whereas ~90% of the transfectants with the cDNA from the control fibroblasts (AG3022; Human Mutant Cell Repository) contained numerous peroxisomes, as seen in the control cells (*13*).

To determine if patient MM inherited the homozygous mutation from her parents, we investigated the nucleotide sequence in this region of their genomes. Genomic DNA fragments corresponding to a portion of the PAF-1 cDNA (nucleotides 340 through 371) were amplified by PCR and cloned. No intron was found in this region. In patient MM, T was substituted for C at position 355, as noted in the cDNA. In the genomic DNA clones isolated from the parents, T was detected in place of C in two and three of six clones, each from the mother and the father, respectively (Fig. 4B). This suggests that both father and mother were heterozygous for the same mutation in PAF-1 and that patient MM inherited the mutation from both parents.

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15. A Japanese girl (MM) who died at 8 months manifested typical findings and a clinical course of ZS, including accumulation of very long chain fatty acids in serum sphingomyelins, absence in liver homogenates of all three peroxisomal β-oxidation enzymes (*13*), and no peroxisomes in all skin fibroblasts immunochemically examined (Fig. 1C). Autopsy revealed macrogyria and polymicrogyria in the brain, hepatosplenomegaly, and many small cysts at the bilateral renal cortices. There was no consanguinity within the patient's family, although her parents were from the same village.
16. Transfected cells were fixed and incubated with rabbit antibody to human catalase or rat catalase, as described [Y. Suzuki, S. Yamaguchi, T. Orii, M. Tsuneoka, Y. Tashiro, *Cell Struct. Funct.* **15**, 301 (1990)]. The antigen-antibody complex was detected with fluorescein isothiocyanate–labeled goat antibody to rabbit immunoglobulin G (Bio-Rad).
17. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
18. Human PAF-1 cDNA was isolated by colony-hybridization screening of a human liver cDNA library constructed in vector pcD2 [C. Chen and H. Okayama, *Mol. Cell. Biol.* **7**, 2745 (1987)]. The probe used was the 0.9-kb Pvu I I–Eco RI fragment of rat PAF-1 cDNA that had been labeled with ^{32}P-labeled deoxycytidine 5′-triphosphate (Amersham) with a random primer labeling kit (Takara Shuzo, Kyoto, Japan). The colony membranes were washed twice sequentially with 2× saline sodium citrate (SSC) and 0.1% SDS for 15 min at room temperature, and with 1× SSC and 0.1% SDS for 15 min at 65°C. Inserts were excised from the vector with Bam HI and subcloned into Bluescript SK(−) (Stratagene). Overlapping deletions in both orientations and nucleotide sequencing were done as described (*8*).
19. Total RNA was isolated from cultured skin fibroblasts by the guanidinium thiocyanate method [J. M. Chirgwin, A. E. Przybyla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1979)]; polyadenylated [poly(A)$^+$] RNA was prepared with oligo(dT)-Latex (Nihon Roche, Tokyo) according to the manufacturer's procedure.
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22. Genomic DNA was prepared by standard procedure from cultured skin fibroblasts from the control, the patient MM, and the blood of the patient's parents [J. Sambrook, E. F. Fritsch, T. Maniatis, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, ed. 2, 1989)]. Primers used to amplify genomic DNA fragments near the mutation site were at nucleotides 321 through 339 and 372 through 391.

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Selective Transmission of Human Immunodeficiency Virus Type–1 Variants from Mothers to Infants

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Multiple human immunodeficiency virus type–1 sequences from the V3 and V4-V5 regions of the envelope gene were analyzed from three mother-infant pairs. The infants' viral sequences were less diverse than those of their mothers. In two pairs, a proviral form infrequently found in the mother predominated in her infant. A conserved N-linked glycosylation site within the V3 region, present in each mother's sequence set, was absent in all of the infants' sequence sets. These findings demonstrate that a minor subset of maternal virus is transmitted to the infant.

A HIGH DEGREE OF GENETIC VARIability in human immunodeficiency virus type–1 (HIV-1) can be found within infected individuals (*1*). Variants arise during retroviral replication by error-prone reverse transcription (*2*). The complex mixture of variants in an infected individual (*3*) may be the result of immunologic pressure for change (*4*), alterations in cell tropism (*5*), and replication efficiency (*6*) among the variants. We analyzed the distribution of distinguishable genotypes transmitted between mother and child to investigate the role of selection in perinatal HIV-1 transmission, a route of infection estimated to occur in 13 to 30% of infants born to HIV-1–infected mothers (*7*).

Sequence sets were examined from one sample of peripheral blood from three mothers and their 2- to 4-month-old infants (*8*). We extracted DNA directly from mononuclear cells and amplified it by polymerase chain reaction (PCR) using nested primer pairs (*9*). The primer sets flanked both the V3 region containing the immunodominant loop (*10*) and the V4-V5 regions that encompass a portion of the CD4-binding domain of the viral envelope gene (*11*). Product DNAs from each individual were cloned, and 5 to 27 clones from each sample were sequenced (*12*).

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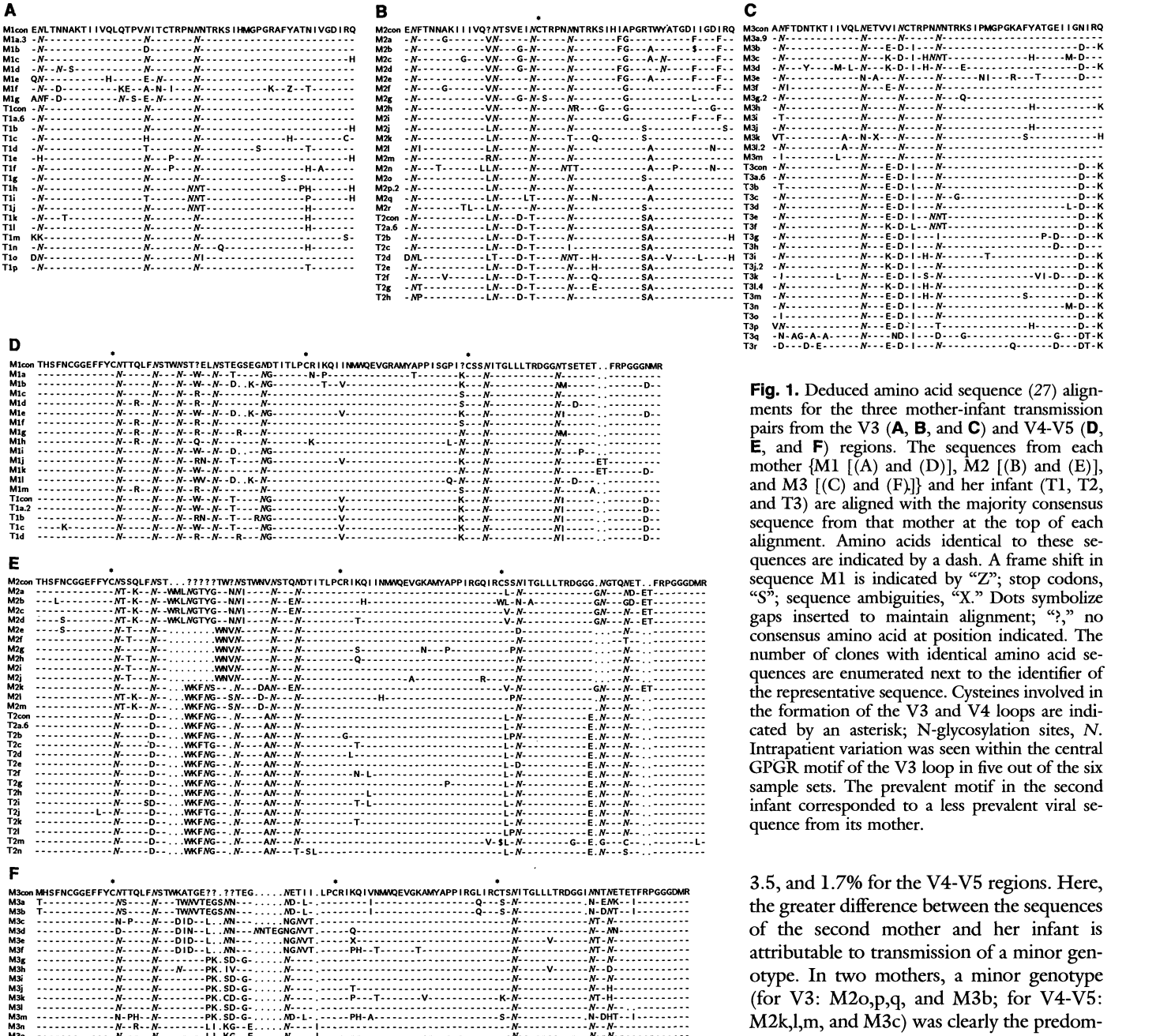

Fig. 1. Deduced amino acid sequence (*27*) alignments for the three mother-infant transmission pairs from the V3 (**A**, **B**, and **C**) and V4-V5 (**D**, **E**, and **F**) regions. The sequences from each mother {M1 [(A) and (D)], M2 [(B) and (E)], and M3 [(C) and (F)]} and her infant (T1, T2, and T3) are aligned with the majority consensus sequence from that mother at the top of each alignment. Amino acids identical to these sequences are indicated by a dash. A frame shift in sequence M1 is indicated by "Z"; stop codons, "S"; sequence ambiguities, "X." Dots symbolize gaps inserted to maintain alignment; "?," no consensus amino acid at position indicated. The number of clones with identical amino acid sequences are enumerated next to the identifier of the representative sequence. Cysteines involved in the formation of the V3 and V4 loops are indicated by an asterisk; N-glycosylation sites, *N*. Intrapatient variation was seen within the central GPGR motif of the V3 loop in five out of the six sample sets. The prevalent motif in the second infant corresponded to a less prevalent viral sequence from its mother.

The coding potential of the envelope open reading frame was maintained in most sequences (Fig. 1) (*13*). Cysteines at positions 296, 385, and 418 (numbered according to the HIV-1 HXB2 clone), involved in disulfide bridge formation of the V3 and V4 region loops (*14*), were perfectly conserved, while cysteine 445 was slightly variable. Inactivating mutations, seen in both the V3 and the V4-V5 regions, occurred only three times (one frame shift and two stop codons) in 41,500 bases, a frequency per nucleotide similar to some (*15*) but not all (*16*) estimates of error rates.

Viral sequences of epidemiologically unlinked mothers and infants differed from selected sequences from the Human Retroviruses and AIDS Database by 10 to 17.3% for V3 and 6.2 to 12.8% for V4-V5, differences comparable to those for other epidemiologically unlinked individuals (*1, 13*). In contrast, the consensus viral sequences from linked transmission pairs differed by 0.5, 6.1, and 3.6% for the V3 region, and 1.7, 3.5, and 1.7% for the V4-V5 regions. Here, the greater difference between the sequences of the second mother and her infant is attributable to transmission of a minor genotype. In two mothers, a minor genotype (for V3: M2o,p,q, and M3b; for V4-V5: M2k,l,m, and M3c) was clearly the predominant variant in their infants (Fig. 1). Comparisons of each pair's sequences showed that specific sequences were highly conserved between each mother and her infant with minimum differences of 0, 3.1, and 0% for the 157 nucleotides (nt) in V3, and 1.7, 2.3, and 0.3% for the 298 nt in V4-V5. Phylogenetic cluster analysis performed separately on all V4-V5 sequence sets supports the observation that the prevalent genotype in the infant was derived from a single form present in its mother (*17*). There was no evidence for transmission of multiple genotypes to the infants.

Despite the conservation of selected sequences between the mothers and their infants, dramatic variation was found among sequences within these sets. In the V3 region, the range of sequence differences be-

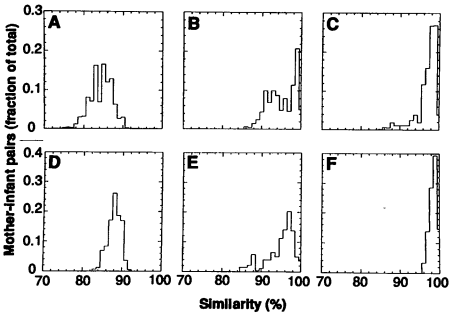

Fig. 2. Distributions of nucleotide sequence similarities from mother-infant pairs. (**A** and **D**) Interpatient sequence similarities between the mothers' sequence sets. (**B** and **E**) Mothers' intrapatient sequence similarities. (**C** and **F**) Intrapatient sequence similarities within the infants' sequence sets. (A), (B), and (C) are comparisons of the V3 region; (D), (E), and (F) are comparisons of the V4-V5 regions. For each comparison, percent similarity (*13*) is rounded off, and the fraction of the total number of mother-infant pairs with a given similarity is represented as a histogram. For intrapatient analysis, each sequence within a set was compared to every other sequence in that set; for interpatient analysis, all sequences from each set were compared to all sequences from the other relevant sets. (A), 941 total comparisons; (B), 490; (C), 639; (D), 585; (E), 276; and (F), 231.

tween the mothers and their infants was 0 to 28%, and the range of nucleotide sequence differences in the V4-V5 regions was 0.3 to 15%. Viral sequence similarity expressed as histograms also shows that sequence diversity within a sample was greater for mothers than for infants (Fig. 2). Although the mothers' sequences varied up to 15% in the V4-V5 regions (range, 85.4 to 100%; quartiles, 93.2 to 97.3%; median, 96.0%), the infants' sequences varied no more than 5% (range, 95.6 to 100%; quartiles, 97.7 to 98.9%; median, 98.7%) (Fig. 2, E and F). The range of the mothers' V3 sequence variation (range, 86.3 to 100%; quartiles, 91.9 to 98.7%; median, 95.3%) was comparable to the infants' (range, 85.1 to 100%; quartiles, 96.3 to 98.8%; median, 97.5%), although the distribution of genotypes from the mothers showed more variability. When we analyzed their viral sequences individually we found most of the infants' V3 sequences differed from one another by less than 4%, with a few differing up to 15% (Fig. 2). Moreover, length polymorphism resulting from insertions, deletions, and duplications was common in each mother's V4-V5 sequence set but absent in each infant's (Fig. 1). The infants' narrow distribution of variants may reflect the brief period between transmission and sampling or viral growth under limited immunologic selection pressure (*18*).

The N-linked glycosylation site [N-X-T or N-X-S sequons (*19*)] proximal to the first cysteine of the V3 loop (amino acid position 295) was strikingly absent from all three of the infants' sequence sets (Fig. 1). It was present in 2 out of 10 sequences from the first mother, 18 out of 19 sequences from the second mother, and 20 out of 23 sequences from the third mother. This site is highly conserved among isolates in the Human Retroviruses and AIDS Database, being present in 27 out of 29 sequences and absent only in two African sequences, ELI and Z321 (*1*). Although the other N-linked glycosylation sites were fairly well conserved within a given patient sample, these sites varied in both number and position between patient samples (Fig. 1). N-linked glycosylation can contribute to the formation of conformational and obscure linear epitopes (*20*) and can influence cell tropism and host range in several viral systems (*19*).

The ratio of synonymous to nonsynonymous substitutions (P_s/P_n) (*21*) in V3 (0.61, 1.90, and 0.90 for the mothers' sequences and 1.3, 0.2, and 1.1 for the infants') and in V4-V5 (0.70, 1.10, and 0.83 for the mothers' sequences and 2.0, 1.7, and 2.2 for the infants') suggests that there is positive selection for change in these regions. The P_s/P_n ratios are low for the envelope gene (<1) compared to the gag gene (6.7) (*15*) and the average (5.3) over a number of mammalian genes (*22*). The lower P_s/P_n ratios for V3 relative to V4-V5 in the infants, as well as the small subset in V3 of divergent sequences (up to 15%) (Fig. 2), suggest that the infants' V3 region is under greater selective pressure for change than the V4-V5 regions. Because the samples were collected from infants at 2 to 4 months of age, before they had developed antibodies (*23*), the presence of maternal antibodies in the infants may be responsible for the difference in selection pressure.

Our data are compatible with several alternative hypotheses. (i) Each infant's viral sequence is derived from an antigenically distinct variant arising in its mother and escaping a critical immune surveillance mechanism during gestation. Amino acid substitutions in B cell (*4, 24*) or cytotoxic T cell (*25*) epitopes can abrogate immune recognition. These escape variants would have a survival advantage that may facilitate their transmission. (ii) The infants' viral sequences are acquired by random transmission of a limited number of virions earlier in gestation (*26*). At transmission this variant could be a major form in a mother but, because of genetic evolution, becomes a minor form by the time of sampling. (iii) The persistent forms of the virus in the infant reflect differences in cell tropism or replicative capabilities relative to the variants recovered from their mothers.

Although each alternative is possible, the relatively narrow distribution of variants and the defined pattern of N-linked glycosylation sites in the infants' sequences support the hypothesis that transmission can involve selection. An effective strategy for vaccine development and immunoprophylaxis may require a diverse array of recognizable epitopes to block viral transmission.

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Cytokine Stimulation of Multilineage Hematopoiesis from Immature Human Cells Engrafted in SCID Mice

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Severe combined immunodeficient (SCID) mice transplanted with human bone marrow were treated with human mast cell growth factor, a fusion of interleukin-3 and granulocyte-macrophage colony-stimulating factor (PIXY321), or both, starting immediately or 1 month later. Immature human cells repopulated the mouse bone marrow with differentiated human cells of multiple myeloid and lymphoid lineages; inclusion of erythropoietin resulted in human red cells in the peripheral blood. The bone marrow of growth factor–treated mice contained both multipotential and committed myeloid and erythroid progenitors, whereas mice not given growth factors had few human cells and only granulocyte-macrophage progenitors. Thus, this system allows the detection of immature human cells, identification of the growth factors that regulate them, and the establishment of animal models of human hematopoietic diseases.

THE HEMATOPOIETIC SYSTEM IS ORganized as a hierarchy, ranging from large numbers of mature differentiated cells to rare pluripotent stem cells capable of extensive self-renewal and differentiation (*1*). Much of our knowledge of the organization and regulation of the hematopoietic system is derived from studies in the mouse where short- and long-term reconstitution assays are available to detect stem cells (*1, 2*). In contrast, our understanding of the biology of the human hematopoietic system is less complete because of a lack of in vivo stem cell assays. Several different approaches for engrafting human hematopoietic cells into immune-deficient mice have been described that employ transplantation of adult bone marrow, mature lymphoid cells, or fetal organs (*3*). These mouse models have already been used for studying a variety of human diseases including leukemia, autoimmunity, and infectious diseases (*3*). However, analysis of the normal developmental program of human hematopoiesis has been limited because engraftment of the murine tissues is low and only lymphoid or macrophage lineages develop.

We have described a system closely modeled on conventional bone marrow transplantation assays employing intravenous injection of adult human bone marrow into immune-deficient mice conditioned with radiation (*4*). Human macrophage progenitors migrate to the murine marrow, increase in number, and are maintained in this environment for several months; however, no mature cells are detected (*4, 5*). Other studies also indicate that the microenvironment of irradiated mice can support the growth of normal human lymphoid cells (*6*) and primary leukemic cells and cell lines (*7*). Human progenitor cells (*8*) and some leukemic cell lines (*9*) can also be maintained on murine stromal cells in vitro.

Because hematopoiesis is regulated by many growth factors, the low engraftment and the absence of multilineage differentiation in immune-deficient mice transplanted

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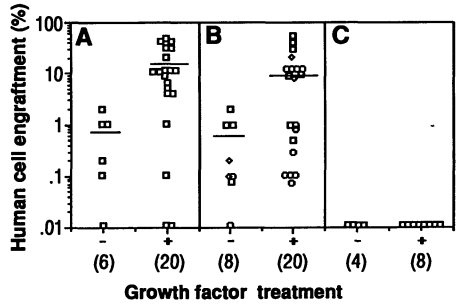

Fig. 1. Summary of the DNA analysis of the bone marrow of SCID mice transplanted with human hematopoietic cells with and without human growth factor treatment. The extent of human cell engraftment of the bone marrow was estimated as described (*13*). The results of 11 different experiments are presented. The horizontal line indicates the mean level of human cells from the number of mice shown (*n*) that were treated with individual or combinations of growth factors (+) or left without growth factors (−). (**A**) Sublethally irradiated SCID mice transplanted with human bone marrow and treated with growth factors for 30 to 40 days. (**B**) Sublethally irradiated SCID mice transplanted with human bone marrow, left untreated for 30 days, then treated with growth factors for an additional 30 days. Mice were transplanted with bone marrow cells from three different donors indicated by the different symbols. (**C**) Sublethally irradiated SCID mice transplanted with ficoll purified human PBLs or RBCs (4×10^7) and treated with growth factors for 21 to 30 days. No human cells were detected in the bone marrow of these mice.