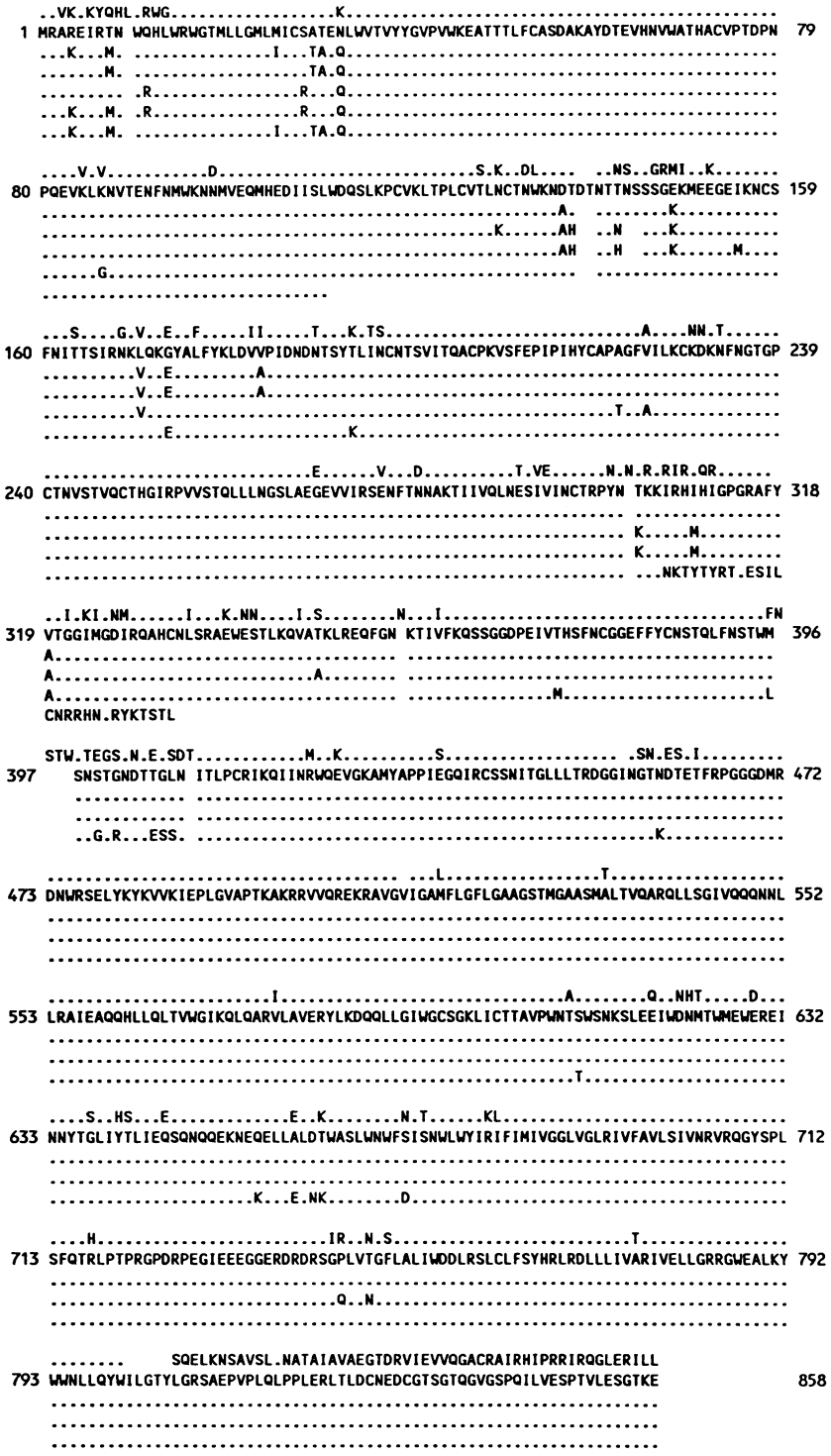

FIG. 2. Envelope protein sequence alignment of JS4 clones. Alignment of the sequences was performed pairwaise with the assistance of a program described elsewhere (28). Sequences were derived from (top to bottom) pHXB2gpt and JS4-6, -22, -24, -26, -15, and -16, and the JS4-6 sequence is numbered. Dots represent sequence homology with JS4-6, and open spaces represent gaps introduced by the alignment program to maximize homology. JS4-16 is terminated at residue 110 because of the presence of a premature termination codon. JS4-15 has an altered reading frame after residue 305 and terminates at 332. The modified carboxy terminus of JS4 clones -6, -22, -24, and -26 compared with that of pHXB2gpt is shown.

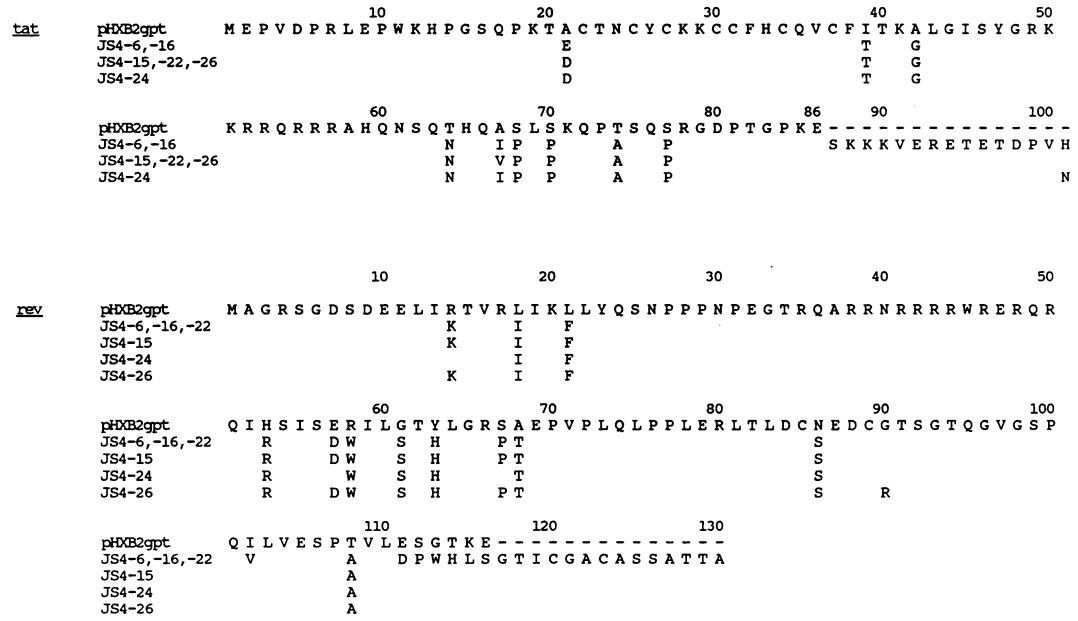

FIG. 3. Comparison of JS4 *tat* and *rev* amino acid sequences. Amino acid substitutions in the JS4 clones, compared to pHXB2gpt, are shown. For *tat*, only JS4-24 showed variation to JS4-6 in the 15-amino-acid extension compared to pHXB2gpt (101H→N). For *rev*, all JS4 clones have the same sequence, 111(D)→130(A), contributed by the pHXB2gpt genome.

infectious outbreak has shown that *env*-defective viruses (prematurely terminated in gp41 prior to the transmembrane spanning domain) represent the major forms in vivo and appear to be capable of transmission and dissemination to a variety of tissues, e.g., brain and lung (18). Indeed, defective HIV-1 genomes are abundant in vivo, as assessed by polymerase chain reaction analyses (10, 18, 20), and defective viruses have a direct role in the pathogenesis of immunodeficiency syndromes in mice and cats (1, 12, 23). Such observations have increased speculations that HIV-1 defective genomes may have a role in AIDS.

Of the JS4 clones possessing an intact *env* reading frame, the variants can be ordered in terms of their similarities. Taking clone 6 as a reference point, clones 22, 24, and 26 show increasing divergence. The variation in amino acid sequence is largely confined to the extracellular (gp120) domain. In particular, this variation clusters within hypervariable domain I (V1), a domain which is defined by conserved cysteine residues at positions 125 and 158 (JS4-6 numbering; Fig. 2). Within this stretch of approximately 30 amino acids the homology between clone 6 and JS4-22, -24, and -26 is 88.2, 76.5, and 73.5%, respectively. It is within this region that the only amino acid deletions and the majority of alterations to potential glycosylation sites can be found. Mutations at positions 129, 137, 142, and 143 in clones 24 and 26 would be predicted to remove the capacity for glycosylation at these sites, as would the change of Asn to Lys at residue 459 for clone JS4-26. A number of HIV-1 isolates have been shown to be composed of viral mixtures by polymerase chain reaction, subcloning, and sequence analysis of *env* gene fragments restricted to V1 (10). The significance of variation clustering to the first hypervariable domain of gp120 is unclear. The nucleotide composition of this portion of the *env* gene is not significantly different from that of the whole, suggesting it does not represent a hot spot for introduction of variation during transcription. Since the domain is defined by cysteine residues (125 and 158; Fig. 2) and is hydrophilic, it may exist as a surface loop on the protein structure and as such provide a target for immunological responses in humans. The literature relevant to such responses, but largely based on animal model systems, has not identified this domain to be of significance for either humoral or T-cell responses.

Outside V1, variation among the six JS4 clones occurs predominantly within or near the signal peptide domain (up to residue 32; Fig. 2). For the clones with intact *env* reading frames (6, 22, 24, 26), there is limited variation within the V3 domain, which is proposed to be immunodominant and exist as a disulfide bonded loop (Cys residues 297 and 332; Fig. 2) (13, 16). No variation is seen within a domain shown to be critical for interaction with CD4, residues 413 to 434 (Fig. 2) (15); however, clone 26 has six substitutions in the V4 domain (limited by Cys residues 385 and 413; Fig. 2) immediately preceding the CD4 binding domain. There is a series of amino acid substitutions in clone 26 (655 to 671; Fig. 2) just prior to the proposed membrane anchor domain of gp41, whereas clone 24 has two substitutions in the proposed cytoplasmic tail of gp41 (21). Which, if any, of these substitutions result in the attenuation of clones 24 and 26 (viruses which showed poor transmission to T cells and monocytes; Table 1) compared with clones 6 and 22 cannot be inferred from the present study.

The *Sal*I-*Bam*HI fragments also encompass the spliced genes *tat* and *rev* (Fig. 1), the translations of which are shown in Fig. 3. The Tat protein is encoded exclusively by RJS-derived genome, and all clones show 9 amino acid substitutions, compared with pHXB2gpt, together with a 15-amino-acid carboxy-terminal extension. The latter has been found in a number of HIV-1 isolates (22). Amino acid substitutions between the JS4 clones are conservative in nature. The Rev protein is derived from the RJS genome to amino acid 110; thereafter, because of frameshift during ligation into pHXB2gpt, there is a 6-amino-acid mismatch and a 14-amino-acid carboxy-terminal extension compared

to pHXB2gpt. Variation between JS4 clones is seen at five positions. Only two of these (67P→S clone 24 and 90G→R clone 26) are nonconservative, and they map to either side of a domain which has been shown to be important for *rev* function (29). Whether these substitutions contribute to the phenotypes of these two clones has not been ascertained.

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