Viral and Immunologic Examination of Human Immunodeficiency Virus Type 1–Infected, Persistently Seronegative Persons

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Persons who were human immunodeficiency virus type 1 (HIV-1)–infected but who remained persistently seronegative (HIPS) on HIV-1 antibody tests were examined through AIDS case surveillance. Six such individuals (HIPS-1 to -4, -7, and -9) were examined to determine whether their persistent seronegativity was attributable to immune dysfunction or infection with atypical HIV. Of the 6, 4 had antibody titers to at least 1 other common pathogen. In vitro stimulation of peripheral blood mononuclear cells from HIPS-4 and HIPS-7 with pokeweed mitogen or phosphorothioate oligodeoxynucleotide (direct B cell mitogen) did not produce HIV-1–specific antibody. Reconstitution experiments with recombinant interleukin (rIL)-4 and rIL-12 also had no impact on antibody production. Virus isolates from HIPS-4 and -9 were R5X4-tropic, whereas HIPS-7 was CCR5-tropic only. Sequence analysis of long terminal repeat, p24, and env gp41 did not reveal any specific mutation, and phylogenetic analysis confirmed that all 6 virus specimens were HIV-1 subtype B. These data suggest that the lack of a detectable antibody response in these patients may be the result of immune dysfunction.

Current methods for detecting human immunodeficiency virus type 1 (HIV-1) infection are based on serologic testing of blood specimens for viral antibodies and antigens. However, blood from HIV-infected persons may be negative by EIA and Western blot for several reasons, including both host and viral factors. During the “window period” of early HIV infection, detectable levels of HIV antibody have not yet developed. This window period is typically 20–25 days but varies depending on the specific EIA reagents used [1]. Blood donations made by recently infected donors have resulted in transfusion-related HIV transmission. To address this problem, HIV p24 antigen testing of all donated blood was implemented in 1996 [2]. The detection of plasma antigenemia permits earlier detection of HIV-1, thus virtually eliminating the risk of transmitting HIV from blood donations.

In addition to the window period of early HIV infection, 2 categories of HIV-infected but seronegative persons have been reported. First, several studies have established that a subset of individuals who seroconvert to HIV lose their antibody response to HIV-1 as the disease progresses, presumably because of a loss of T cell help [3–6]. Second, a minor subset of HIV-infected persons who rapidly progress to AIDS never develops a B cell response because their primary infection is so fulminant [7]. Among the various host factors that may explain seronegative HIV infection are hypoglobulinemia, B cell function defects, and iatrogenic factors, such as immunosuppression related to the administration of chemotherapeutic agents [8, 9]. Virologic factors include infection with highly divergent HIVs (groups O and N) that may not be detectable by standard EIA. These infections can cause false-negative serologic results due to significant diversity in the immunodominant epitopes of the envelope protein. Some of the problems associated with group O detection have been eliminated by the addition of group O–specific peptides in the screening tests [10].

In contrast to typical antibody detection among individuals infected with HIV-1, there have been a few reports of patients with a documented history of negative HIV-1 serology despite HIV infection and AIDS symptoms [11–13]. Recently, we identified several such persons who had AIDS-defining symptoms and virally detectable HIV infection but who remained per-
sistently seronegative for HIV-1 antibody during the course of their clinical care [14]. Although rare, such HIV-1–infected seronegative cases represent a unique opportunity to try to understand the basis of the lack of HIV-specific antibody response.

Material and Methods

Study cases. The case definition of persons who are HIV-infected who remain persistently seronegative (HIPS) has been described elsewhere [14]. HIPS describes a person who has HIV-1 infection, documented by positive p24 antigen, DNA and/or RNA polymerase chain reaction (PCR), or viral culture; at least 2 negative HIV-1 antibody screening test results from independent laboratories on 2 different specimens collected on different dates (with the exception in our study of HIPS-9, for whom only one time point was available but who had late-stage HIV disease); and the presence of at least one AIDS-defining opportunistic illness [15] at the time the samples were collected. Because of the latter requirement, recently infected patients who have negative screening results because they have not yet produced detectable antibody are excluded. There is usually an interval of several years between HIV infection and seroconversion and the development of an AIDS-defining opportunistic illness [16]. Cells and plasma from 6 HIPS cases (HIPS-1 to -4, -7, and -9) were available for the present study. The case histories and clinical presentations of these individuals have been described elsewhere, and matching numbers (e.g., case 1 is HIPS-1) were established for continuity [14].

Antibody detection. Each plasma sample was screened for anti–HIV-1 antibody with an EIA kit (Genetic Systems, Redmond, WA) according to the manufacturer’s instructions. Antibodies to the immunodominant region of env gp41 were examined by use of a peptide-based assay. In brief, a 44mer consensus group M env gp41 peptide, WGIKQLQARVLAVERYLKDQQLLGIWGCSGKLICTTAVPWNASW, was coated at 5 μg/mL to 96-well plates (Immunlon 2; Dynatech, Chantilly, VA) at 4°C for 24 h. After blocking of each sample, serum was added (1:100 dilution), and samples were incubated for 18 h, and then alkaline phosphatase–conjugated human antibodies were added. The cutoff was determined as the mean + 3 SD of 10 HIV-1–negative control sera.

An HIV-1 Western blot (Cambridge Biotech, Worcester, MA) was also done according to manufacturer’s instructions. Plasma aliquots were treated separately, to disrupt immune complexes by association reagent (Vironostika HIV-1 Antigen Base Dissociation; Organon Teknika, Durham, NC) as specified. Each plasma sample was also screened for HIV-1–specific IgA antibodies by use of an affinity-purified, biotin-conjugated goat anti-human serum IgA at 1:1000 (ICN, Aurora, OH).

Antigen-capture assay and plasma HIV-1 RNA levels. Each plasma sample was screened for HIV-1 p24 antigen with an EIA kit (Coulter, Hialeah, FL) according to the manufacturer’s instructions. HIV-1 RNA in each plasma sample was quantified by use of the Amplicor HIV-1 Monitor test kit (Roche, Branchburg, NJ) as instructed. Results are reported as HIV-1 RNA copies per milliliter.

In vitro proliferation and antibody production. Peripheral blood mononuclear cells (PBMC) from each sample were cultured in triplicate at 10^6 cells/mL in 96-well round-bottom microtiter plates. Cultures were incubated for 144 h at 37°C in a 7% CO2 humidified atmosphere. Cells were cultured in the absence (control) or presence of pokeweed mitogen (PWM; 1:500; Gibco, Gaithersburg, MD) or phosphorothioate oligodeoxynucleotide (1 μg/mL). Medium used throughout these studies consisted of RPMI 1640 supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), 2 mM L-glutamine, and 10% (v/v) heat-inactivated fetal calf serum (FCS). After 144 h, 125 μL of supernatant was removed from each well, and triplicates were pooled. All cultures received a terminal 16-h 0.5 μCi/well pulse of tritiated thymidine ([3H]Tdr; New England Nuclear Life Sciences Products, Boston, MA). Cultures were harvested on glass-fiber filters that were processed for liquid scintillation counting (Beckman Instruments, Fullerton, CA). Results are presented as net counts per minute.

In vitro antibody production was measured by HIV-1 EIA according to the manufacturer’s instructions. Results (optical density) are presented as sample/cutoff. Detection of gag p24 antigen production was also measured from the collected supernatants by HIV-1 p24 antigen EIA (Coulter) according to the manufacturer’s instructions.

Virus isolation and infection studies. Virus isolates were established by cocultivation of patient PBMC with phytohemagglutinin–stimulated CD8 cell–depleted uninfected donor PBMC. Culture supernatants were collected, filtered through 0.22-μm filters, tested for reverse transcriptase (RT) activity, and, if RT positive, aliquoted, and stored at −70°C. Cell culture–adapted T-tropic (LAI) and M-tropic (BAL) laboratory isolates of HIV-1 were used as controls.

Infection of GHOST-4 cells coexpressing various chemokine receptors. GHOST-4 cells (kindly provided by Dan Littman, NYUMC, NY), which are HOS cells transduced with the human CD4 gene, maintained by G418 selection and further modified by introduction of the various coreceptor genes by infection with the pBABEpuro vector, were utilized to further elucidate specific coreceptor usage by HIV-1 primary isolates. The GHOST-4 cells expressing CXCR4, CCR5, CCR4, CCR3, CCR2B, CCR1, BOB, and BONZO were maintained in Dulbecco’s modified Eagle medium (DMEM) with 10% FCS, 1 μg/mL puromycin, 100 μg/mL hygromycin B, and 500 μg/mL G418. The media for the parental GHOST-4 cells did not contain puromycin. The GHOST-4 cells were plated at 4 × 10^4 cells/well in 24-well plates and infected with 40,000 RT counts of virus stock, as described elsewhere [17]. All cultures were washed 3× with 1.5 mL of PBS after a 6- to 18-h incubation with virus. The cultures were maintained in 2 mL of the DMEM described above. Supernatants (1 mL) were collected from the cultures every 3–4 days and tested for p24 antigen (Coulter).

PCR. HIV-1–specific RT–PCR was done on total RNA recovered from plasma samples. RNA was prepared from 200 μL of plasma by use of the QIAamp blood kit (QIagen, Chatsworth, CA) according to the manufacturer’s protocol. After extraction, RNA was reconstituted in diethylpyrocarbonate-treated water. Thermal cycling conditions for RT–PCR were as follows: 42°C, 15 min for 1 cycle; 99°C, 5 min for 1 cycle; and 5°C, 5 min for 1 cycle. All 20 μL of the cDNA reaction from the RT step was added to the DNA PCR. Thermal cycling conditions for PCR were as follows: 95°C, 2 min for 1 cycle; denaturation, 95°C, 30 s; annealing, 55°C, 30 s;
extension 72°C, 1 min for 35 cycles; and 72°C, 7 min for 1 cycle. gag p24 and env gp41 immunodominant-region PCR amplification were done as described elsewhere [18]. The standard primers for env gp41 immunodominant-region amplification were as follows: RT–PCR, JH41 (5′-CAGCGGAGCACKTATG; nt 7815–7833, according to HIV-1MN) and JH38 (5′-GGTGARTATCTCCTKCAAC; nt 8322–8343); nested PCR env57F (5′-CTGGYATAGTGCARCARCA; nt 7878–7896) and Menv19R (5′-TGCCTKCCTAAC; nt 8382–8363); nested PCR p24#2 (5′-TGTGWAGCTTGYTCRGCTC; nt 1727–1709) and p24#3 (5′-AGRACYTTRAAYGCATGGGT; nt 1243–1262) for env gp41 immunodominant-region PCR amplification were as follows: first round PCR, p24#1 (5′-AGYCAAAATTAYCCYATAGT; nt 1180–1199) and p24#7 (5′-CCCTGCRATGCTGTCATCA; nt 1849–1832); nested PCR p24#2 (5′-AGRACYTTRAAYGCATGGGT; nt 1243–1262) and p24#5 (5′-TGTGWAGCTTGYTCRGCTC; nt 1727–1709).

The standard primers for nef and long terminal repeat (LTR) sequencing Reaction Kits (Perkin Elmer, Foster City, CA) with AmpliTaq DNA polymerase FS and an automated sequencer (ABI model 370; Applied Biosystems, Foster City, CA). Direct sequencing of PCR-amplified fragments represents a consensus sequence or the predominant virus species present. Sequence data were analyzed by use of Sequencher 3.0 software (Gene Codes, Ann Arbor, MI). Nucleic acid sequences were trimmed to equivalent lengths and aligned with representative HIV-1 group M and group O subtypes available in the Los Alamos database. The neighbor-joining method included in the Phylip 3.5c software package (University of Washington, Seattle) was used to construct the HIV-1 gp41 phylogenetic tree. Six DNA sequences were submitted to GenBank; each strain name is followed by the GenBank accession number: HIPS-1, AF157471; HIPS-2, AF157470; HIPS-3, AF157472; HIPS-4, AF157469; HIPS-7, AF157468; and HIPS-9, AF157473.

Results

Persistent seronegativity by Western blot analysis. As of January 1998, through AIDS case surveillance, we had identified 9 HIPS cases with clinical AIDS. A detailed analysis of 6 cases was undertaken to identify a reason for the lack of an HIV-specific antibody response. All 6 HIPS cases had low CD4 cell counts and various opportunistic infections [14]; however, all were p24-antigen positive and had plasma virus loads ranging from 17,000 to >4 million copies/mL (table 1). Because of the p24 antigenemia, it is possible that weak antibodies might be complexed as immune complexes. We performed Western blot analysis of HIPS samples to detect IgG antibodies before and after immune-complex dissociation after basic treatment of plasma. Western blot analysis revealed that 3 of 6 HIPS samples had weak p24 antibody reactivity before dissociation (HIPS-1, -4, and -9). Immune-complex dissociation treatment of plasma from the 3 predissociation-negative specimens demonstrated that 1 (HIPS-2) had weak p17 antibodies only, 1 (HIPS-3) had weak p17 and p24 antibodies, and the other sample (HIPS-7) had weak p24 reactivity. Thus, although all 6 samples had weak gag antibodies either before or after dissociation, none revealed any unmasked antibodies to envelope proteins. Recent studies have shown that some persons who are highly exposed to HIV-1 but who remain persistently seronegative have IgA HIV-1-specific antibodies [19]. None of the HIPS specimens revealed any HIV-1-specific IgA responses in the plasma (table 1).

In vitro antibody production. We next examined the HIPS plasma samples for antibodies reactive to the immunodominant region of env gp41 by peptide-based EIA. None of the HIPS patients had detectable antibody to env gp41 group M-specific peptide (table 1), whereas all HIV-1–positive controls, representing group M subtypes A–G, had detectable antibody to this peptide (data not shown). Furthermore, the lack of HIV-1–specific antibodies in these samples was not due to an inherent inability to mount an antibody response because most had detectable antibody titers to other common pathogens [14].

In vitro antibody production. We next examined in vitro antibody production in response to various mitogens, such as PWM (T dependent) or phosphorothioate oligodeoxynucleotide mitogen (Oligo30; T-independent B cell mitogen) in the presence or absence of recombinant interleukin (rIL)-4 and rIL12. To validate the assay, we used PBMC from an HIV-1–infected long-term nonprogressor (LTNP; >14 years of in-
In vitro T cell proliferative responses (A, C, E) and antibody production (B, D, F) in human immunodeficiency virus (HIV)–infected, persistently seronegative (HIPS) persons; HIV-infected, long-term nonprogressor (LTNP); and HIV type 1 (HIV-1)–seronegative person (control). Peripheral blood mononuclear cells were cultured in absence or presence of pokeweed mitogen (PWM), with and without recombinant interleukin (rIL)-4 and rIL-12, for 144 h. Supernatants were collected for HIV-1–specific antibody production prior to terminal 16-h pulsing with tritiated thymidine. Data are reported as net cpm for proliferation (A, C, E) and index (S/C, sample over cutoff) for HIV-1–specific antibody production (B, D, F).
Figure 2. Replication characteristics and coreceptor usage of human immunodeficiency virus–infected, persistently seronegative (HIPS) virus isolates. Primary peripheral blood mononuclear cells (PBMC) and PM-1 and GHOST-4 cell lines transfected with CD4 and various chemokine receptors (CCR-1, CCR-2b, CCR-3, CCR-4, CCR-5, CXCR-4, BONZO, and BOB) were infected with viral stocks generated from HIPS patients or from laboratory-adapted LAI strain. Infection was monitored by p24 production for up to 14 days. Data are expressed as p24 production (ng/mL) on day 8 of infection.

These PBMC demonstrated proliferative responses to PWM and PWM plus rIL-4 and rIL-12 (figure 1A). A parallel analysis of culture supernatants from the LTNP demonstrated the presence of HIV-1–specific antibodies (figure 1B). As expected, an HIV-negative control specimen did not reveal HIV-specific antibody production (figure 1B). Likewise, in vitro stimulation with T-independent antigen, Oligo-30, resulted in HIV-1–specific antibody production by the LTNP, although no T cell proliferative responses were detectable (data not shown). A similar analysis of HIPS-4 and HIPS-7 demonstrated proliferative responses to PWM (figure 1C, E); however, no antibody could be detected in the culture supernatants (figure 1D, 1F). No PBMC were available to complete a similar analysis on the other HIPS samples. Reconstitution experiments with rIL-4 and rIL-12 also failed to induce HIV-1–specific antibodies, although a marked increase in proliferation was observed (figure 1). The inability to detect antibodies in the culture supernatants was not due to immune complexes because none of the stimulation conditions induced detectable p24 production (data not shown).

Coreceptor specificities of HIPS isolates. We next examined the replication characteristics and coreceptor specificities of virus isolates derived from HIPS cases. Isolates derived from HIPS-4, -7, and -9 are replication competent and infect both human PBMC and the PM-1 cell line (figure 2). HIPS-4 and -9 are syncytium-inducing isolates, whereas HIPS-7 is nonsyn-
cytium inducing. Analysis of GHOST-4 cell lines coexpressing various coreceptors demonstrated that HIPS-7 can utilize only CCR5, whereas HIPS-4 and -9 can utilize both CCR5 and CXCR4 (figure 2). None of the isolates could utilize CCR1, CCR2b, CCR3, or CCR4, but HIPS-4 could use BOB (GPR15) and BONZO (STRL33; figure 2).

**Virologic characteristics of HIPS.** Sequence analysis of the partial genome was done to define any unique characteristic of the structural and functional gene regions (gp41, p24, and integrase) or regulatory gene regions (nef, LTR) that may reveal infection with atypical HIV-1 that escapes immune detection. env gp41 sequences were derived from viral RNA that was recovered from plasma from HIPS cases. We recently established that phylogenetic analysis of env gp41 provides a reliable region to conduct subtyping of HIV-1 [20]. Phylogenetic analysis of HIPS-derived gp41 sequences indicated that they all clustered with HIV-1 subtype B (figure 3). Sequence divergence among the 6 HIPS samples ranged from 8% to 14.5%, which is within the expected distribution range for subtype B (6%–16%). Analysis of the translated DNA sequences of the env immunodominant region in the gp41 revealed conservation of the AVERY peptide, cysteine loop region, and three conserved tryptophans and maintenance of the glycosylation site in cluster I; and conservation of the ectodomain region, 2 conserved tryptophans, and maintenance of the glycosylation site in cluster II (figure 4A). All strains were very similar to 2 prototype HIV-1 subtype B strains, HIV-1MN and HIV-1HXB2, and the consensus subtype B sequence. Likewise, no specific mutation could be identified in gag p24 (figure 4B) or in integrase (data not shown) when compared with consensus subtype B sequence.

A comparative analysis of the LTR of HIPS-2, -7, and -9 with HIV-1MN and consensus subtype B revealed no variation in the many enhancer or promoter elements reported to influence viral gene expression, including nuclear factor of activated T cells, upstream stimulatory factor, T cell–specific transcription factor–1α, NF-κB, TATA box, Sp1, and TAR/Initiator complex (figure 5). As expected, the 3 samples from HIPS had 2 copies of the NF-κB enhancer similar to that for the B subtype consensus [21]. Furthermore, the TAR 3-bp bulge and 6-bp loop were conserved. Analysis of vpr and nef (HIPS-2, -3, -7, and -9) showed no common substitution or deletion differing from HIV-1MN and HIV-1HXB2 (data not shown). Taken together, these data suggest that the samples from HIPS had no specific changes in the structural or regulatory regions examined that could explain lack of antibody responses.

**Discussion**

We and others have previously described several HIV-1–infected patients with AIDS who remain persistently seronegative by antibody screening tests [7, 11–14, 22, 23]. In this report, we describe a detailed analysis of virologic and immunologic immune responses in 6 persons who were infected with HIV-1, had low CD4 cell counts, and presented with several opportunistic infections despite having a documented history of persistently negative results on Food and Drug Administration–approved, licensed EIA kits for antibodies to HIV-1 through the late stage of HIV infection. The most plausible
A. gp41

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Figure 4. Amino acid sequence alignment of envelope gp41 (A) and gag p24 (B) of 6 human immunodeficiency virus–infected, persistently seronegative (HIPS) strains. Amino acid identity between sequences is represented by dot, and deviations from subtype B consensus are indicated. A, In cluster I of gp41, 3 conserved tryptophans, immunodominant region, cysteine loop, and glycosylation site are highlighted. In cluster II, glycosylation site, ectodomain region, and 2 conserved tryptophans are highlighted. B, Amino acid alignment of gag p24. 5' gag p24 cleavage site is 34 amino acids upstream, as indicated.

Explanation for lack of antibody (i.e., window-period infection) was excluded on the basis of longitudinal specimens all being seronegative and the late stage of HIV disease.

Analysis of antibodies to specific HIV-1 structural proteins revealed that 3 of the 6 specimens from HIPS cases had weak antibodies to p24 only, whereas none revealed antibodies to the envelope glycoprotein. This reactivity to p24 may be nonspecific since cross-reactivity to gag p24 in the general population (blood donors) has been observed by others [24, 25]. Previous studies have also established the loss of gag-specific antibodies late in disease [3–6], but in HIPS cases, no envelope-specific antibody could be detected as well, suggesting the absence of antibody rather than the loss of HIV-specific antibodies. Because all HIPS patients had high plasma viremia (on the basis of p24 levels and virus load), we hypothesized that HIV-specific antibodies may be masked by immune complexes. However, basic dissociation of immune complexes revealed weak gag (p17 or p24) reactivity, and, once again, no specific antibody to envelope glycoproteins could be detected. Furthermore, an env gp41 peptide–based assay also failed to reveal env-specific antibodies in the plasma samples from HIPS patients. Likewise, no HIV-1–specific IgA antibodies were detectable in any of the HIPS cases.

It has been well established that the humoral immune response, specifically neutralizing antibody, plays an important role in HIV-1 infection [26, 27]. Therefore, the lack of an antibody response in HIPS may adversely affect HIV disease progress. For example, 3 of 6 previously reported HIPS cases, including HIPS-7 [7, 12, 13], had rapid clinical progression of HIV-1 disease, suggesting that either the lack of antibody production after primary infection led to quick onset of disease or the rapid progression of HIV disease led to no detectable antibody production. Previous studies of experimental infection of macaques with simian immunodeficiency virus have shown...
Figure 5. Alignment of long terminal repeat sequences from human immunodeficiency virus–infected, persistently seronegative (HIPS) samples. Nucleotide identity between sequences is represented by dot, and deviations from subtype B consensus are indicated. Blackened box indicates that no nucleotide was identified. Enhancer and promoter elements are indicated on top of highlighted boxes. USF, upstream stimulatory factor.
that, although infrequent, some animals do not seroconvert but rapidly progress to AIDS and death [28–30]. We were unable to ascertain the source or spread of infection for most of the HIPS patients; therefore, duration from infection to the onset of AIDS could not be discerned.

In vitro antibody production from 2 samples from HIPS cases failed to reveal production of HIV-1–specific antibodies, whereas PBMC from an HIV-1–infected LTNP revealed antibody production. Reconstitution experiments with rIL-4 and rIL-12 also failed to induce antibody production in HIPS cases, although both patients had marked proliferative responses to mitogen in the absence or presence of cytokines. Recently, it has been demonstrated that antigen-specific T cell responses correlate with disease status but are lost as the disease progresses [31]. Although no study has addressed in vitro–specific antibody production as it relates to disease, once the immune system has failed, maintaining antigen-specific T cell responses and T-dependent B cell responses would be difficult. In fact, in vitro antibody production by an HIV-1–infected patient who had been on antiretroviral therapy demonstrated no antibody production (data not shown), whereas an LTNP who has maintained T cell helper responses demonstrated HIV-1–specific antibody production. Because most of the HIPS cases are in the late stage of disease, their lack of antibody production may be explained by a lack of specific T helper cell activity resulting from the rapid depletion of CD4 cells or may simply reflect an immune system that is incapable of mounting an effective humoral immune response. Alternatively, the repeated exposure to HIV antigen may have led to the induction of B cell tolerance in these HIPS patients.

An imbalance in the Th1 and Th2 responses may also contribute to the immune dysfunction. Progression to AIDS is characterized by a loss of T helper function and IL-2 and interferon-γ production [32, 33]; also, there is concomitant B cell activation, humoral response augmentation, and increased production of IL-4 and IL-10. Because most of the HIPS samples had antibody titers to other common pathogens, no generalized humoral immune dysfunction was observed, even though 2 specimens (from HIPS-4 and -7) were HIV positive, had strong proliferative responses and CD4 cell levels <500/mL, lacked in vitro antibody production, and had no augmented humoral responses on reconstitution with rIL-4 and rIL-10 [14]. In addition, 1 patient (HIPS-1) demonstrated seroconversion within 2 weeks of initiation of antiretroviral treatment [14].

Sequence analysis of the partial genome and examination of cellular tropism of the virus isolates from the HIPS individuals demonstrated no unusual characteristics. Replication kinetics of virus isolates derived from 3 of the samples, including HIPS-7, were similar to that in normal human PBMC and the T cell line PM-1. Coreceptor usage analysis revealed that HIPS-7 utilized CCR5 only, whereas HIPS-4 and -9 were R5X4-tropic. The ability of primary virus isolated from HIPS-7 to utilize only CCR5 corroborates previous results in which an independent isolate from the same patient was shown to have the predominant nonsyncytium-inducing phenotype [7]. However, the exclusive CCR5-using phenotype of the HIPS-7 primary virus differs from what was expected for a rapid progressor [34, 35]. Such lack of coreceptor adaptation may be due to an unusual rapid disease progression (death occurred 10 months after initial presentation). As expected, late in disease with AIDS, HIPS-4 and -9 had broadly tropic R5X4-using viruses with a syncytium-inducing phenotype [34, 35].

Phylogenetic analysis of the env gp41 immunodominant region shows that the viruses of the HIPS individuals are HIV-1 subtype B, the dominant strain in the United States. Although recent studies have demonstrated that recombinant viruses may account for up to 10% of all HIV-1 genomes [36], this was not the case in our study: subtype B sequences were obtained from all regions of the genome. Moreover, the regulatory elements, which can play an important role in genetic divergence and pathogenic potential, do not differ from other HIV-1 B subtype sequences [21]. Taken together, these data strongly suggest that the lack of detectable antibody response in these cases was caused by a defect in the host immune system rather than by a viral characteristic. This hypothesis is further supported by the fact that the infected partner of HIPS-7 [14] and 2 other persons [11, 22] who were infected with the same virus strain as the seronegative partner had detectable antibody responses.

Progression to AIDS varies among infected individuals, and various immunologic and virologic mechanisms most likely account for the difference. For example, 2 HIPS patients (HIPS-7 and -8) had HIV-1–infected partners, and in each instance, the HIV-infected partner’s virus was similar to the that of the seronegative partner, linking the partners with the same virus. The virus was shown to be transmitted from one human host to another [12, 13], but there was a distinct contrast in the response of each host to the same virus, and this supports the remarkable nature of the virus in each instance. A dysfunctional immune response or the immunologic escape of viral variants may account for the inability of the HIPS patients to develop an antibody response to the viral antigens.

In conclusion, our findings demonstrate that the lack of a detectable antibody response may be a result of immune dysfunction. Whether the immune dysfunction is due to an imbalance in the Th1 and Th2-type responses, a selective loss of memory cells, a defect in the antigen-presenting cell, an induction of suppressor cells and factors, or an elaboration of cytokines that exhibit immunoregulatory properties is unknown.

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