Human immunodeficiency virus (HIV) primary infection is commonly associated with clinical symptoms. Seroconversion usually occurs within 2–4 months, which is followed by alterations in the immune system, including the progressive depletion of CD4 lymphocytes [1]. Serologic screening of HIV infection is highly sensitive (as shown in intravenous drug users, prostitutes, high-risk homosexuals, and hemophiliacs). HIV antibody responses often decline during the final stage of AIDS. However, immunosilent HIV-1 infection is extremely rare, and the persistent absence of an HIV-specific humoral response in HIV-infected subjects has been reported in only 3 cases [2–4]. We analyzed the clinical and laboratory findings of a woman resident of the Ivory Coast who died of AIDS but remained HIV-seronegative, even though she was demonstratively infected by clade A HIV-1.

### Subjects and Methods

#### Subjects.

A 38-year-old woman (IC2) was married to a man (IC1) known to have been HIV-seropositive since 1987. Both lived in Ivory Coast and reported unprotected sexual intercourse. Table 1 summarizes the serologic, virologic, and clinical features of subject IC2.

**HIV isolation in cell culture.** HIV isolation was done as described previously [5] using cocultivation of the patient's peripheral blood mononuclear cells (PBMC) or plasma with CD8-depleted cells from a healthy donor. Cells were stimulated using protein A (10 μg/mL) for 2 days and cultivated in RPMI medium with 10% fetal calf serum and 10% Tcell growth factor (Biotest, Dreieich, Germany). Virus isolations from IC1 and IC2 were never done at the same time to preclude the possibility of cross-contamination.

**Molecular detection and quantification of HIV.** HIV-1 RNA was quantified in plasma using quantitative reverse transcription–polymerase chain reaction (RT-PCR: Monitor HIV; Roche, Nutley, NJ) and bDNA hybridization (Quantiplex HIV; Chiron, Emeryville, CA) according to manufacturers' instructions.

PCR-based detection of HIV-1 was done with DNA either directly purified from isolated peripheral blood lymphocytes (PBL) or after in vitro amplification of HIV in the presence of PBL from a healthy donor. DNA was extracted using the QiAamp blood kit (Qiagen; Chatsworth, CA). Two nested PCR assays were used, one with a target within the \( \text{pol} \) gene and the other with a target within the \( \text{env} \) gene. For the \( \text{pol} \) gene assay, the outer primers were HPOL4235 and HPOL4538; the inner primers were HPOL4481 [6]. For the \( \text{env} \)-specific assay, the outer primers were \( 5'\text{V3CB} (5'-\text{GTACAATGTACACATGGAAT}-3') \) and \( 3'\text{V3CB} (5'-\text{GTAGAAAATT±CCCCTCCAC}-3') \), and the inner primers were \( 5'\text{J5CB} (5'-\text{TGTGAATGG±CAGTCTAGCA}-3') \) and \( 3'\text{J3CB} (5'-\text{TGGATTACAAATATTTCTGGG}-3') \). Thermal cycling conditions were as described by Fransen et al. [6], except that the annealing temperature was 55°C. Stringent precautions were taken to exclude any contamination with PCR-generated products.

**Sequence determination of PCR products.** PCR products were cloned using TA cloning (Invitrogen, San Diego). Several transformants were selected, and both strands of DNA plasmids were sequenced using a 373A DNA automatic sequencer (Applied Biosystems, Foster City, CA). Representative sequences were analyzed by use of Genetics Computer Group (Madison, WI) software.

**Ex vivo phenotypic analysis.** Fluorescent staining of freshly isolated PBMC was done as described [7], and the phenotype of lymphocytes from subject IC2 was compared with those of >80 control donors. Monoclonal antibodies specific for surface antigens CD4,
CD8 conjugated to fluorescein isothiocyanate, CD16, or CD38 conjugated with phycoerythrin were from Becton Dickinson (San Jose, CA). Isotypically matched negative control antibodies were used. All samples were fixed with 1% paraformaldehyde and immediately analyzed by flow cytometry (FACScan; Becton Dickinson).

Results

Lack of HIV-specific humoral response despite immunologic and clinical AIDS features. Because subject IC2’s husband was HIV-seropositive, she was regularly tested by commercial ELISA and Western blot (WB) assay for HIV-1 and HIV-2 serologic reactivity beginning in 1989. As summarized in table 1, no specific HIV antibodies were ever detected in subject IC2. As shown in figure 1, WB analyses of sera obtained during 1991–1995 were negative for all HIV-1 proteins, except p24. Antibodies against p24 were detected beginning in April 1995. This response was not considered specific for HIV seropositivity, because p24 is occasionally detected in sera from uninfected persons. Moreover, p24 was not observed with the radioimmunoprecipitation assay (RIPA) that detects antibodies against native proteins. Under the same experimental procedures, WB analysis of HIV-1–specific antibodies produced by subject IC1 was typically positive, including antibodies directed against gp120 and gp160 (figure 1).

In September 1994, subject IC2 developed lung interstitial opacity, suggestive of bacillosis, associated with fever and significant weight loss, which was rapidly followed by infections typical of AIDS (table 1). At that time, she was profoundly immunodeficient, although her immune status was normal when previously tested in 1992. Complete depletion of CD8 T cells bearing the CD38 activation marker, which is known to be expanded during the AIDS stage [8]. A dramatic expansion of NK lymphocytes (48% of CD16+ cells) was de-
Figure 1. A. Western blot analysis of HIV-specific antibodies in sera from subjects IC-1 and IC-2 collected at dates at bottom of strips (New Lav Blot; Sanofi Diagnostics Pasteur, Marne-la-Coquette, France) used at 1/100 dilution. C− is negative control. Location of HIV-specific polypeptides is indicated. B, Radioimmunoprecipitation assay (RIPA) results of HIV isolates from subjects IC-1 and IC-2. RIPA was done essentially as described previously [5], except that HIV proteins from IC-2 isolate were labeled in infected MT2 cells incubated in presence of 35S-labeled methionine and 35S-labeled cysteine (250 mCi/mL). Proteins from labeled HIV isolate were immunoprecipitated in presence of sera from IC-2 or IC-1 (collection dates shown at top). C+, positive control (sample from HIV-1 infected person); C−, negative controls (sera from 2 HIV-seronegative persons). gp120 and p25 polypeptides and protein markers (in kDa) are indicated.

Virologic studies. Early attempts (in August 1991) to isolate HIV from subjects IC1 and IC2 were unsuccessful. However, no depletion of CD8 T cells was done at that time, decreasing the sensitivity of the isolation procedure. HIV-1 was isolated from IC1 in February 1993, but no attempt was made for IC2 at that time, since she was considered uninfected because of her HIV seronegativity. HIV-1 was successfully isolated from both PBMC and plasma from subject IC2 on 5 July 1995 and reisolated on 24 July 1995. The presence of HIV-specific genetic sequences in DNA purified from the PBMC isolated from IC2 was evaluated by PCR. We obtained a specific PCR amplification on 11 July 1995 using consensus primers within the pol gene [6]. Two weeks later, similar PCR experiment was negative, although HIV was isolated by coculture.

The virus from IC2 was metabolically labeled in vitro and tested for reactivity against antibodies in sera from IC1, IC2, and control donors. The results clearly indicate that the virus isolate from IC2 was recognized by antibodies from IC1 and from another HIV-positive person (figure 1B). In particular, the HIV-1 gp160 and p25 antigens were strongly immunoprecipitated. In contrast, sera from subject IC2 did not immunoprecipitate proteins from her own virus, confirming the lack of HIV-specific humoral response.

The detection of p24 antigenemia in June 1995 suggested that subject IC2 was infected by HIV despite negative serologic results. Indeed, as reported above, HIV-1 was isolated at that time from blood samples, and p24 antigenemia follow-up until the subject’s death indicated a very high virus load beginning in August (table 1). The plasma virus load was determined using two different commercial assays—the Monitor HIV RT-PCR assay from Roche and the Quantiplex HIV bDNA hybridization assay from Chiron. It is of interest that the results with these 2 tests were always discordant (table 1). These discrepancies were confirmed on samples obtained on 21 August and 19 September: The plasma virus load determined by the bDNA assay was higher than the upper limit of quantification. These same discrepancies were observed with plasma samples from subject IC1 (3 November 1995 sample: 26,600 and 0 genome equivalents/mL with bDNA and RT-PCR, respectively; 19 January 1996 sample: 110,900 and 129 genome equivalents/mL with bDNA and RT-PCR, respectively).

Molecular characterization of virus isolates from subjects IC1 and IC2. The PCR products obtained either directly from
freshly isolated PBMC or from cells infected with the viruses isolated in vitro from blood samples from IC1 and IC2 were cloned, and their nucleotide sequences determined. Virus isolated from IC1 and IC2 within the V3-encoding region of the gp120 at the nucleotide level was 95% identical. When the sequences from IC1 and IC2 isolates were compared with consensus sequence of clade A viruses, the homology was 90% and 89%, respectively. Comparison with consensus sequences from other clades (B–H and O) showed a lower level of identity (61%–85%). The identity within the same region among viruses isolated from IC1 and IC2 at the amino acid level was ≳86%. In addition to this high homology, the V3 regions deduced from all determined sequences showed an unusual insertion of the tripeptide GPG at the amino side of the loop apex. When considered together, these results indicate that isolates from IC1 and IC2 are clade A, which is dominant in the Ivory Coast [10], and argue for HIV transmission from subject IC1 to his wife, IC2.

Discussion

Very few cases of clinical AIDS in HIV-infected but seronegative persons have been reported [2–4]. In the first two studies, the infecting virus was not characterized, the source of infection was not determined, and RIFAs were not done. Therefore, the possibility that commercial serologic tests were not able to detect antibodies against a very divergent virus cannot be excluded, as reported for infections by O strains of HIV-1 [11]. Much like findings in the third study [4], the virus of subject IC2 was isolated and characterized. Furthermore, in the present study, we showed that IC2’s HIV proteins were recognized by sera from her husband and by other HIV-1–positive sera. The lack of humoral response observed in IC2 was HIV-specific, since she was normogammaglobulinemic and had mounted a humoral response against a series of microbial agents (table 1). The possibility of a transient seroconversion followed rapidly by a seroreversion was considered. However, an extensive study of ≳2.5 million persons showed no evidence for true seroreversion of HIV-1 antibodies [12]. Based on these observations, the possibility of a seroreversion for patient IC2 is very unlikely. The lack of antibodies against HIV in IC2 could be explained by a lack of specific helper T cell activity, resulting from rapid depletion of CD4 T cells. Another possibility is that repeated exposure to small amounts of HIV may lead to the induction of a B cell tolerance. This case also raises the issue of the absolute character and duration of protection of seronegative HIV-exposed persons who exhibit HIV-specific cell-mediated immune response in the absence of HIV infection [13]. The HIV-specific cell-mediated immunity could not be studied in IC2 because so few lymphocytes were available. Although the exact date of the subject’s HIV infection could not be determined, her immunologic and clinical parameters were consistent with rapid evolution of the disease.

Because subject IC2 was infected with a clade A virus, which is prevalent in Africa, the present study raises questions about the sensitivity of the molecular tests designed to measure plasma virus load. Although there is usually good correlation between results obtained by bDNA and RT-PCR, the clade A viruses from the 2 subjects in this study were poorly recognized by a commercial PCR kit but correctly detected by bDNA. Similarly, a failure to amplify an HIV-1 variant from clade G [14] and HIV-1–positive samples collected in Ivory Coast [11] has been reported. Clearly, more studies are needed to evaluate the respective sensitivity of these quantitative assays for patients infected with viruses of African origin.

In conclusion, the immunologic and virologic results of the subjects described strongly suggest that the lack of HIV-specific humoral response in subject IC2 was host related rather than related to a specific HIV strain. We believe cases such as this are probably rare, but not exceptional. Regular sex partners of HIV-seropositive persons and of intravenous drug users should be followed regularly by both antibody testing (WB and ELISA) and immunologic parameters (absolute CD4 lymphocyte numbers and CD4:CD8 ratio). When clinical or biologic data suggest an HIV infection, direct detection of the virus is recommended.

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References

Detection of Infection with Human Immunodeficiency Virus Type 1 before Seroconversion: Correlation with Clinical Symptoms and Outcome

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Early (pre-seroconversion) infection with human immunodeficiency virus type 1 (HIV-1) was identified in 50 of 267 participants in the Multicenter AIDS Cohort Study. These 50 men had a positive EIA result, which detected IgM antibody (n = 35), p24 antigen, or serum HIV RNA (n = 15) at their last “seronegative” visit. At that visit, the mean CD4 lymphocyte number (890/mm³ vs. 1038/mm³) was significantly lower than in men who subsequently seroconverted but had no evidence of early infection. The decline in CD4 cells was slower and the duration of AIDS-free time longer in the 19 men who were symptomatic in comparison to the 31 asymptomatic men with early infection, but differences were not significant.

Identification and treatment of early infection due to human immunodeficiency virus type 1 (HIV-1) has been advocated as a means of reducing viral replication and favorably altering disease progression [1, 2]. As a significant number of persons with primary infection are asymptomatic [3], it has been suggested that symptomatic incident HIV-1 infection is associated with more rapid immunologic deterioration and clinical progression [4–7].

The “seronegative” phase of primary infection generally lasts between 2 and 6 weeks [3]. Recently, an EIA capable of detecting IgM as well as IgG antibody has been shown to reduce the window period between inoculation and seroconversion by ~1 week [8]. The use of techniques to detect viral p24 antigen and RNA in serum also decreases the time between establishment and diagnosis of infection with HIV-1 [9–11]. We used assays to identify persons with early HIV infection and sought to determine whether symptomatic primary infection was associated with clinical progression.

Methods

Details of the Multicenter AIDS Cohort Study (MACS), a prospective investigation of the natural history of HIV-1 infection among homosexual and bisexual men, have been published [12]. The participants included in this analysis were the 267 men with incident infection occurring within an interval of <7 months (median, 6; range, 4–7) between the last seronegative and first seropositive visit identified using first-generation EIAs. Serum obtained at the last “seronegative” visit was retested to determine the prevalence of early infection in these 267 participants.

Symptomatic early infection was defined by self-reports of fatigue, new skin rash, fever, tender or enlarged lymph nodes, weight loss, diarrhea, and night sweats occurring during the intervisit interval. Questions relevant to symptoms persisting for 2 weeks were answered by all 267 men. Midway through the study, ques-