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Monocyte-lymphocyte fusion induced by the HIV-1 envelope generates functional heterokaryons with an activated monocyte-like phenotype

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ABSTRACT

Enveloped viruses induce cell-cell fusion when infected cells expressing viral envelope proteins interact with target cells, or through the contact of cell-free viral particles with adjoining target cells. $CD4^+$ T lymphocytes and cells from the monocyte-macrophage lineage express receptors for HIV envelope protein. We have previously reported that lymphoid Jurkat T cells expressing the HIV-1 envelope protein (Env) can fuse with THP-1 monocytic cells, forming heterokaryons with a predominantly myeloid phenotype. This study shows that the expression of monocytic markers in heterokaryons is stable, whereas the expression of lymphoid markers is mostly lost. Like THP-1 cells, heterokaryons exhibited FcγR-dependent phagocytic activity and showed an enhanced expression of the activation marker ICAM-1 upon stimulation with PMA. In addition, heterokaryons showed morphological changes compatible with maturation, and high expression of the differentiation marker CD11b in the absence of differentiation-inducing agents. No morphological change nor increase in CD11b expression were observed when an HIV-fusion inhibitor blocked fusion, or when THP-1 cells were cocultured with Jurkat cells expressing a non-fusogenic Env protein, showing that differentiation was not induced merely by cell-cell interaction but required cell-cell fusion. Inhibition of TLR2/TLR4 signaling by a TIRAP inhibitor greatly reduced the expression of CD11b in heterokaryons. Thus, lymphocyte-monocyte heterokaryons induced by HIV-1 Env are stable and functional, and fusion prompts a phenotype characteristic of activated monocytes via intracellular TLR2/TLR4 signaling.

1. Introduction

Cell-cell fusion is conspicuous in physiological and pathological situations, such as development of metazoans, placentation, organ repair by stem cells, malignant transformation, viral infections, and reactions to tissue injury. Products of fusion between cells from different types are called heterokaryons, in opposition to syncytia, which originate from fusion of homologous cells. Major biological implications of cell-cell fusion are functional synchronization and cellular reprogramming [1]. Enveloped viruses can induce cell-cell fusion when infected cells expressing viral envelope proteins interact with target cells, or through the contact of cell-free viral particles with adjoining target cells [2,3].

Fused cells are frequently observed in cultures of human leukocytes infected with HIV-1 isolates from patients with HIV-1 infection [4]. In vivo, multinucleated cells expressing viral antigens are a hallmark of HIV-1 and SIV infections in the nervous system, and the presence of these cells is associated with severe neuropathology [5–9]. Infected multinucleated cells have been also observed in lymph nodes of asymptomatic HIV-1 infected individuals [10–17]. Expression of myeloid markers indicates that multinucleated cells may originate from dendritic cells [11,13,16] or macrophages [5,10,12,14,15,17,18]. Cell-cell fusion can be induced by interaction of membrane-bound HIV-1 envelope protein (Env) with the CD4 molecule and a coreceptor on a target cell. The interaction of the gp120 Env subunit with CD4 and with a molecule belonging to the family of chemokine receptors, induce conformational changes in the gp41 Env subunit, which then induce fusion of the cell membranes [2,3]. The origin and role of multinucleated cells in HIV-1 pathogenesis are not clear.

Human monocytes express CD4 and chemokine receptors [19], and are precursors of macrophages and dendritic cells. Monocytes are susceptible to infection by HIV-1 both in vitro [19] and in vivo [20],

Abbreviations: HIV-1, human immunodeficiency virus type 1; MFI, mean fluorescence intensity; FcγR, receptor for the Fc portion of IgG; Env, HIV-1 envelope protein; SIV, simian immunodeficiency virus; PMA, phorbol 12-myristate 13-acetate; DiI, 1,1'-dioctadecyl-3, 3,3', 3'-tetramethylindocarbocyanine perchlorate; DiO, 3,3'-dioctadecyloxacarbocyanine perchlorate; SRBC, Sheep red blood cells

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and they may contribute to viral dissemination and generation of virus reservoirs after differentiation [21,22]. On the other hand, $CD4^+$ T lymphocytes are the main targets of HIV-1, and their depletion in blood is related to AIDS. Given that $CD4^+$ monocytes and T lymphocytes can come in close proximity at sites of inflammation, it is possible that HIV Env expressed by infected cells, or virus particles, promote fusion between these cells.

THP-1 is a human monocytic $CD4^+$ cell line that can be induced to differentiate into a macrophage-like phenotype by PMA, and is widely used as a model of the monocyte/macrophage transition. THP-1 cells are susceptible to infection by HIV-1, even without activation by PMA [23,24]. We showed before that non-differentiated THP-1 cells are able to fuse with a HIV-1 Env-expressing lymphoid T cell line (Env^+ Jurkat cells), forming multinucleated heterokaryons. Heterokaryons did not arise when THP-1 cells were cocultured with Env^+ Jurkat cells expressing a mutant non-fusogenic gp41 protein, showing that a functional Env protein was necessary for the formation of heterokaryons. Participation of CD4 and Env in cell-cell fusion was further demonstrated by the inhibition of fusion by nanomolar concentrations of an anti-CD4 monoclonal antibody, or by the HIV-1 fusion inhibitor T-20, which specifically targets the fusogenic conformation of gp41 [25]. Some hours after formation, heterokaryons showed a decreased expression of lymphoid markers while preserving the expression of myeloid markers. The myeloid phenotype was predominant even when heterokaryons incorporated several lymphoid cells per each monocyte [26].

This study examined the stability of the lymphocyte-monocyte heterokaryon phenotype as well as some of its functional features. Thus, the expression of lymphocytic (CD3 and CD28) and myeloid (CD32 and CD68) markers in heterokaryons was analyzed during 3 days in cocultures of THP-1 and Env^+ Jurkat cells, and their phagocytic capacity and response to activation with PMA was determined. Since heterokaryons showed morphological changes compatible with differentiation in the absence of activating agents, we also evaluated the expression of the monocyte differentiation marker CD11b by the heterokaryons, as well as the effect of blocking of TLR4 and TLR2 signaling.

2. Materials and methods

2.1. Cell culture

Env^+ HXBc2(4) and 522F/Y Jurkat cells were obtained from the AIDS Research and Reference Reagent Program, Division of AIDS, NIAD, NIH. These cell lines contain an inducible tetracycline-dependent transactivator and are transfected with the *env* and *rev* genes from the HIV-1 HXBc2 strain coupled to a cytomegalovirus promoter and to tetracycline operator sequences [27]. This construction allows the expression of Env (gp120/gp41) after tetracycline removal from the culture medium. HXBc2(4) cells express a fusion competent Env protein, whereas the 522F/Y cell line do not fuse with target cells due to an F/Y substitution at position 522 in gp41 [27,28]. The THP-1 cell line was purchased from ATCC (ATCC TIB-202).

HXBc2 and 522F/Y cells were grown in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% of inactivated fetal bovine serum (Gibco) (RPMI-10), 200 μg/ml of geneticin and hygromycin, and 1 μg/ml of tetracycline. Cell cultures were maintained at 37 °C and 5% of CO_2. To induce Env expression, cells were washed with PBS and cultured in medium without tetracycline. After 24 h, cells were labeled with DiO as indicated below, and stained cells were kept in an incubator overnight before fusion experiments. Thus, fusion experiments were performed 48 h after induction of Env expression.

THP-1 cells were grown in RPMI 1640 supplemented with 10% of inactivated fetal bovine serum (Gibco) with 600 μg/ml of penicillin, 600 μg/ml of streptomycin, 2 mM L-glutamine, 100 μM non-essential amino acids and 1 mM of sodium pyruvate. Cells were maintained at a density of 0.5×10^6 cells/ml.

2.2. Cell-cell fusion assay

Fusion partners were stained with the red fluorescent DiI and green fluorescent DiO lipophilic dyes, obtained from Molecular Probes (Eugene, OR). DiI and DiO stock solutions were made by dissolving 3 mg/ml of dye in DMSO, and working solutions were prepared by diluting stocks 1:10 in the same solvent. Labeling of cells with DiI and DiO was performed as described before [29]. Cells were labeled the day before fusion experiments. Briefly, cells were adjusted to $5–15\times10^6$ cells/ml in RPMI-10 and 10 μl of DiO working solution were added to Env^+ HXBc2 or 522 F/Y cells (after 24 h of tetracycline deprivation), and 15 μl of DiI working solution were added to THP-1 cells. Cells suspensions were covered from light and incubated at room temperature for 10 min with gently shaking. Cells were then washed twice with 10 ml of RPMI-10 and resuspended in the same medium. Labeled cell cultures were incubated overnight at 37 °C and 5% of CO_2.

DiO-labeled Env^+ Jurkat and DiI-labeled THP-1 cells (which will be hereinafter called DIO-Env^+ Jurkat and DiI-THP-1 cells, respectively) were cocultured at a 1:1 ratio (4×10^5 cells of each type) in a 48- flat bottom well plate in a total volume of 800 μl. For the fusion inhibition assays, DiO-labeled Env^+ Jurkat cells were pre incubated for 30 min with 60 nM of the HIV fusion inhibitor peptide T-20 before addition of THP-1 cells. Unlabeled, as well as single DiO- and DiI-labeled cells were placed in separated wells to use them as controls for adjusting FACS settings. Each condition was tested in duplicate.

After the indicated times, cells were collected from wells, centrifuged to remove the culture medium and resuspended in 0.4 ml of FACS flow buffer. Cells were analyzed by flow cytometry using unlabeled and single labeled cells for setting's adjustment. A region of viable cells was selected and analyzed in FL-1 vs. FL-2 dot plots, where the percentage of single green-, single red-, and double fluorescent cells (unfused Env^+ Jurkat cells, unfused THP-1 cells, and heterokaryons, respectively) was determined on 10,000 events in an Attune cytometer (BD) equipped with red and blue lasers.

2.3. T-20 fusion inhibitor

T-20 is a synthetic 36 amino acid peptide comprising the heptad repeat region 2 (HR2) of gp41, and is complementary to gp41 HR1. It interferes specifically with the fusion process by impeding the assembly of the gp41 fusogenic conformation [25]. Fuzeon, the N-acetylated derivative of T-20 was obtained from Trimeris/Roche (Lexington, MA/Basel, Switzerland) and dissolved in PBS at 98 μg/ml.

2.4. Immunophenotyping

Cells were collected from plates after the indicated times in coculture, washed with 1 ml of PBS, resuspended in binding buffer and incubated with APC conjugated monoclonal antibodies against the CD3, CD28, CD32, or CD68 receptors (BD Pharmingen, San José, CA), and analyzed by flow cytometry as described before [26]. APC conjugated monoclonal antibodies against ICAM-1 and CD11b were purchased from eBioscience (San Diego, CA). The mean fluorescence intensity (MFI) index was calculated as the ratio of the geometric mean fluorescence intensity of the specific monoclonal antibody and the geometric mean fluorescence intensity of the isotype-matched control antibody.

2.5. Phagocytosis assay

SRBC were opsonized with anti-SRBC IgG (IgG-SRBC) for the measurement of FcγR-mediated phagocytosis as previously described [30] with some modifications. Briefly, SRBC (1.2×10^9 in PBS) were coated with 250 μg/ml of sulfo-NHS-biotin (Pierce, Rockford, IL) for 20 min at 4 °C. Next, they were washed and incubated with a sub-hemagglutinating dilution of rabbit anti-SBRC IgG for 30 min at room