

# Up-Regulation of Integrins $\alpha 2 \beta 1$ and $\alpha 3 \beta 1$ Expression in Human Foreskin Fibroblast Cells after *In-Vitro* Infection with Herpes Simplex Virus Type 1

Shapour Shahgasempour<sup>\*1</sup>, Ali Shamsi Shahomabadi<sup>2</sup>, Farah-Dokht Fateminasab<sup>2</sup> and Abbas Barin<sup>3</sup>

<sup>1</sup>National Research Institute of Tuberculosis and Lung Disease, Shaheed Beheshti University of Medical Sciences, Darabad; <sup>2</sup>Dept. of Immunology, Iran University of Medical Sciences; <sup>3</sup>Dept. of Health Sciences, Tehran University, Tehran, Iran

## ABSTRACT

The interaction of Herpes Simplex Virus type 1 (HSV-1) with human fetal foreskin fibroblast (HFFF) cell was studied using a recent isolate of HSV-1 which was propagated in Hep-2 cells. HFFF cells were challenged with HSV-1 with a multiplicity of infection (MOI) of 1 virus/cell for 24 hours. Flow cytometric analysis demonstrated that HSV-1 challenged HFFF cells expressed increased levels of  $\alpha 2 \beta 1$ ,  $\alpha 3 \beta 1$  (a collagen and laminin receptor) and LFA-1 (a  $\alpha 2 \beta 1$  integrin, ligand for intercellular adhesion molecule-1, ICAM-1) integrins without a significant change in the expression of  $\alpha 5 \beta 1$  (a fibronectin receptor) as compared to the mock-challenged cells. In addition, virus challenged HFFF cells showed increased expression of ICAM-1 and HLA class I antigen when compared to mock-infected cells. These results suggest that HSV-1 is capable of inducing functional changes in human foreskin fibroblast cells. The altered expression of cell-matrix and cell-cell adhesion molecules resulting from HSV-1 infection could contribute to the immunopathological diseases associated with HSV-1 infection *in vivo*. *Iran. Biomed. J.* 5 (2 & 3): 55-59, 2001

**Keywords:** Fibroblasts, HSV-1, Integrin receptors, Adhesion molecules

## INTRODUCTION

Receptors play a crucial role in transduction of a variety of external stimuli and signals into a coordinated physiological response. The receptors in the plasma membrane where components of the extracellular matrix proteins (ECM) adhere are known as integrins [1]. Integrins are a group of heterodimeric cell surface receptors that bind to the ECM proteins such as fibronectin, collagen, vitronectin and laminin with cytoskeletal components, vinculin, talin, actin and tropomyosin. Integrins are distributed nearly on all tissues and cell types. They participate in cell-matrix and cell-cell adhesion processes involved in embryonic development, hemostasis, thrombosis, immune defense, cancer and wound healing [1]. Therefore, factors such as virus infection, that can affect the interaction of cells with the ECM, could consequently influence the normal function of the cells contributing to disease conditions. The striking aspect of the phenotype of the cells infected with

Herpes Simplex Virus type 1 (HSV-1) *in vitro* is the formation of syncytium with concomitant rounding [2]. The molecular mechanism of this phenomenon is unknown but molecules involved in cell-cell and cell-matrix interactions may be implicated. Most *in vitro* studies have attributed these morphological changes after HSV-1 infection to a reduction in host cell protein synthesis and an alteration in the cytoskeleton of the infected cells [2, 3]. While, others have shown that the reduced binding of the HSV-1-infected cells to ECM are due to the suppression in the synthesis of the ECM proteins [4]. Furthermore, studies have revealed that HSV-1 infected cells show increased adherence for leukocytes. It is assumed that this increased adhesion of leukocytes could be due to the increased expression of adhesion molecules.

Hence, to elucidate the molecular mechanism by which HSV-1 might contribute to the rounded phenotype appearance of cells and increased leukocyte adhesion, we studied the expression of integrin receptors, intercellular adhesion molecule-1

\*Corresponding Author.

(ICAM-1) and HLA-ABC after HSV-1 infection on HFFF cells. Here, we present data suggesting that HSV-1 infection of human fetal foreskin fibroblast (HFFF) cell increase the expression of  $\alpha 2$ ,  $\alpha 3$ , and LFA-1 without affecting the expression of  $\alpha 5\beta 1$  integrins. Also, an increase in the cell surface expression of ICAM-1 and HLA-ABC antigens was observed at 24 h post-challenge.

## MATERIALS AND METHODS

**Cell culture.** HFFF cells obtained from the Pasteur Institute of Iran (Tehran) and subcultured using trypsin/EDTA. The subcultures were grown in RPMI supplemented with 10% heat-inactivated fetal calf serum (FCS), 110  $\mu$ g/ml streptomycin and 60  $\mu$ g/ml penicillin and 2 mM L-glutamine.

**Virus stock preparation.** Hep-2 cells were obtained from the Pasteur Institute of Iran (Tehran) were infected with HSV-1 at 70-80% confluence. When the monolayers exhibited 90% cytopathic effect, the supernatant was collected and centrifuged at 5,000 rpm for 20 min and then mixed with an equal volume of 70% sorbitol and stored in 1 ml aliquots at  $-80^{\circ}\text{C}$  until use. Virus titer was determined, as described previously, by the tissue culture infectious dose 50 (TCID<sub>50</sub>) method [5, 6]. Mock stocks were prepared similar to the virus stock except it was prepared from uninfected monolayers.

**Flow cytometric analysis of integrin and adhesionmolecule expression.** Briefly, mock-stocks and HSV-1 challenged HFFF monolayers were dislodged from the tissue culture flasks and suspended in a small volume of PBS containing 10% FCS and 0.1%  $\text{NaN}_3$  and then were incubated for 20 min. After three washes, cells were incubated with anti-  $\alpha 2$ , -  $\alpha 3$ , -  $\alpha 5$ , -LFA-1, -ICAM-1 (Calbiochem, Switzerland) and anti-HLA class I (Dako, Denmark) monoclonal antibodies in PBS containing 10% FCS at  $4^{\circ}\text{C}$  for 45 min. Cells were then washed three times and incubated with 1/20 dilution of FITC-conjugated goat anti-mouse antibody (Dako, Denmark) in PBS containing 10% FCS and 0.1%  $\text{NaN}_3$  at  $4^{\circ}\text{C}$  for 30 min, followed by three washes. Ten to twenty thousand cells were analyzed by a FACSCalibur (Becton-Dickinson, USA) cell sorter equipped with a 15 m-W argon-ion laser. Negative controls consisted of cells treated with isotype-matched antibodies [6, 7].

**Statistical analysis.** Results were expressed as the mean  $\pm$  SD and the statistical significance was analyzed using student's *t*-test. The fluorescent intensity of antigen expression was determined by the Cell Quest software supplied by the instrument manufacturer.

## RESULTS

**Isolation and identification of HSV-1.** A clinical isolate was obtained from the blister of a 45-year-old man. HSV-1 infection was confirmed by an anti-HSV-1 and anti-HSV-2 antibodies (Dako, Denmark) conjugated to FITC in the infected Hep-2 cell culture.

**Modulation of integrin expression.** HFFF cells were infected with HSV-1 with a multiplicity of infection (MOI) of 1 virus/cell for 24 h and then the expression of  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 5$ , and LFA-1 integrins on mock- and HSV-1 infected HFFF was investigated using flow cytometry. The results presented in Table 1 demonstrate that the infection of HFFF with HSV-1 appears to up-regulate the cell surface expression of  $\alpha 2$ ,  $\alpha 3$  and LFA-1 integrins on infected cells as compared to mock challenged cells. On the other hand, the expression of  $\alpha 5$  showed no significant alteration when compared to control cells after 24 h post-infection.

**Alteration of adhesion molecule expression.** Flow cytometric analysis revealed that there was a statistically significant increase in the level of ICAM-1 antigen expression by 24 h post-infection on HSV-1 infected cells when compared to mock-challenged cells (Fig. 1).

In addition, flow cytometric analysis of the expression of HLA class I antigen on mock- and HSV-1 infected cells at 24 h post-infection revealed a significant increase in the level of HLA class I antigen expression on HSV-1 challenged cells (Fig. 1).

## DISCUSSION

The aim of the present study was to set up an *in vitro* experimental system for the evaluation of the expression of integrin receptors and adhesion molecules on HSV-1 infected HFFF cells. The use of this kind of *in vitro* system will enable us to understand the molecular mechanism of the pathogenicity of viral infection.

**Table 1.** Levels of expression of integrins and adhesion molecule and HLA class I antigen in HSV-1 infected human fetal foreskin fibroblasts.

Antigen	Cell surface expression (mean fluorescence intensity)	
	Uninfected HFFF	HSV-1-infected HFFF
2 1	499.80	1242.40
3 1	90.98	546.40
a5b1	301.85	304.28
LFA-1	61.74	70.15
ICAM-1	90.57	332.80
HLA-ABC	409.17	591.61

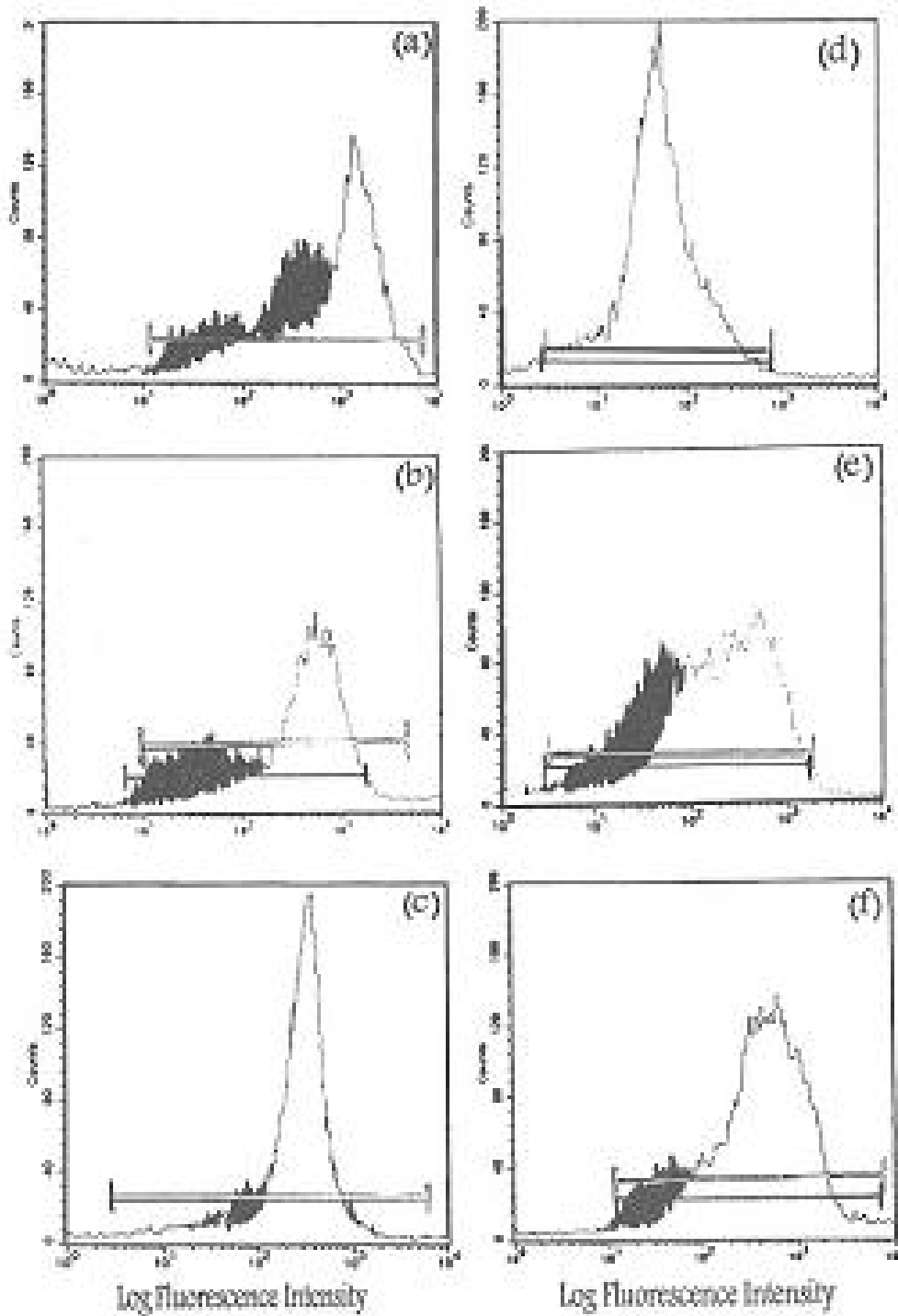
The data presented here suggest that the infection of HFFF cells with HSV-1 selectively alter the expression of integrin receptors. Our data show that HSV-1 infection of human foreskin fibroblasts results in an increase in the cell surface expression of integrins 2 1, 3 1 and LFA-1 while having no effect on the expression of integrin a5b1. The increased expression in 2 1 and 3 1 and LFA-1 at the surface of infected cells could be due to an increase in either  $\alpha$  or  $\beta$  chain biosynthesis. Normally, the  $\alpha$  chain is synthesized in excess compared with a  $\beta$  chain, and then the two chains associate intercellularly before transport to the cell surface [8]. The altered expression of these molecules on the challenged cells could be due to several factors. Firstly, the up-regulation of 2 1, 3 1 and LFA-1 integrins could be attributable to production of soluble factor(s) either present in the virus inoculum or released into the medium during the course of HSV-1 infection, since integrin expression can be modulated by cytokines. For example, interleukin-1 (IL-1) and tumor necrosis factor (TNF-) up-regulate a1b1 expression in human skin fibroblasts by increasing the expression of a  $\beta$  chain without affecting the  $\alpha$  chain [5, 9]. In addition, it is likely that HSV-1 infection may induce a factor(s), particularly growth factor(s) such as basic fibroblast growth factor (bFGF), and/or transforming growth factor- $\beta$  (TGF- $\beta$ ) that particularly affect the a chain synthesis of the integrin heterodimer [5,10,11]. Hence, it would be necessary to use virus-free supernatants from HSV-1 infected fibroblasts to test this possibility.

A second possible explanation for the altered

expression of integrin receptors could be due to the binding of HSV-1 to its receptors or release of viral proteins within the virus particle. Finally, viral replication (gene expression) within the infected cells results in the production of proteins that, either directly or indirectly, alter integrin expression selectively. Further studies with UV-inactivated virus are required to address the above-mentioned possibilities.

Numerous studies have shown that infection of various cell types with HSV or cytomegalovirus (CMV) results in the increased adherence of leukocytes [7, 12, 13]. Since adhesion molecules and HLA are the main receptors that mediate homotypic and heterotypic cell-cell interactions, we studied the expression of ICAM-1 and HLA class I molecules on the HSV-1 infected fibroblasts. We report here that HSV-1 induces up-regulation of ICAM-1 and HLA class I on the surface of challenged fibroblasts at 24 h post-infection. It is expected that the interaction between ICAM-1 and HLA class I with their ligands, LFA-1 and CD8 respectively, play a role in the increased adhesion of cells to HSV-1 infected cells. Recently, a similar result was reported by Stuart *et al.* [13] demonstrating that the infection of human corneal fibroblasts with HSV-1 increased the cell surface expression of ICAM-1, suggesting that the up-regulation was not a direct result of productive infection, but of other mechanisms. We try to show whether the increased expression of ICAM-1 and HLA class I are due to the production of soluble factor(s) and/or due to viral gene expression. The up-regulation of ICAM-1 and HLA class I antigens has been shown to play important roles in initiation and amplification of immune responses and inflammation, not only as the mediators of the leukocyte recognition of the infected cells but also as the markers of many diseases [7, 14, 15].

Whilst the importance of these observations reported in this paper is yet to be understood, integrins and adhesion molecules play important roles in cell-substratum and cell-cell adhesion processes. Thus, changes in the expression of these molecules by HSV-1 may have important consequences with respect to the development of certain HSV-induced diseases.



**Fig. 1.** Flow cytometric analysis of integrin receptors and adhesion molecules on mock- and HSV-1-infected HFFF cells. HFFF cells were harvested after 24 h post-infection with mock or HSV-1 and were analyzed by flow cytometry after immunofluorescence staining using (a) anti- 2 , (b) anti- 3 1, (c) anti-LFA-1, (d) anti- 5 1, (e) anti-ICAM-1 and (f) anti-HLA-ABC antibodies. Filled histogram represents mock-infected and red histogram represents HSV-1-infected HFFF cells.

## REFERENCES

1. Hynes, R.O. (1992) Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* 60: 11-25.
2. Macarak, J., Friedman, N.M. and Kefalides, N.A. (1985) Herpes simplex virus type 1 infection of endothelium reduces collagen and fibronectin synthesis. *Lab. Invest.* 53: 280-286.
3. Kaner, R.J., Iozzo, R.V., Ziaie, Z. and Kefalides, N.A. (1990) Inhibition of proteoglycans synthesis in human endothelial cells after infection with herpes simplex virus type 1 *in vitro*. *Am. J. Respir. Cell Mol. Biol.* 2: 423-431.
4. Ziaie, Z., Friedman, H.M. and Kefalides, N.A. (1986) Suppression of matrix protein synthesis by herpes simplex virus type 1 in human endothelial cells. *Collagen Rel. Res.* 6: 333-350.
5. Shahgasempour, S., Woodroffe, S.B., Sullivan-Tailyour, G. and Garnett, H.M. (1997) Alteration in the expression of endothelial cell integrin receptors  $\alpha 5\beta 1$  and  $\alpha 2\beta 1$  and  $\alpha 6\beta 1$  after *in vitro* infection with a clinical isolate of human cytomegalovirus. *Arch. Virol.* 142: 125-138.
6. Shahgasempour, S., Woodroffe, S.B. and Garnett, H.M. (1998) Modulation of HLA class I antigen and ICAM-2 on endothelial cells after *in vitro* infection with human cytomegalovirus. *Immunol. Cell Biol.* 76: 217-221.
7. Shahgasempour, S., Woodroffe, S.B. and Garnett, H.M. (1997) Alterations in the expression of ELAM-1, ICAM-1 and VCAM-1 after *in vitro* infection of endothelial cells with a clinical isolate of human cytomegalovirus. *Microbiol. Immunol.* 41: 121-129.
8. Warren, A.P., Owens, C.N., Borysiewicz, L.K. and Patel, K. (1994) Down-regulation of integrin  $\alpha 1\beta 1$  expression and association with cell rounding in human cytomegalovirus-infected fibroblasts. *J. Gen. Virol.* 75: 3319-3325.
9. Heino, M.E., Ignatz, R.A., Hemler, M.E., Crouse, C. and Massague, J. (1989) Regulation of cell adhesion receptors by TGF- $\beta$ : Concomitant regulation of integrins that share a common  $\beta 1$  subunit. *J. Biol. Chem.* 264: 380-388.
10. Defilippi, P., Bozzo, C., Geuna, M., Rossino, P., Silengo, L. and Tarone, G. (1992) Modulation of extracellular matrix receptors (integrins) on human endothelial cells by cytokines. In: *Angiogenesis*. (Steiner, R., Weisz, P.B. and Langer, R eds.). pp: 193-197.
11. Grundy, J.E., Pahal, G.S. and Akbar, A.N. (1993) Increased adherence of CD2 peripheral blood lymphocytes to cytomegalovirus-infected fibroblasts is blocked by anti-LFA-3 antibody. *Immunol.* 78: 413-420.
12. Lewandowski, G. (1997) Immunohistochemical examination of intracerebral T cell and adhesion molecule induction in herpes simplex virus-infected mice. *Brain Behav. Immun.* 11: 254-272.
13. Stuart, P.M., Usui, N., Randhawa, R.S., Laycock, K.A., Fleming, T.P. and Pepose, J.S. (1997) Differential effects of HSV-1 and CMV infection on adhesion molecule expression on human corneal keratocytes. *Curr. Eye Res.* 16: 496-502.
14. Ito, M., Watanabe, M., Ihara, T., Kamiya, I.T. and Sakura, M. (1995) Increased expression of adhesion molecules (CD54, CD29 and CD44) on fibroblasts infected with cytomegalovirus. *Microbiol. Immunol.* 39(2): 129-133.
15. Meng, H., Marchese, M.J. and Garlick, J.A. (1995) Mast cells induce T-cell adhesion to human fibroblasts by regulating intercellular adhesion molecule and vascular cell adhesion molecule-1 expression. *J. Invest. Dermatol.* 105: 789-795.