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Quasispecies tropism and compartmentalization in gut and peripheral blood during early and chronic phases of HIV-1 infection: possible correlation with immune activation markers

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Abstract

HIV quasispecies was analysed in plasma and proviral genomes hosted by duodenal mucosa and peripheral blood cells (PBMC) from patients with early or chronic infection, with respect to viral heterogeneity, tropism compartmentalization and extent of immune activation. Seventeen HIV-1-infected combined antiretroviral therapy naive patients were enrolled (11 early infection and six chronic infection). V3 and *nef* genomic regions were analysed by ultra-deep pyrosequencing. Sequences were used to infer co-receptor usage and to construct phylogenetic trees. As markers of immune activation, plasma sCD14 and soluble tumour necrosis factor receptor II (sTNFRII) levels were measured. Median diversity of HIV RNA was lower in patients with early infection versus chronic infection patients. Overall, direct correlation was observed between V3 diversity and X4 frequency; V3 diversity of HIV RNA was inversely correlated with CD4 T-cell count; median sCD14 and sTNFRII values were similar in early and chronic patients, but X4 frequency of HIV RNA was directly correlated with plasma sCD14. The proportion of patients harbouring X4 variants and median intra-patient X4 frequency of proviral genomes tended to be higher in chronic infection than early infection patients. More pronounced compartmentalization of proviral quasispecies in gut compared with PBMC samples was observed in patients with early infection compared with chronic patients. The loss of gut/PBMC compartmentalization in more advanced stages of HIV infection was confirmed by longitudinal observation. More studies are needed to understand the pathogenetic significance of early HIV quasispecies compartmentalization and progressive intermixing of viral variants in subsequent phases of the infection, as well as the role of immune activation in tropism switch.

Keywords: Compartmentalization, co-receptor usage, gut mucosa, HIV quasispecies, immune activation markers, viral diversity
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Introduction

Infection with HIV-1 typically involves the interaction between the viral envelope glycoprotein gp120 and the CD4 molecule, followed by interaction with a chemokine receptor, usually CCR5 or CXCR4. CCR5-using (R5) viruses predominate in the early stages of HIV-1 infection, whereas a switch towards CXCR4 usage (X4 viruses) may occur at later stages. While the propensity to X4 switch varies according to HIV clade, the presence of X4 variants is invariably associated with a faster disease progression [1]. Several studies, based on either phenotypic tests or bulk sequencing, suggest that up to 19.2% of acutely infected patients may harbour X4 variants [2–6]. Thanks to the newly introduced ultra-deep pyrosequencing (UDPS), which allows high-resolution analysis of viral quasi-species, recently it has been shown that the proportion of patients with primary infection harbouring X4 variants is larger than previously anticipated [7]. In addition, these technologies allow us to quantify the relative frequency of even rare variants

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with different co-receptor usage, as compared with the dominant virus population [8–11]. In a recent study, about half of acutely infected patients were shown to harbour X4 strains by UDPS, with highly variable intra-patient frequency, and worsened clinical course in those with a high burden of X4 variants [7].

In the first phases of the infection, independently of the route of transmission, early and massive viral replication occurs in gut-associated lymphoid tissue, leading to irreversible mucosal CD4 T-cell depletion. A complex network of HIV-1-target cells is established during this phase, leading to a progressive filling of reservoirs and to viral dissemination in the body [12].

There is growing evidence that compartmentalization may be an early event during the natural history of HIV-1 infection [13,14]. Some pathogenetic models suggest that a status of immune hyperactivation represents one of the main predictors of disease progression [15]. Recently, van Marle *et al.* [16] showed discordant results for compartmentalization of virus replication within different gut locations and peripheral blood mononuclear cells (PBMC) of chronically infected patients naive to antiretroviral therapy, depending on the genomic region analysed (*nef* and *pol*). In another report on gut-associated lymphoid tissue versus PBMC, compartmentalization of viral quasispecies was highlighted on the basis of different drug resistance-associated mutations [17]. In other studies, no evidence of compartmentalization was observed between gut and peripheral blood and even within different gut regions (colon versus ileum) in chronically HIV-1-infected individuals [18]. At the same time, evidence of cross-infection between gut-associated lymphoid tissue and PBMC during combined antiretroviral therapy (cART) has been suggested as a possible mechanism for HIV persistence [19].

The present study was aimed at studying, by UDPS, the tropism and compartmentalization of HIV-1 quasispecies in gut and peripheral blood in cART-naive patients with early infection compared with chronically infected patients, analysing possible correlations between the presence of X4 variants and the extent of immune activation.

Methods

Patients

Seventeen HIV-1 infected patients, for whom there was clinical indication to perform a duodenal biopsy for diagnostic purposes, were consecutively enrolled in the study. Eleven subjects had a primary HIV infection, defined by a negative or indeterminate HIV-1 Western Blot with simultaneous positive plasma HIV viraemia or by a positive HIV-1 antibody test preceded by a documented negative test within the preceding 180 days (early infected patients); six patients had an HIV-1 infection documented for ≥3 years (chronic patients). At the moment of biopsy, all patients with primary infection were in Fiebig stage VI. At the same time-point, blood samples were also collected (PBMC and plasma). All patients had acquired HIV infection by a sexual route and were naive to cART at enrolment. One early patients who underwent cART after enrolment, was analysed at subsequent time-points (6 and 12 months from enrolment). The study was approved by the Institutional Ethics Committee, and the patients agreed to participate by signing an informed consent.

UDPS and data analysis

Total DNA extraction from PBMC and from duodenal biopsies that were first homogenized in cell lysis buffer (Qiagen, Hilden, Germany) using a pestle, was performed using the extraction kit 'DNA blood' (Qiagen). Plasma HIV-1 RNA was extracted using the QIAamp Viral RNA kit (Qiagen). The number of templates actually undergoing UDPS analysis was evaluated by a commercial real-time PCR for HIV-1 RNA in plasma samples (Abbott real-time HIV, Abbott Molecular Inc., Des Plaines, IL, USA), and by a quantitative real-time PCR targeting the long terminal repeat region for HIV DNA [20].

V3 amplification was performed by nested PCR. Briefly, two rounds of 30 cycles (94°C for 2 min, 94°C for 30 s, annealing at 60°C for 30 s, extension at 68°C for 30 s and final elongation at 68°C for 5 min) were carried out using a proofreading DNA polymerase (Platinum® Taq DNA Polymerase High Fidelity; Invitrogen, by Life Technologies, Monza, Italy). First- and second-round primers were described in ref. [7]. Nef amplification was performed by a nested PCR, as described in ref. [16]. For plasma samples, the first round of both *env* and *nef* included a one-step RT-PCR, using a Platinum quality proofreading reverse transcriptase (Invitrogen). Unique in-house-designed stretches of eight nucleotides (Multiplex Identifiers), were used to tag each sample. To maximize the genetic heterogeneity to be amplified and sequenced, for each sample the amplicons from at least four replicate PCR were pooled, representing, for viral RNA, the content of 1 mL of plasma, and, for DNA, the content from 2×10^6 to 6×10^6 PBMC. To minimize the occurrence of artefacts attributable to template re-sampling, a minimum of 1200 templates were analysed by UDPS, on the basis of viral and proviral load (Table 1).

The UDPS was carried out with the 454 Life Sciences platform (GS-FLX, Roche Applied Science, Monza, Italy) as described previously [7], using Titanium chemistry. For V3 amplicons the correction pipeline and the evaluation of experimental error necessary to establish the sensitivity

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