Available online at www.sciencedirect.com
ScienceDirect
Virus Research 123 (2007) 178–189
Virus Research
www.elsevier.com/locate/virusres

The tyrosine kinase inhibitor genistein blocks HIV-1 infection in primary human macrophages

Tzanko S. Stantchev [a], Ingrid Markovic [b], William G. Telford [c], Kathleen A. Clouse [b], Christopher C. Broder [a,*]

[a] *Department of Microbiology and Immunology, F. Edward Hébert School of Medicine, Uniformed Services University Bethesda, 4301 Jones Bridge Road, MD 20814, USA*
[b] *Center for Drug Evaluation and Research, Food and Drug Administration, Bethesda, MD, USA*
[c] *Experimental Transplantation and Immunology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA*

Received 24 July 2006; received in revised form 6 September 2006; accepted 7 September 2006
Available online 9 October 2006

Abstract

Binding of HIV-1 envelope glycoprotein (Env) to its cellular receptors elicits a variety of signaling events, including the activation of select tyrosine kinases. To evaluate the potential role of such signaling, we examined the effects of the tyrosine kinase inhibitor, genistein, on HIV-1 entry and infection of human macrophages using a variety of assays. Without altering cell viability, cell surface expression of CD4 and CCR5 or their abilities to interact with Env, genistein inhibited infection of macrophages by reporter gene-encoding, β-lactamase containing, or wild type virions, as well as Env-mediated cell-fusion. The observation that genistein blocked virus infection if applied before, during or immediately after the infection period, but not 24 h later; coupled with a more pronounced inhibition of infection in the reporter gene assays as compared to both β-lactamase and p24 particle entry assays, imply that genistein exerts its inhibitory effects on both entry and early post-entry steps. These findings suggest that other exploitable targets, or steps, of the HIV-1 infection process may exist and could serve as additional opportunities for the development of new therapeutics. Published by Elsevier B.V.

Keywords: HIV; Macrophage; Tyrosine kinase; Genistein; Entry; Fusion; Receptor; CD4; CCR5; CXCR4; Envelope glycoprotein; gp120; Infection

1. Introduction

HIV-1 envelope glycoprotein (Env) interacts with CD4 and subsequently with a member of the chemokine receptor family, predominantly CCR5 and/or CXCR4, to gain access to the intracellular environment (reviewed in Berger et al., 1999). It is now well established that during the interactions with its receptors, the HIV-1 Env gp120 elicits various intracellular signaling events both in primary cells and cell lines (reviewed in Popik and Pitha, 2000; Stantchev and Broder, 2001), which are similar, but not identical to that caused by chemokines (Freedman et al., 2003; Liu et al., 2000).

Often given as an example of G-protein coupled, 7 transmembrane domain receptors (GPCR), that associates exclusively with pertussis toxin (PT) sensitive $G_{\alpha I}$ proteins, the chemokine receptors may also couple with other subclasses of G_{α} molecules, such as $G_{\alpha q}$ or $G_{\alpha s}$. The mode of these interactions is complex and may depend on the chemokine receptor molecule itself, the cell type or even the activation state of the cell. In addition to modulation of intracellular cAMP levels, chemokine receptors are also able to activate various non-receptor tyrosine kinases in both PT-sensitive and/or insensitive modes (reviewed in Rodriguez-Frade et al., 2005; Stantchev and Broder, 2001). The signaling pattern of CD4 is less complex and has been primarily associated with activation of the CD4 cytoplasmic tail-associated Src related tyrosine kinase, $p56^{lck}$. Interestingly however, a recent study has shown that CD4 is able to induce Ca^{2+} influx, tyrosine phosphorylation and activation of phospholipase C gamma (PLC-γ), phosphatidylinositol 3-kinase (PI-3K) and up-regulation of the stress activated protein kinases (SAPK) branch of the mitogen activated protein kinases (MAPKs) pathway in promonocytic cell lines, which like primary monocytes/macrophages do not express $p56^{lck}$ (Graziani-Bowering et al., 2002).

* Corresponding author. Tel.: +1 301 295 3401; fax: +1 301 295 1545.
E-mail address: cbroder@usuhs.mil (C.C. Broder).

0168-1702/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.virusres.2006.09.004

In macrophages gp120 binding caused an elevation of intracellular Ca^{2+} and interestingly the response to HIV-1 Envs from CCR5 using (R5) strains, which efficiently replicate in these cells, was markedly higher and more sustained in comparison to Envs derived from CXCR4 using (X4) strains (Arthos et al., 2000; Freedman et al., 2003). R5 Envs were also more potent than X4 ones in the activation of an array of genes in resting $CD4^+$ lymphocytes, that would subsequently favor the productive infection of these cells (Cicala et al., 2006). Furthermore, the gp120 induced Ca^{2+} elevation and activation of proline rich tyrosine kinase Pyk2 in macrophages occurred in a PT-independent manner (Freedman et al., 2003). Recently, CCR5 dependent activation of the Src kinase Lyn, in response to gp120 from the R5 strain JR-FL, has been recently described in macrophages as well (Tomkowicz et al., 2006). In addition, some of these processes such as the up-regulation of PI-3K, c-Jun and certain MAPKs in primary macrophages, following gp120 stimulation, have been described as events that are likely downstream effects of Ca^{2+} and/or tyrosine kinase signaling (Del Corno et al., 2001; Freedman et al., 2003; Lee et al., 2003; Tomkowicz et al., 2006). At present, the gp120 elicited signaling is usually associated with augmentation of HIV-1 replication in already infected cells (Cicala et al., 2002; Kinter et al., 2003) or the production of several pro-inflammatory cytokines, which in auto or paracrine fashion may alter the activation status of the cell, and hence HIV-1 infection (reviewed in Freedman et al., 2003). However, any direct role of gp120 induced signaling in the establishment of infection, such as with CCR5 dependent strains in macrophages, has yet to be shown.

Because of the multiple domains of GPCRs involved in their G protein dependent and independent signaling processes (Hall et al., 1999; Pierce et al., 2002), the generation of chemokine receptors completely devoid of any tyrosine kinase signaling properties, while at the same time maintaining normal cell surface expression-levels and gp120 binding ability have been difficult if not an impossible task. Also, gp120 has the potential to induce tyrosine kinase activation via both CD4 and CCR5 or CXCR4, suggesting the possibility that an impaired signaling through one of the receptors may be compensated for by the other. Finally, during progeny virion budding, HIV-1 itself may incorporate into its membrane a variety of different molecules including proteins which may subsequently interact with their counterparts on the host cell membrane (reviewed in Tremblay et al., 1998), resulting in tyrosine kinase activation and facilitation of virus fusion (Liao et al., 2000; Tardif and Tremblay, 2003). Here, we sought to explore the significance of tyrosine kinase signaling in the establishment of HIV-1 infection in a primary cell target by examining the effects of the broad spectrum tyrosine kinase inhibitor genistein using a battery of virus entry and infection assays.

2. Materials and methods

2.1. Reagents

The tyrosine kinase inhibitor, genistein, was obtained from Biomol (PA) and initially resuspended in hybridoma grade, endotoxin negative DMSO (Sigma–Aldrich (MO)) to initial concentration of 20 mg/ml. Potential endotoxin contamination of the genistein was excluded by the Kinetic Turbidimetric Method (test performed by Associates of Cape Cod, Inc., Falmouth, MA). The pNL4-3 HIV-1 backbone plasmids encoding the luciferase (Luc) or green fluorescence protein (GFP) reporter gene and pSV7d-Ba-Lgp160 were provided by Dr. R. Doms (University of Pennsylvania). The plasmids pSV7d-JR-FLgp160, pcDNA3.1(Zeo)-Ba-Lgp160, pDK38 (pLNCX2-CD4-Stag), pCG-VSV-G and pMM310 (pcDNA3.1(Zeo)-Vpr-BlaM) β-lactamase were supplied by Dr. G. Quinnan (Uniformed Services University) (USUHS), Dr. T.R.Fouts (University of Maryland), Dr. D. Khetawat (USUHS), Dr. P. Cannon (Childrens Hospital, LA) and Dr. M. Miller (Merck & Co., Inc., West Point, PA), respectively. The wild-type CD4-encoding recombinant vaccinia virus vCB3 was previously described (Broder et al., 1993). Recombinant human JR-FL gp120 (rgp120) was produced in BS-C-1 cells by recombinant vaccinia virus and purifying the protein by affinity chromatography using lentil lectin Sepharose 4B (Amersham Pharmacia Biotech, NJ) as previously described (Earl et al., 1994). pYK-JRCSF (Cann et al., 1990; Koyanagi et al., 1987), HIV-$1_{Ba\text{-}L}$ (Gartner et al., 1986) and HIV-$1_{92/UG/024}$ (the UNAIDS Network for HIV Isolation and Characterization and DAIDS, NIAID) were received through the AIDS Research and Reference Reagent Program, NIAID, NIH. The pAdVAntage vector and the Luciferase Assay System were purchased from Promega Corporation (Madison, WI). The CCF2/AM Beta lactamase Loading Kit (GeneBLAzer Reporter Assay) and Calcein AM were ordered from Invitrogen Corporation (CA). FuGENE 6 and Complete Mini protease inhibitor tablets were obtained from Roche Diagnostics. HIV-1 p24 and TNFα ELISA Kits were purchased from Beckman Coulter GmbH (Germany).

2.2. Cells and culture conditions

The 293T cells were obtained from Dr. G Quinnan (USUHS) and maintained in Dulbecco's modified Eagle's medium (Quality Biologicals, Gaithersburg, MD), 10% bovine calf serum (BCS), 2 mM L-glutamine, and antibiotics (DMEM-10) at 37 °C in a humidified 5% CO_2 atmosphere. Peripheral blood mononuclear cells (PBMC) were isolated from human blood following leukapheresis of HIV-1 seronegative donors and subsequent density gradient centrifugation; monocytes were purified by countercurrent centrifugal cell elutriation as previously described (Gerrard et al., 1983). Macrophages were prepared from elutriated monocytes by differentiation in 100 mm square Petri dishes (Bibbi Sterilin Ltd., Stone Staffs, UK) in DMEM supplemented with 10% human AB serum from several different sources, 2 mM L-glutamine and antibiotics (Broder et al., 1994; Lazdins et al., 1990). Macrophages were obtained after 7–14 days differentiation without exogenous growth factors and were either used immediately after the differentiation period or kept frozen in liquid nitrogen. The day before the experiment, the frozen cells were thawed, washed, centrifuged, resuspended in DM-10, put in the desired plate format and incubated at 37 °C overnight.