Occupational HIV infection in a research laboratory with unknown mode of transmission: a case report

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ABSTRACT

A lab-worker was infected with HIV-1 in a biosafety level-2 of containment, without any apparent breach. Through full-genome sequencing and phylogenetic analyses, we could identify the source of infection in a replication-competent clone, unknowingly contaminating a safe experiment. Mode of transmission remains unclear. Caution is warranted when handling HIV-derived constructs.

Keywords: HIV-1; occupational exposure; laboratory safety; HIV transmission

INTRODUCTION

HIV-1-based lentiviral vectors are widely used in research laboratories to produce viral particles for gene-delivery, drug-screening, and HIV replication studies [1,2]. These safe, non-replication-competent viral particles derive from simultaneous transfection of producer cells with HIV-1 defective expression constructs and autologous/heterologous envelope-encoding plasmids, such as for HIV-1 Env, or vesicular stomatitis virus glycoprotein (VSV-G), the latter being commonly used for its pantropism [3]. Their recognized safety [4] has allowed handling of these non-replication-competent viruses or pseudoviruses in a biosafety level (BSL)-2 conditions [5,6].

Here we describe a case of HIV-1 infection, which occurred during HIV-1 pseudoviruses production in a BSL-2 containment, due to unaware contamination of these procedures with a replication-competent recombinant clone.

CASE REPORT

A blood donor resulted HIV-1-positive at routine screening. Previous tests (the latest 7 months before) were negative. At first clinical assessment, investigating the HIV-acquisition route, no classical risk factors emerged. However, during the six months preceding HIV-1 diagnosis, the subject worked in a research laboratory, with the exclusive task of producing pseudoviruses by co-transfecting 293T cells with HIV-1 NL4-3 *env*/*nef*-defective, green fluorescein protein-labeled vector, and JRFL Env- or VSV-G-encoding plasmid in a BSL-2 containment. No access to BSL-3 was allowed and reported. No major or minor laboratory accidents (percutaneous injury, medium splash, breaking of gloves, eye or skin contact with cell-derived medium, etc.) were recalled.

METHODS

Ethical statement. After Institutional and Ethics Committee approval, the patient gave written informed consent for all the analyses and the publication of this report.

Viro-immunological parameters. Plasma HIV-RNA and CD4+ cell count were evaluated every 4 months from diagnosis. Total HIV-DNA was measured at month 12 [7].

HIV-1 sequencing. HIV-1 from plasma and peripheral blood mononuclear cells (PBMCs) was sequenced and analyzed at different time-points by bulk and ultra-deep pyrosequencing (UDPS).

Phylogenetic analysis. Maximum-likelihood methods (ML) [8], using the full-length HIV-DNA sequence (month 12), were applied to define the infectious source. Viral evolution was evaluated through dN/dS ratio, number of Env-glycosylation sites, and co-receptor usage at different time-points.

Viral isolation, phenotypic determination, and neutralizing activity assessment. Thirty-six months post-diagnosis the virus was isolated from patient's PBMCs, and phenotypically characterized on U87.CD4+ cells expressing CCR5 and CXCR4 [9]. Patient's neutralizing antibody response was assessed by testing the virus for sensitivity to patient's sera collected at different time-points, and to a panel of monoclonal antibodies (MoAbs) [10].

Immunological characterization. At month 36, cytometric analyses were performed on HIV peptides-stimulated and unstimulated PBMCs.

Full methodology in Supplementary Text, Tables S1-S2.

RESULTS

Viral sequence characterization. At HIV-1 diagnosis, Western blot was positive to Env, Pol, Gag, and low-reactive to p31. CD4+ cell count and plasma HIV-1 RNA were 577 cells/μL and 119 (2.1 $\log_{10}$) copies/mL, respectively (Figure S1).

The full-length sequencing showed the presence of a whole HIV-1 genome. The ML phylogenetic trees inferred from *gag-pol*, *vif-vpr-vpu-tat/rev* (first-exon), and *nef* sequences demonstrated that in these three regions the patient's virus formed well-supported clusters with NL4-3 (bootstrap: 100%, 100%, 97.8% respectively; Figure 1). The high homology with NL4-3 was confirmed by the extremely low genetic distance between the two strains: 0.004±0.001 for *gag-pol*, 0.008±0.003 for *vif-vpr-vpu-tat*/*rev* (first-exon), and 0.006±0.003 for *nef*. The few amino acid changes from NL4-3 reference sequence in accessory genes are shown in Table S3.

In contrast, the ML tree obtained by the full-length *env* and *tat*/*rev* (second-exon) revealed that the patient's virus formed a well-supported cluster with JRFL (bootstrap: 99.7%; genetic distance: 0.017±0.002; Figure 1).

Although the phylogenetic results showed a high homology between the patient's virus and the HIV-1 constructs he/she was supposed to have handled, the presence of *nef* in the patient's virus suggested that the source of infection should not have been the recombination between HIV-1 NL4-3 *env*/*nef*-defective vector and JRFL Env-encoding plasmid, but more likely an unaware use (due to contamination or labeling error) of a full-length infective clone.

A retrospective evaluation of the clones present in the laboratory at that time identified an HIV-$1_{\text{NL4-3/JRFL}}$ plasmid, with a backbone of NL4-3, and an *env* of JRFL, handled in the BSL-3 of the same laboratory by other researchers for other experimental purposes. We presume that this plasmid erroneously but unknowingly entered in the BSL-2.

Tropism characterization. Serial plasma and PBMCs V3 UDPS showed predominant R5-viruses; only at month 36 two minority species emerged, with a false-positive-rate (FPR) <10% (below the cut-off to predict R5-tropism) and the mutation S11D, at position important for coreceptor binding (Table S4). This mutation was also present in the virus isolated from patient's PBMCs at month 36 (Figure S2). This virus exhibited an R5 phenotypic tropism in U87 cells, despite a FPR of 8.1% at genotypic assay, suggesting that this single mutation, in this particular backbone, is not probably enough to determine X4 phenotype.

Virus sensitivity to autologous serum and monoclonal antibodies. Patient's sera were unable to neutralize the patient's primary virus, but neutralized the pseudovirus JRFL at high titers. The neutralization assay performed with MoAbs showed a high sensitivity of the patient's virus to gp120-directed MoAbs PG9 and PG16 but less to b12, 2G12 or the gp41-directed MoAbs 2F5 and 4E10 (Table S5). The pseudovirus JRFL showed an inverse pattern of neutralization sensitivity. This may suggest an escape of autologous virus through evolution. Indeed, *env* sequencing at different time-points showed a substantial C2/V3 *env* modifications (defined by the increasing dN/dS ratio, which varied from 0.00 at month 4 to 2.00 at month 36, and by the heterogeneity of V3 quasispecies) (Table S4). Serial *gag* and *nef* sequencing also revealed a genetic evolution (dN/dS ratio: 0.00 and 0.01 at month 12, and 0.22 and 0.63 at month 36, respectively), and the emergence of amino acid changes at critical residues for Cytotoxic-T-Lymphocyte-escape (Table S3).

Immunological characterization. Peripheral immune activation, higher in the patient than in healthy controls, the skewed distribution of CD4+ and CD8+ sub-populations observed in the patient, and the higher percentage of TNFα/IFNγ-producing CD8+ cells perforin and granzyme-expressing CD8+ cells observed after stimulation with Gag and Env predicted a high cytotoxic effector potential, and did not differentiate this case from typical clinical course of HIV infection (Table S6).

Clinical follow-up. At month 36, CD4+ cell counts fell below 400 cells/μL and plasma HIV-RNA increased to 20,208 (4.3 log_{10}) copies/mL. Following the guidelines of that time, antiretroviral therapy with tenofovir/emtricitabine/rilpivirine was started; viral suppression was achieved at week 10, and maintained thereafter (Figure S1).

DISCUSSION

Here we describe for the first time an HIV-1 infection of a laboratory worker with a replication-competent recombinant clone, which was erroneously and inadvertently introduced in a BSL-2 containment during routine experiments of HIV-1 pseudoviruses production. The relevant novelty, which differentiates this case from the other rare laboratory accidents (where laboratory workers consciously had handled infectious viral cultures) [11] is that the patient described here was supposed to exclusively manage constructs that were *per se* non-infectious, with restrictions for BSL-2, and that an unaware contamination by a recombinant clone caused the infection.

Initial viral load and CD4+ counts dynamics depicted a slow progression during the first 2.5 years. The subsequent viro-immunological deterioration, the successful escape from host immune system, and the marked genetic evolution (documented with the modification in *gag*, *nef* and *env*, including the heterogeneity of V3 quasispecies) reveal that a pure laboratory recombinant clone has adapted to a human host encountered for the first time, thus determining a progressive HIV-1 infection.

This case is disturbing for the unexplained route of virus transmission. An in-depth retrospective examination of the events did not recall any accident nor any type of evident minor exposure to the source of infection. In this respect, we wonder if VSV-G may have played a favouring role. It was previously demonstrated that the plasmid encoding for VSV-G allows to obtain pantropic viral particles able to deliver the expression construct to a wide range of mammalian and non-mammalian target cells [3], thus dramatically enhancing viral infectivity. Previous studies have demonstrated that HIV-1 produced in cells infected with VSV-G gives rise to phenotypically mixed virions with an expanded host range [12,13]. Thus, it is conceivable that if the infectious HIV-$1_{NL4\text{-}3/JRFL}$ plasmid, instead of the HIV-1 NL4-3 *env*/*nef*-defective vector, was erroneously transfected with VSV-G plasmid into producer cells, hybrid virions containing the VSV-G and the full HIV-1 genome might have been generated. This could have expanded viral tropism, augmented viral infectivity, and extremely facilitated infection, so that even unperceived droplets of supernatant of a high-level replicating hybrid viral culture, getting inadvertently in contact with mucous membranes, may have caused an accidental, unaware infection.

Despite intensive investigation, the lack of identification of the “breach-point” that led to contamination, and the mode of transmission that remains obscure hinder the full comprehension of this accident.

Notwithstanding these limitations, this case warns of potential hazard in research laboratories where multiple HIV-derived constructs are managed: BSL-2 are often large spaces occupied by many laboratory personnel working on different lists of projects (also conducted in BSL-3) and sharing laboratory equipment. A precise risk assessment and procedures’ control, applied to every research project, could contribute to minimize biohazards and prevent at best laboratory exposures.

NOTES:

AUTHORS' CONTRIBUTIONS

A.S. & C.A. equally contributed to this work, and followed the case by clinical and scientific point of view. Both carried out analysis and interpretation of data and both drafted the manuscript. G.S. and M.T. performed viral isolation, phenotypic determination, and neutralizing activity assessment. A.B. (Ada Bertoli) carried out acquisition of bulk sequencing data. E.B. performed VSV neutralization assay. M.C.B., F.C., L.C. carried out ultra-deep pyrosequencing and bioinformatic analyses. M.B., D.T., A.B. (Alessandra Bandera) carried out experiments for immunological characterization and corresponding data interpretation. A.S., C.A., F.C.S., C.F.P., A.G. participated in study conception and design. G.S., F.C.S., C.F.P., A.G. substantially contributed to critical revision of the manuscript. All the authors accepted the final version of the manuscript.

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Conflicts of interest related to this manuscript: Alessandro Soria: no conflict; Claudia Alteri: no conflict; Gabriella Scarlatti: no conflict; Ada Bertoli: no conflict; Monica Tolazzi: no conflict; Emanuela Balestra: no conflict; Maria Concetta Bellocchi: no conflict; Fabio Continenza: no conflict; Luca Carioti: no conflict; Mara Biasin: no conflict; Daria Trabattoni: no conflict; Alessandra Bandera: no conflict; Francesca Ceccherini-Silberstein: no conflict; Carlo Federico Perno: no conflict; Andrea Gori: no conflict.

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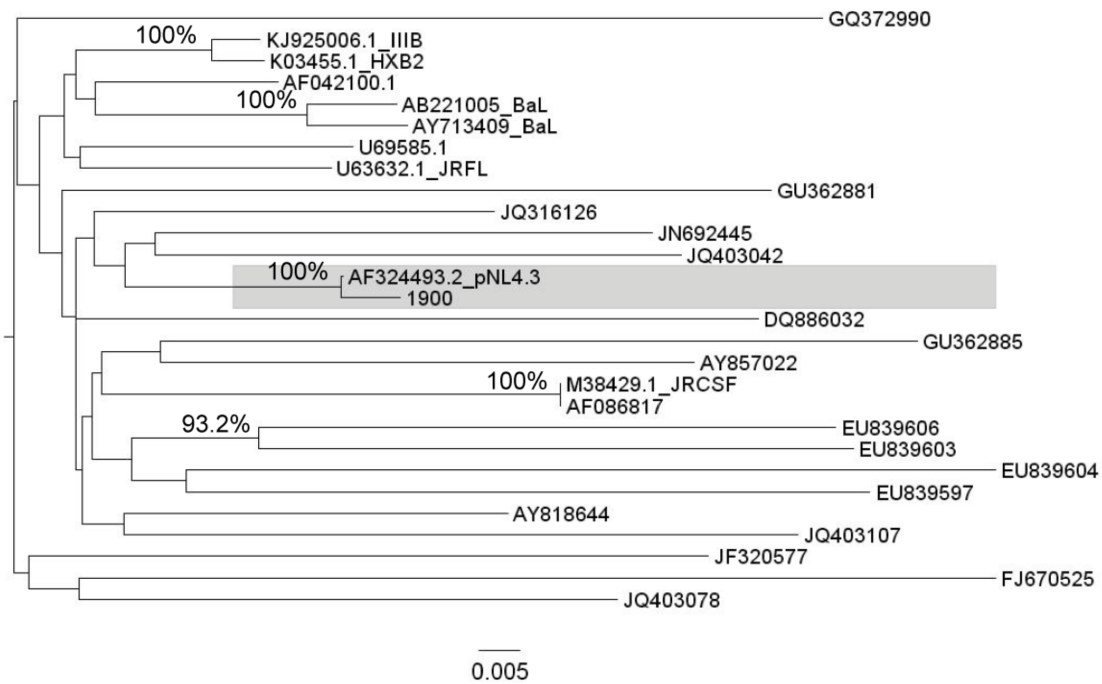
FIGURE LEGENDS

Figure 1. Phylogenetic trees based on: **panel A**: full-length sequences encoding for the long terminal repeat (LTR)-5’ and the structural proteins Gag-Pol (nt: 5,007), corresponding to nt 89 to nt 5,096 in reference strain HXB2 (accession number: K03455.1); **panel B**: full-length sequences encoding for the regulatory proteins Vif, Vpr, Vpu, Tat/Rev (first-exon) (nt: 1,145), corresponding to nt 5,097 to nt 6,224 in reference strain HXB2 (accession number: K03455.1); **panel C**: full-length sequences encoding for the regulatory protein Nef and the LTR-3’ (nt: 762), corresponding to nt 8,797 to nt 9,559 in reference strain HXB2 (accession number: K03455.1); **panel D**: full-length sequences encoding for the Env polyprotein (nt: 2,463), and Tat/Rev (second-exon), corresponding to nt 6,225 to nt 8,796 in reference strain HXB2 (accession number: K03455.1).

The Maximum Likelihood tree was inferred by using PhyML program. Only bootstrap supports >70% are shown. The tree was rooted using a midpoint rooting. The gray box defines the cluster involving the patient’s virus, identified by the number 1900.

Figure 1

A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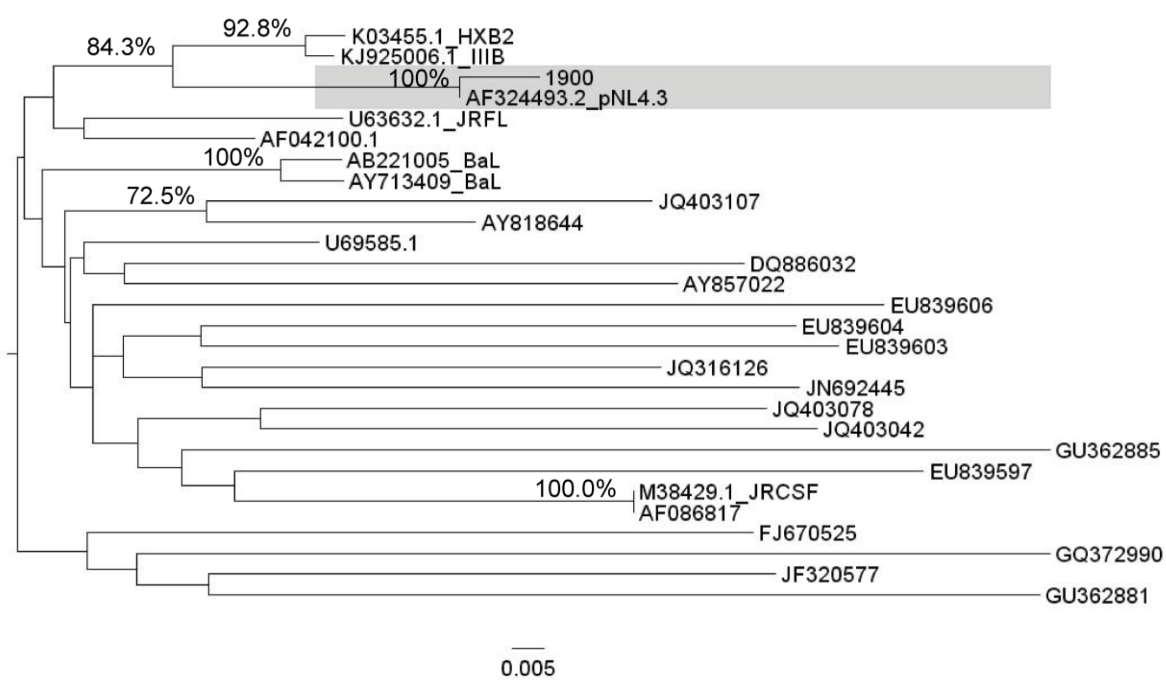
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