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Isolation of human immunodeficiency virus-type 1 (HIV-1) clones with biological and molecular properties of the primary isolate

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Abstract

We developed a new biological cloning system for HIV-1 isolates using the U87.CD4 cell lines that express different chemokine receptors. We demonstrate that our method is sensitive and specific because the clones isolated had the same coreceptor usage and genotype as viruses of the primary isolate. We evaluated our cloning system by isolating 27 biological clones from two primary HIV-1 R3R5X4 isolates. Three HIV-1 phenotypes (R3R5X4, R3R5 and R5) were identified in isolate 29 and two (R3R5X4 or R5X4) in isolate 31. Each phenotype was distinguished by a unique genotype. Sequencing of 20 molecular clones from each isolate did not reveal additional genotypes. One of the three genotypes identified from isolate 29 was not found by molecular cloning of the original isolate, suggesting high specificity and sensitivity of the biological cloning system in isolating minor virus populations. Our results suggest that the new cloning approach can be used as an alternative to the existing method for isolating biological clones in PBMC.

Keywords: Biological cloning; HIV-1; Coreceptors

Introduction

Human immunodeficiency virus type 1 (HIV-1) infects cells of the T-cell and macrophage lineages (Dalgleish et al., 1984; Folks et al., 1986; Gartner et al., 1986; Klatzmann et al., 1984). At the cell surface, the viral envelope (Env) glycoprotein (gp) 120 interacts with CD4 and a chemokine coreceptor. This interaction results in a series of conformational changes in both the gp120 and gp41 which allows the virus to enter the cell (Berger, 1997). HIV-1 has been defined as being fast or slow replicating in peripheral blood mononuclear cells (PBMC) (Asjo et al., 1986; Fenyo et al., 1988) or as having the capacity to induce syncytia in PBMC or MT-2 cells (called syncytium inducing, SI and non-syncytium inducing, NSI, respectively) (Tersmette et al., 1988). The molecular basis for the phenotypic differences of HIV-1 is due to the ability of the virus to use different coreceptors. Nowadays, it is common that HIV-1 isolates are classified as belonging to different phenotypes by their ability to use different chemokine receptors as coreceptors (Berger et al., 1998; Choe et al., 1996; Deng et al., 1996; Doranz et al., 1996; Feng et al., 1996). Early in infection, HIV-1 most often uses CCR5 as coreceptor and these viruses are called R5 viruses. Later in the infection, coreceptor usage of viruses may change to CXCR4 and these viruses are termed X4 viruses. The viruses may also broaden their coreceptor repertoire into using a combination of CCR5, CXCR4 and other minor chemokine receptors, mostly CCR3. In infected individuals, a switch of coreceptor usage is often associated with an accelerated loss of $CD4^+$ T-cells and progression towards clinical symptoms characteristic of acquired immunodeficiency syndrome (AIDS) (Bjorndal et al., 1997; Connor et al., 1997; Scarlatti et al., 1997; Yu et al., 1998).

The viral population of an infected individual consists of a swarm of genetically related viruses which are termed quasi-species. Quasi-species likely arise by at least two mechanisms; first, the viral replication machinery is error prone because the viral reverse transcriptase lacks proof reading ability, and second, there is continuous rapid virus turn over. While

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extensive genetic analyses have been carried out, less is known about the impact of quasi-species on the biological variability of HIV-1. Early work showed that SI (CXCR4-using) isolates are composed of both SI and NSI phenotypic variants (Schuitemaker et al., 1992). In agreement with these observations, we have previously shown that viruses with different coreceptor usage can be selected by limited passage in single coreceptor expressing cells (Bjorndal et al., 1997; Scarlatti et al., 1997). Coreceptor evolution plays an important role in HIV-1 pathogenesis and there is a clear correlation between appearance of CXCR4 usage and progression towards AIDS (Bjorndal et al., 1997; Karlsson et al., 1994; Koot et al., 1993).

In-depth studies on biological clones, rather than primary isolates, are important and may reveal features of the pathogenic process. It is common practice to isolate HIV-1 biological clones using PHA-stimulated peripheral blood mononuclear cells (PBMC) (Schuitemaker et al., 1992). Although PBMC express the major coreceptors of HIV-1, they are present at unequal densities on the cell-surface. CXCR4 is expressed earlier after stimulation and at higher levels than CCR5, whereas CCR3 is only expressed at low levels (Bleul et al., 1997). This uneven coreceptor density may lead to a positive selection for X4 viruses at the expense of viruses with other coreceptor preference in PBMC.

To avoid selection due to uncontrolled coreceptor availability, we have developed an alternative method for isolating biological clones using the U87.CD4-system. Isolation of biological clones is performed by dilutions of viral isolates in the presence of only one coreceptor at a time.

Results presented here, using two multitropic R3R5X4 primary HIV-1 isolates, show that our method accurately identifies viral clones having the same biological and molecular properties as the primary isolates. In addition, our approach is faster and less labor intensive than conventional cloning in PBMC.

Results

Cloning strategy and biological properties of the isolates

In comparison to PBMC, syncytia formation is easily detected in U87 cells. Cultures showing 5 or less syncytia per well were defined as having low degree of syncytia formation (LDSF). As is described below, the LDSF definition allowed us to successfully identify biological clones without determining classical "end-points" that is common practice in PBMC-based cloning. Two HIV-1 primary isolates (29 and 31) were first diluted and passaged in four parallel wells on U87.CD4 cells expressing either CCR3, CCR5 or CXCR4 coreceptors. Cultures were analyzed for syncytia formation and RT activity at day 6 (for CCR5 and CXCR4 expressing cells) or at day 10 (for CCR3 expressing cells).

After first passage, the isolates reached LDSF at different dilutions on the different cell lines (Figs. 2 and 3). Isolate 29 scored highest both by RT and syncytia formation on CCR5-expressing cells (Fig. 2) whereas isolate 31 showed highest titer on CXCR4-expressing cells (Fig. 3). Isolate 29 infected CCR3-expressing cells to about the same extent as CXCR4-expressing cells (Fig. 2), whereas isolate 31 infected CCR3-expressing cells at comparatively lower dilutions (Fig. 3).

Isolation of biological clones

Virus supernatants from the first passage were subjected to a second selection step by infection of the three U87.CD4 coreceptor expressing cell lines. Proviral DNA was obtained from cells in the second selection step. A 300-bp region containing the V3 region of *env* was amplified and genotypes were determined by direct sequencing (Fig. 1). Direct sequencing of isolate 29 showed many heterogenic sites (Fig.

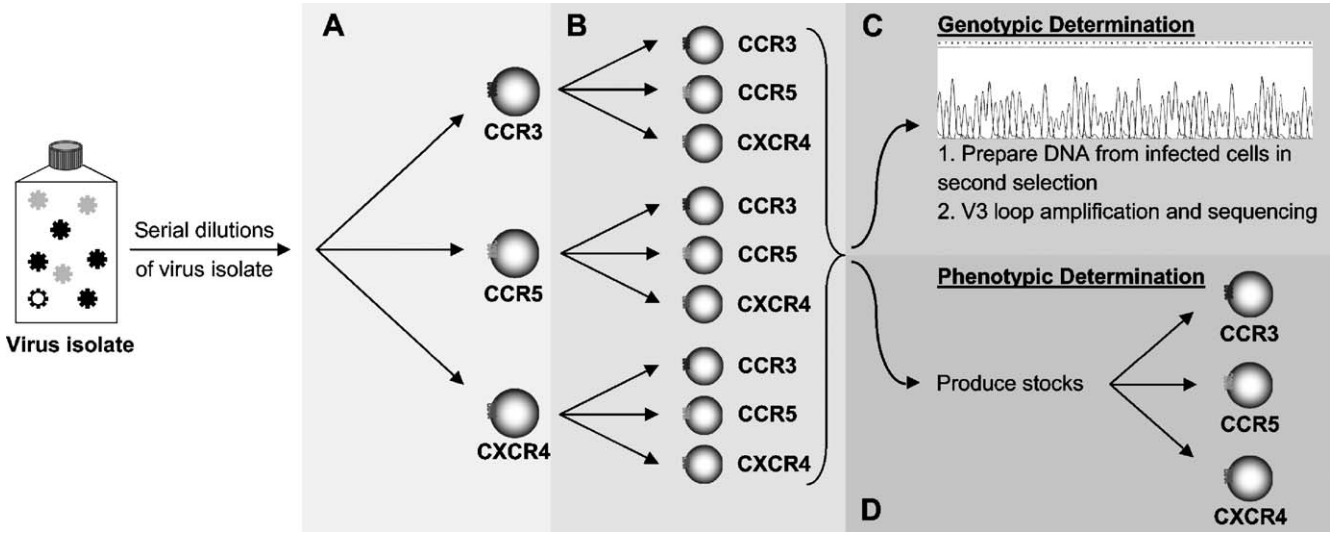

Fig. 1. Schematic picture of the selective biological cloning system. (A) First selection step: in the first selection step, virus isolates were diluted and passaged 1–3 times on U87.CD4-CCR3, -CCR5 and -CXCR4 expressing cells. (B) Second selection: supernatants from first selection step were used to infect U87.CD4-CCR3, -CCR5 and -CXCR4-expressing cells in a second selection step. (C) Genotypic determination: to confirm clonality, V3 region from proviral DNA, obtained after second selection, was amplified and sequenced. (D) Phenotypic determination: confirmed biological clones were grown on PBMC and the phenotype was finally determined by infection of U87.CD4 cell lines.