Available online at www.sciencedirect.com
ScienceDirect
Virus Research 123 (2007) 138–146
Virus Research
www.elsevier.com/locate/virusres

In vitro cell fusion between CD4$^+$ and HIV-1 Env$^+$ T cells generates a diversity of syncytia varying in total number, size and cellular content

N. López-Balderas [a], L. Huerta [a,*], C. Villarreal [b], E. Rivera-Toledo [a], G. Sandoval [a], C. Larralde [a], E. Lamoyi [a]

[a] *Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Apartado Postal 70228, Ciudad Universitaria, Distrito Federal, C.P. 04510, Mexico*
[b] *Instituto de Física, Universidad Nacional Autónoma de México, Ciudad Universitaria, Distrito Federal, Mexico*

Received 16 May 2006; received in revised form 22 August 2006; accepted 23 August 2006
Available online 2 October 2006

Abstract

Syncytia formation in HIV infections is driven by the virus fusion-active molecules (Env) interacting with membrane components of hosts cells. HIV-syncytia are usually interpreted as pathogenic entities and although they may potentially vary in size, numbers and types of constituent cells, little is known about the extent and significance of their diversity. Here, we describe numerically the cell population dynamics and the diversity of syncytia produced in the in vitro cell-fusion between two Jurkat T cell lines, one CD4$^+$ and the other Env$^+$. Cell-fusion partners were differentially stained with the lipophilic DiI and DiO, or with the cytoplasmic CMFDA and CMTMR tracers and syncytia showing double fluorescence were counted in a flow cytometer. The total number of syncytia formed, their size, cellular complexity and ratio of CD4$^+$/Env$^+$ cells recruited, varied significantly in relation with time of reaction and initial proportions of fusion partners. The considerable structural diversity of syncytia formed, in so limited an in vitro cell fusion reaction, suggests that a greater heterogeneity may be formed in the natural course of disease. Identification of the main determinants of syncytia diversity allows for a detailed study of the relation between the syncytia structure and function.

Keywords: Cell fusion; Env; Virus envelope; Syncytia; Multinucleated cells; Giant cells; Flow cytometry; HIV

1. Introduction

Fusion of single cells leads to the formation of syncytia (also referred as multinucleated giant cells or polykaryons): a collection of two or more nuclei inside a single, continuous outer cell-membrane (Ogle et al., 2005). Complex physiological tasks, like those of the syncytium trophoblast (Gude et al., 2004) and regulation of embryonic development (Shemer and Podbilewicz, 2003), are accomplished by cells organized as syncytia: a hint of syncytia being perhaps optimal forms of cell organization to face complex demands. In pathological processes, syncytia of mononuclear cells are conspicuous histological entities often associated with chronic inflammation in a variety of infections involving viruses, bacteria and parasites (Fais et al., 1997; Murch et al., 1982). The role of syncytia in the host's management of the infection's challenge is not clear and may vary in each instance. Differences in size and cellular composition raise the hypothesis that the net biological significance of cell fusion (i.e., harmful or useful) is associated with the syncytia's cellular composition, as well as with the nature and intensity of the challenge.

Syncytia are identified in cultures of HIV-infected T cells (Lifson et al., 1986; Sodroski et al., 1986) and found in lymphoid and brain tissues from HIV-1 infected individuals, where they may function as viral reservoirs or participate in the pathogenic mechanisms underlying CD4$^+$ T cell depletion and immunodeficiency (Amendola et al., 1996; Budka, 1986; Castedo et al., 2003; Frankel et al., 1996; Koenig et al., 1986; Orenstein, 2000; Rinfret et al., 1991). Detection of syncytium-inducing virus in the circulation of HIV-infected patients is associated with an increased rate of CD4$^+$ lymphocyte depletion and progression to AIDS (Blaak et al., 2000; Connor et al., 1993; Miedema et al., 1994) and the effect of serum antibodies on cell fusion associates with the clinical status of HIV-infected individuals (Huerta et al., 2005). Notwithstanding the pathogenic capacity that syncytia may have in HIV infection, the heterogeneity in time course, clinical manifestations and response of the patients

* Corresponding author. Tel.: +52 555 622 38 54; fax: +52 555 622 33 69.
E-mail address: leonorhh@biomedicas.unam.mx (L. Huerta).

0168-1702/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2006.08.009

to treatment (Hogan and Hammer, 2001a, b) hint to a network of complex events in operation during infection. The large numbers of structurally and functionally different syncytia that can be constructed by the combinatorial of different lymphoid and myeloid cells, infected and normal, may significantly contribute to the heterogeneity of pathogenesis in HIV infections.

The precise molecular mechanism of the HIV-mediated fusion reaction is under intense investigation. It involves the binding of receptors on the surface of lymphoid cells (CD4 and co-receptors) to fusion active molecules of viral origin (Env), followed by the proteins reorganization to form a sort of fusion machine incorporated in the membranes of the bound cells (Chen and Olson, 2005; Gallo et al., 2003). In contrast with the advanced understanding at the molecular level, the cell population dynamics (i.e., the time associated changes in the numbers of reacting cells and of the fusion products) involved in syncytia formation in HIV infection has been scarcely explored. The requirement of an initial binding step suggests that cell fusion follows the principles of mass action and hence the number and composition of the cell fusion products (i.e., syncytia) should be influenced by time of reaction, by the relative abundance of cell fusion partners and by the number of receptors on cell membranes. Differences in these parameters, along with the type and physiological state of the fusing cell partners, should produce quantitative and qualitatively diverse syncytia.

We tried constructing different HIV-related lymphoid syncytia to study their potentially different functions. In order to approach a quantitative description of the kinetics of the cell-fusion reaction in HIV infection, a reliable FACS-based method was developed to measure the cells and syncytia populations involved in the fusion of Env^+ with $CD4^+$ Jurkat cells when cocultured in vitro; the first mimicking an HIV infected cell and the second an uninfected $CD4^+$ lymphocyte (Huerta et al., 2002, 2005, 2006). Herein, we show that the cell fusion reaction approaches a maximum in a few hours and does not include all the cells in the coculture. Significant differences were observed in the total number of syncytia produced as well as their size, granularity and the ratios of fusion partners incorporated ($r = CD4^+/Env^+$), which varied in relation with the initial proportions of the cell fusion partners and the coculture time. The calculation of r is based on a simple mathematical treatment of flow cytometry data at different coculture times.

2. Materials and methods

2.1. Cells

Jurkat E6-1 and transfected cell lines HXBc2(4) (Env^+ cells) and 522F/Y containing an inducible tetracycline-dependent transactivator and the *env* gene from the HIV-1 HXBc2 strain coupled to a cytomegalovirus promoter and to tetracycline operator sequences (Cao et al., 1996), were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAD, NIH. HXBc2 (4) cell line express a functional gp120/gp41 glycoprotein, while 522F/Y cell line contains a mutant gene with a F/Y substitution at position 522 in gp41.

2.2. Fluorescent dyes

Membranes were stained with lipophilic red fluorescent DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate) and green fluorescent DiO (3,3′-dioctadecyloxacarbocyanine perchlorate). Cytoplasmic staining was performed with CellTracker Green CMFDA (5-chloromethylfluorescein diacetate), and CellTracker Orange CMTMR (5-(and-6)-((((4-chloromethyl) benzoyl) amino) tetramethylrhodamine. DNA staining was performed with Hoechst 33342. All dyes were obtained from Molecular Probes (Eugene, OR).

2.3. Cell culture

Env expressing Jurkat cell lines were grown in RPMI medium (Gibco BRL, Rockville, MD) containing 10% fetal bovine serum (Gibco BRL) (RPMI-10), 200 μg/ml of G418, 200 μg/ml of hygromycin, and 1 μg/ml of tetracycline. To induce *env* expression, cells were washed with PBS and cultured for 3 days in medium without tetracycline before fusion experiments (Cao et al., 1996). E6-1 cells were maintained in the same medium without the mentioned antibiotics.

2.4. Cell labelling and fusion assay

Labelling of cells with DiI and DiO was performed as described before (Huerta et al., 2002, 2006). Briefly, 11 μl of DiI or 20 μl of DiO working solutions were added to 1 ml of cells adjusted to 5–15 × 10^6 cells/ml in RPMI-10, and incubated 15 min at room temperature protected from light. After washing two times with 10 volumes of RPMI-10, cells were resuspended in the same medium (adding hygromycin and G418 to transfected lines) and maintained overnight. For standard fusion experiments, 0.2 × 10^6 labelled cells of each type were cocultured in a serum-free medium (AIM-V medium, Gibco BRL) and incubated at 37 °C, with 5% CO_2 for 5 or 8 h as indicated. For kinetic experiments, the proportion of fusion partners and incubation times were as indicated in the Figures. Finally, cells were collected from wells, washed with 3 ml of PBS, resuspended in 300 μl of FACSs buffer (Becton Dickinson) and analyzed immediately. Labelling with the cytoplasmic probes CellTracker Green CMFDA and CellTracker Orange CMTMR was similarly performed. Briefly, 1 ml of $CD4^+$ or Env^+ cells adjusted to 5–15 × 10^6 cells/ml in AIM-V medium was incubated with 5 μM of CMTMR or with 0.3 μM of CMFDA for 30 min at 37 °C and 5.0% CO_2, then washed with fresh prewarmed medium and incubated for another 30 min at 37 °C. After washing with 10 volumes of AIM-V medium, cells were resuspended in RPMI-10 (adding hygromycin and G418 to transfected lines) and maintained overnight.

2.5. FACS and apoptosis analyses

Flow cytometry analysis was performed on 10,000 events captured in a FACScalibur flow cytometer (Becton Dickinson, San Jose, CA) using the CellQuest software (Becton Dickinson). Loosely aggregated cells were dissociated by gently pippeting