Short Communication

Transcriptional downregulation of DC-SIGN in human herpesvirus 6-infected dendritic cells

Hironari Niiya, Taichi Azuma, Lei Jin, Naoyuki Uchida, Atsushi Inoue, Hitoshi Hasegawa, Shigeru Fujita, Mikiko Tohyama, Koji Hashimoto and Masaki Yasukawa

First Department of Internal Medicine and Department of Dermatology, Ehime University School of Medicine, Shigenobu, Ehime 791-0295, Japan

Correspondence
Masaki Yasukawa
yasukawa@m.ehime-u.ac.jp

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DC-SIGN expressed on dendritic cells (DCs) efficiently binds and transmits various pathogens, including human immunodeficiency virus, to lymphoid tissues and permissive cells. Consequently, alteration of DC-SIGN expression may affect susceptibility and resistance to pathogens. The present study shows that infection with human herpesvirus 6 (HHV-6) induces downregulation of DC-SIGN expression on immature DCs. Expression levels of DC-SIGN mRNA and intracellular protein appeared to decrease following infection with HHV-6, indicating that downregulation of surface DC-SIGN occurs at the transcriptional level. Downregulation of DC-SIGN was not induced by inoculation of UV-inactivated HHV-6 or culture supernatant of HHV-6-infected DCs, indicating that replication of HHV-6 in DCs is required for downregulation of DC-SIGN. The present study demonstrates for the first time that expression of DC-SIGN is altered at the transcriptional level by virus infection.

Human herpesvirus 6 (HHV-6) exhibits tropism mainly for leukocytes, including T lymphocytes, monocytes/macrophages and dendritic cells (DCs) (Dockrell, 2003). Among them, DCs are considered to be the professional antigen-presenting cells, possessing the ability to activate the immune response by capturing antigens in peripheral tissues and migrating to secondary lymphoid organs, where they sensitize naive T lymphocytes to the antigens. It has been reported that various viruses, including HHV-6, induce phenotypic and functional alterations of DCs (Salio *et al.*, 1999; Ho *et al.*, 2001; Kakimoto *et al.*, 2002).

DC-SIGN, also called CD209, has recently been identified as a cellular adhesion molecule on DCs by the generation of antibodies that inhibit binding to intracellular adhesion molecule-3 (ICAM-3) (Geijtenbeek *et al.*, 2000a). DC-SIGN is a type II transmembrane protein that belongs to the C-type lectin family. The interaction of DC-SIGN with ICAM-3 mediates the early transient clustering of DCs and T lymphocytes necessary for T lymphocyte activation. Another important function of DC-SIGN is interaction with ICAM-2. The interaction of DC-SIGN with ICAM-2 mediates rolling along endothelial linings and transmigration of DCs into the periphery (Geijtenbeek *et al.*, 2000b).

DC-SIGN has also been identified as the human immunodeficiency virus 1 (HIV-1) *cis*- and *trans*-receptor on DCs (Geijtenbeek *et al.*, 2000c). DC-SIGN binds HIV-1 and transmits it very efficiently to neighbouring permissive target cells. It has been reported that DC-SIGN also interacts with various micro-organisms, including Ebola virus (Alvarez *et al.*, 2002), hepatitis C virus (Pohlmann *et al.*, 2003), human cytomegalovirus (Halary *et al.*, 2002), dengue virus (Tassaneetrithep *et al.*, 2003), *Leishmania* (Colmenares *et al.*, 2002), *Mycobacterium tuberculosis* (Geijtenbeek *et al.*, 2003), *Candida albicans* (Cambi *et al.*, 2003) and *Schistosoma mansoni* (van Die *et al.*, 2003). These recent findings strongly suggest that alteration of DC-SIGN expression may affect the immune response as well as susceptibility to and resistance against various pathogens. In the present study, we examined the effect of HHV-6 on the expression of DC-SIGN and the importance of this molecule in HHV-6 infection of DCs.

The U1102 strain of HHV-6A and the Z29 strain of HHV-6B were used in the present study. Immature DCs were generated from peripheral blood monocytes by culturing in the presence of granulocyte–macrophage colony-stimulating factor and IL4, as described previously (Romani *et al.*, 1996). Immature DCs were inoculated with HHV-6 at an approximate m.o.i. of 1 $TCID_{50}$.

Replication of HHV-6 was examined quantitatively by real-time PCR, as described previously (Locatelli *et al.*, 2000). Sequences of the primers were 5′-TTTGCAGTCATCACG-ATCGG-3′ and 5′-AGAGCGACAAATTGGAGGTTTC-3′. The sequence of the fluorogenic probe was 5′-AGCCACAG-CAGCCATCTACATCTGTCAA-3′.

The expression of cell-surface molecules on DCs was examined by flow cytometric analysis using the following monoclonal antibodies (mAbs): anti-CD80, anti-CD83, anti-CD86 and anti-DC-SIGN (clone AZND1) (all from Immunotech). Hybridomas producing anti-DC-SIGN mAbs 3G2 and 6D1 were established in our laboratory by immunizing mice with human *DC-SIGN* gene-transfected K562 cells (K562-DC-SIGN), which were established as follows. The whole coding region of the *DC-SIGN* gene was obtained by RT-PCR as described previously (Baribaud *et al.*, 2001). The cDNA for *DC-SIGN* was transfected stably into an erythroleukaemia cell line, K562, by use of the pCXN2 vector (Niwa *et al.*, 1991). The mAbs 3G2 and 6D1 specifically recognize K562-DC-SIGN but not parent K562 cells. Expression of intracellular DC-SIGN was examined as follows. Mock-infected and HHV-6-infected DCs were cultured in the presence of Brefeldin A overnight and the cells were then fixed with 3·0 % formaldehyde, permeabilized with 0·05 % saponin and incubated with FITC-conjugated anti-DC-SIGN mAb AZND1. After being washed, the cells were analysed with a FACSCalibur (Becton Dickinson).

Expression of mRNA for *DC-SIGN* was examined by semi-quantitative RT-PCR and Northern blot analysis. Sequences of the primers for PCR were 5′-CTGCAACTCCTCTCC-TTCAC-3′ and 5′-TCGTTCCAGCCATTGCCACT-3′.

The role of DC-SIGN in *cis*- and *trans*-infection of HHV-6 was examined with K562-DC-SIGN cells and anti-DC-SIGN mAbs. Immature DCs were incubated with anti-DC-SIGN mAbs for 1 h and inoculated with HHV-6. After incubation for 1 h, DCs were washed extensively and cultured for 5 days. HHV-6 copy numbers in DCs treated with and without anti-DC-SIGN mAbs were determined by real-time RT-PCR as described above. To examine *trans*-infection of HHV-6, K562-DC-SIGN and parent K562 cells were inoculated with HHV-6. After incubation for 1 h, the cells were washed extensively and frozen and thawed. These cell lysates were added to cord blood lymphocytes that had been stimulated with phytohaemagglutinin (PHA) and the lymphocytes were cultured for 5 days. HHV-6 copy numbers were determined as described above.

Fig. 1(a) shows phenotypic changes of surface molecules on immature DCs induced by HHV-6B infection. As we reported previously (Kakimoto *et al.*, 2002), the surface expression levels of CD80, CD83 and CD86 increased following infection with HHV-6B. Expression of surface DC-SIGN appeared to decrease significantly after infection with HHV-6B. Downregulation of DC-SIGN appeared to require the replication of HHV-6, since UV light-inactivated HHV-6 showed no effect on DC-SIGN expression (data not shown). The kinetic study showed that downregulation of DC-SIGN on HHV-6-infected DCs was first detectable 3 days after HHV-6 infection. Similar results were also obtained in HHV-6A-inoculated immature DCs (data not shown). Flow cytometric analysis revealed that the expression level of intracellular DC-SIGN in DCs also decreased following infection with HHV-6 (Fig. 1b). As shown in Fig. 1(c), the downregulation of DC-SIGN in HHV-6-infected DCs was confirmed by Western blot analysis using whole-cell lysates.

To investigate the level at which the expression of DC-SIGN was downregulated in HHV-6-infected DCs,

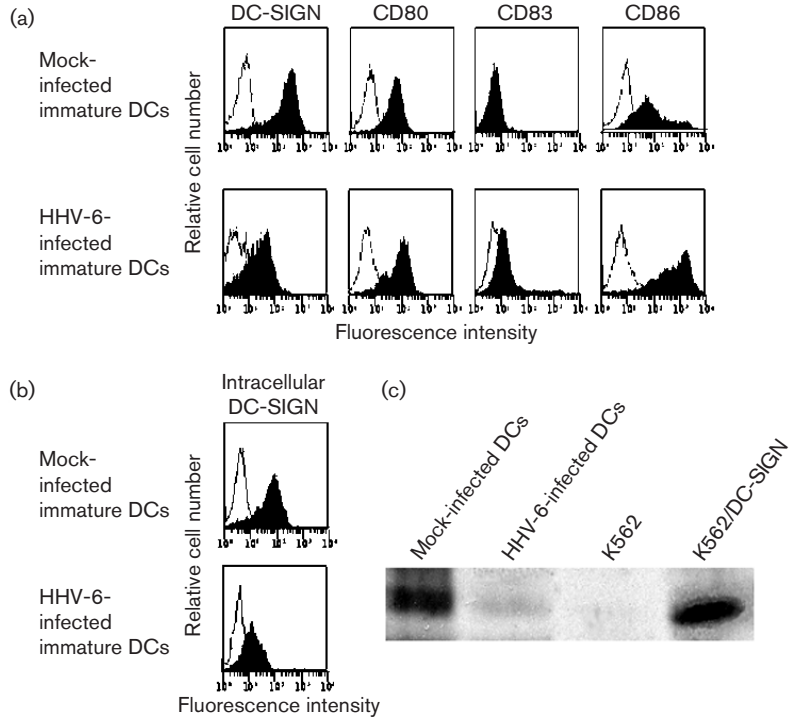

Fig. 1. Downregulation of surface and intracellular DC-SIGN by HHV-6 infection. (a) Flow cytometric analysis of cell-surface molecules on mock-infected and-HHV-6-infected immature DCs. Staining with negative-control Abs is shown as open histograms. (b) Flow cytometric analysis of intracellular DC-SIGN in mock-infected and HHV-6-infected DCs. (c) Western blot analysis of DC-SIGN protein in mock-infected DCs, HHV-6-infected DCs, K562 cells and K562-DC-SIGN cells. Whole-cell extracts were subjected to Western blotting using anti-DC-SIGN mAb.

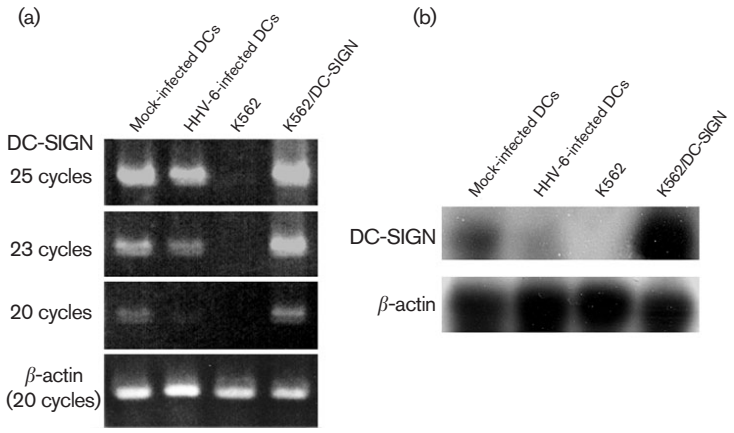

Fig. 2. Transcriptional downregulation of DC-SIGN by HHV-6 infection. Semi-quantitative RT-PCR of mRNA (a) and Northern blot analysis of RNA (b) for *DC-SIGN* in mock-infected DCs, HHV-6-infected DCs, K562 cells and K562-DC-SIGN cells.

semi-quantitative RT-PCR and Northern blot analysis of mRNA for *DC-SIGN* was performed. As shown in Fig. 2(a, b), the expression level of mRNA for *DC-SIGN* decreased significantly following infection with HHV-6, indicating that downregulation of DC-SIGN by HHV-6 infection is induced at the transcriptional level.

DC-SIGN has been reported to act as a receptor for *trans*-infection by various pathogens. In the present study, we investigated the possibility that HHV-6 infects *in cis* or *in trans* via binding with DC-SIGN. Although CD46, a cellular receptor for HHV-6 (Santoro *et al.*, 1999), is expressed on K562 cells, K562-DC-SIGN cells, as well as parent K562 cells, appeared to be resistant to HHV-6 infection. Furthermore, none of the three anti-DC-SIGN mAbs, AZND1, 3G2 and 6D1, which react to different epitopes on DC-SIGN, was able to inhibit HHV-6 infection of immature DCs, which are permissive to HHV-6 infection. In addition, K562-DC-SIGN cells did not transfer HHV-6 to PHA-stimulated cord blood lymphocytes, which are highly sensitive to HHV-6 infection and replication (detailed data not shown). These data indicated that DC-SIGN is not necessary for binding of HHV-6 on the cell surface.

The present study demonstrates for the first time that viral infection directly alters expression of DC-SIGN, which is an important molecule for *trans*-infection by various pathogens, including HIV. It has recently been reported that the expression of DC-SIGN is affected by cytokines: DC-SIGN expression is IL4 dependent and negatively regulated by IFN-α, IFN-γ and transforming growth factor β (Relloso *et al.*, 2002). It has also been reported that HHV-6 infection induces and inhibits production of various kinds of cytokine (Flamand *et al.*, 1991; Mayne *et al.*, 2001; Smith *et al.*, 2003). Taken together, it seems possible that cytokines produced by DCs following infection with HHV-6 might inhibit DC-SIGN expression. However, this possibility seems unlikely, because the repeated addition of culture supernatant from HHV-6-infected DCs did not affect the expression level of DC-SIGN (data not shown). In addition, expression of DC-SIGN was not altered by inoculation of inactivated HHV-6, strongly suggesting that replication of HHV-6 in DCs is essential to induce transcriptional downregulation of DC-SIGN.

Downregulation of DC-SIGN induced by HHV-6 infection may provide new insights into the role of HHV-6 in the pathogenesis of various infectious diseases, especially HIV infection. We reported previously that HHV-6 infection induces downregulation of CXCR-4, resulting in resistance of $CD4^+$ T lymphocytes to X4 HIV infection (Yasukawa *et al.*, 1999; Hasegawa *et al.*, 2001). It was reported that HHV-6 suppressed R5- but not X4-tropic HIV-1 replication in lymphoid tissue (Grivel *et al.*, 2001). Downregulation of DC-SIGN induced by HHV-6 infection may render DCs resistant to HIV infection. On the other hand, HHV-6 infection was reported to induce upregulation of CD4 (Lusso *et al.*, 1991), which is the main receptor for HIV. In addition, HHV-6 was shown to induce *trans*-activation of the HIV long terminal repeat and cell death (Ensoli *et al.*, 1989). Therefore, the interaction between HHV-6 and HIV appears to be complex. Further *in vivo* studies using HHV-6-permissive animals are necessary to clarify this important issue.

References

Alvarez, C. P., Lasala, F., Carrillo, J., Muniz, O., Corbi, A. L. & Delgado, R. (2002). C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. *J Virol* **76**, 6841–6844.

Baribaud, F., Pohlmann, S., Sparwasser, T. & 13 other authors (2001). Functional and antigenic characterization of human, rhesus macaque, pigtailed macaque, and murine DC-SIGN. *J Virol* **75**, 10281–10289.

Cambi, A., Gijzen, K., de Vries, J. M. & 7 other authors (2003). The C-type lectin DC-SIGN (CD209) is an antigen-uptake receptor for *Candida albicans* on dendritic cells. *Eur J Immunol* **33**, 532–538.

Colmenares, M., Puig-Kroger, A., Pello, O. M., Corbi, A. L. & Rivas, L. (2002). Dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN, CD209), a C-type surface lectin in human DCs, is a receptor for *Leishmania amastigotes*. *J Biol Chem* **277**, 36766–36769.

Dockrell, D. H. (2003). Human herpesvirus 6: molecular biology and clinical features. *J Med Microbiol* **52**, 5–18.

Ensoli, B., Lusso, P., Schachter, F., Josephs, S. F., Rappaport, J., Negro, F., Gallo, R. C. & Wong-Staal, F. (1989). Human herpes virus-6 increases HIV-1 expression in co-infected T cells via nuclear factors binding to the HIV-1 enhancer. *EMBO J* **8**, 3019–3027.

Flamand, L., Gosselin, J., D'Addario, M., Hiscott, J., Ablashi, D. V., Gallo, R. C. & Menezes, J. (1991). Human herpesvirus 6 induces interleukin-1 beta and tumor necrosis factor alpha, but not interleukin-6, in peripheral blood mononuclear cell cultures. *J Virol* **65**, 5105–5110.

Geijtenbeek, T. B., Torensma, R., van Vliet, S. J., van Duijnhoven, G. C., Adema, G. J., van Kooyk, Y. & Figdor, C. G. (2000a). Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell* **100**, 575–585.

Geijtenbeek, T. B., Krooshoop, D. J., Bleijs, D. A., van Vliet, S. J., van Duijnhoven, G. C., Grabovsky, V., Alon, R., Figdor, C. G. & van Kooyk, Y. (2000b). DC-SIGN–ICAM-2 interaction mediates dendritic cell trafficking. *Nat Immunol* **1**, 353–357.

Geijtenbeek, T. B., Kwon, D. S., Torensma, R. & 9 other authors (2000c). DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell* **100**, 587–597.

Geijtenbeek, T. B., Van Vliet, S. J., Koppel, E. A., Sanchez-Hernandez, M., Vandenbroucke-Grauls, C. M., Appelmelk, B. & Van Kooyk, Y. (2003). Mycobacteria target DC-SIGN to suppress dendritic cell function. *J Exp Med* **197**, 7–17.

Grivel, J. C., Ito, Y., Faga, G., Santoro, F., Shaheen, F., Malnati, M. S., Fitzgerald, W., Lusso, P. & Margolis, L. (2001). Suppression of CCR5- but not CXCR4-tropic HIV-1 in lymphoid tissue by human herpesvirus 6. *Nat Med* **7**, 1232–1235.

Halary, F., Amara, A., Lortat-Jacob, H. & 7 other authors (2002). Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. *Immunity* **17**, 653–664.

Hasegawa, A., Yasukawa, M., Sakai, I. & Fujita, S. (2001). Transcriptional down-regulation of CXC chemokine receptor 4 induced by impaired association of transcription regulator YY1 with c-Myc in human herpesvirus 6-infected cells. *J Immunol* **166**, 1125–1131.

Ho, L. J., Wang, J. J., Shaio, M. F., Kao, C. L., Chang, D. M., Han, S. W. & Lai, J. H. (2001). Infection of human dendritic cells by dengue virus causes cell maturation and cytokine production. *J Immunol* **166**, 1499–1506.

Kakimoto, M., Hasegawa, A., Fujita, S. & Yasukawa, M. (2002). Phenotypic and functional alterations of dendritic cells induced by human herpesvirus 6 infection. *J Virol* **76**, 10338–10345.

Locatelli, G., Santoro, F., Veglia, F., Gobbi, A., Lusso, P. & Malnati, M. S. (2000). Real-time quantitative PCR for human herpesvirus 6 DNA. *J Clin Microbiol* **38**, 4042–4048.

Lusso, P., De Maria, A., Malnati, M., Lori, F., DeRocco, S. E., Baseler, M. & Gallo, R. C. (1991). Induction of CD4 and susceptibility to HIV-1 infection in human $CD8^+$ T lymphocytes by human herpesvirus 6. *Nature* **349**, 533–535.

Mayne, M., Cheadle, C., Soldan, S. S. & 7 other authors (2001). Gene expression profile of herpesvirus-infected T cells obtained using immunomicroarrays: induction of proinflammatory mechanisms. *J Virol* **75**, 11641–11650.

Niwa, H., Yamamura, K. & Miyazaki, J. (1991). Efficient selection for high-expression transfectants with a novel eukaryotic vector. *Gene* **108**, 193–199.

Pohlmann, S., Zhang, J., Baribaud, F. & 7 other authors (2003). Hepatitis C virus glycoproteins interact with DC-SIGN and DC-SIGNR. *J Virol* **77**, 4070–4080.

Relloso, M., Puig-Kroger, A., Pello, O. M. & 7 other authors (2002). DC-SIGN (CD209) expression is IL-4 dependent and is negatively regulated by IFN, TGF-β, and anti-inflammatory agents. *J Immunol* **168**, 2634–2643.

Romani, N., Reider, D., Heuer, M., Ebner, S., Kampgen, E., Eibl, B., Niederwieser, D. & Schuler, G. (1996). Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *J Immunol Methods* **196**, 137–151.

Salio, M., Cella, M., Suter, M. & Lanzavecchia, A. (1999). Inhibition of dendritic cell maturation by herpes simplex virus. *Eur J Immunol* **29**, 3245–3253.

Santoro, F., Kennedy, P. E., Locatelli, G., Malnati, M. S., Berger, E. A. & Lusso, P. (1999). CD46 is a cellular receptor for human herpesvirus 6. *Cell* **99**, 817–827.

Smith, A., Santoro, F., Di Lullo, G., Dagna, L., Verani, A. & Lusso, P. (2003). Selective suppression of IL-12 production by human herpesvirus 6. *Blood* **102**, 2877–2884.

Tassaneetrithep, B., Burgess, T. H., Granelli-Piperno, A. & 10 other authors (2003). DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J Exp Med* **197**, 823–829.

van Die, I., van Vliet, S. J., Nyame, A. K., Cummings, R. D., Bank, C. M., Appelmelk, B., Geijtenbeek, T. B. & van Kooyk, Y. (2003). The dendritic cell-specific C-type lectin DC-SIGN is a receptor for *Schistosoma mansoni* egg antigens and recognizes the glycan antigen Lewis x. *Glycobiology* **13**, 471–478.

Yasukawa, M., Hasegawa, A., Sakai, I. & 8 other authors (1999). Down-regulation of CXCR4 by human herpesvirus 6 (HHV-6) and HHV-7. *J Immunol* **162**, 5417–5422.