Acta Microbiologica et Immunologica Hungarica 65 (2), pp. 119–125 (2018)
DOI: 10.1556/030.65.2018.010
First published online March 7, 2018

HUMAN HERPESVIRUS 6A AND 6B AND NK CELLS

ROBERTA RIZZO and DARIO DI LUCA*

Department of Medical Sciences, Section of Microbiology, University of Ferrara, Ferrara, Italy

(Received: 11 December 2017; accepted: 4 January 2018)

Human herpesvirus 6 (HHV-6) comprises two viral species, HHV-6A and HHV-6B, closely related but differing for pathogenic and biological characteristics. Both viral species are predominantly lymphotropic, infecting T lymphocytes and other lymphoid cells, including natural killer (NK) cells. The interactions between HHV-6 and NK cells have been scarcely studied, but it has become clear that NK cells are not only crucial in immune protection during the early phases of infection, but also that HHV-6 infection can affect NK cell functions. In this report, we shortly summarize the interactions between HHV-6 and NK cells.

Keywords: HHV-6A, HHV-6B, NK cells

Human Herpesvirus 6A (HHV-6A) and HHV-6B Infection

HHV-6 was first isolated in 1986 [1]. Soon it became evident that viral isolates fall into two distinct groups, which, although very similar from a genetic point of view, can be easily distinguished on the basis of biological, immunological, and cell tropism characteristics [2]. Originally, the two viral groups were designated as HHV-6A and HHV-6B variants, but recently, it was recognized that each variant has such specific characteristics to be classified as different viral species [3]. HHV-6A and HHV-6B have collinear genomes, over 90% aminoacid identity, and conserved epitopes cross-reactive to monoclonal antibodies. However, there are species-specific monoclonal antibodies; the two viruses have different *in vitro* cell tropism and more importantly have different epidemiology and pathogenic associations. HHV-6 research has often suffered from lack of discrimination between HHV-6A and HHV-6B, and potentially conflicting or negative results published in the scientific literature may be ascribed to the biological and pathogenic differences between the two species.

*Corresponding author; E-mail: ddl@unife.it

HHV-6B primary infection occurs in the early childhood (between 6 months and 2 years of age) and is associated with exanthem subitum (2–3 days of fever followed by rash) and other benign febrile diseases, whereas HHV-6A primary infection has still to be precisely characterized: it is thought to occur later in life and is possibly asymptomatic, potentially due to the fact that existing HHV-6B protective immunity exerts its effects also on HHV-6A [4]. HHV-6 (mostly -6B) is frequently detected in saliva and salivary glands [5] and therefore it is possible that the main route of transmission is represented by the salivary route. Other routes of transmission (i.e., blood transplantation and breast feeding) have occasionally been reported. In addition, HHV-6, mainly HHV-6A, might be transmitted by the sexual route [6, 7].

HHV-6A and -6B have a preferential tropism for lymphoid cells (T cells, monocytes/macrophages) but *in vitro* and *in vivo*, they can also infect other cell types (endothelial cells, fibroblasts, NK cells, glial cells, astrocytes, hepatocytes, etc.) [8], with different cell target and/or efficiency of infection for the two viral species. In fact, the two viruses use different cell receptors to recognize and attach to the cell membrane. HHV-6A uses CD46, a molecule expressed on the surface of all human cells [9], whereas HHV-6B uses CD134/OX40 [10], present only on specific cell types.

Infection with HHV-6B, and less frequently HHV-6A, is widespread in the human adult population and seropositivity rates are over 90%. However, since serological assays do not allow to discriminate between HHV-6B and HHV-6A, it is not possible to determine the effective seroprevalence of each virus in the human population.

Like all herpesviruses, HHV-6 establishes latent lifelong persistent infections in the host, most frequently in lymphoid cells but also in the brain and bone marrow, as proven by the PCR detection of viral DNA in peripheral blood lymphocytes or different tissues from healthy adults. Interestingly, most of these HHV-6 positive cases contain HHV-6B DNA, while HHV-6A is very infrequently detected.

During latency, HHV-6 does not replicate and expresses only few genes; therefore, the absence of transcription from lytic genes in the presence of latency-associated mRNAs (i.e., U94) is indicative of true latency [11]. Reactivations occur frequently, particularly in immunocompromised patients. HHV-6 reactivation in transplant recipients can be associated with several different clinical conditions, including encephalitis, graft failure and graft-versus-host disease, pneumonia, cognitive dysfunction, thrombotic microangiopathy, and human cytomegalovirus reactivation. Reactivation is also supposed to play a role in drug reaction with eosinophilia and systemic symptoms, a serious adverse drug reaction [12].

Both variants of HHV-6 have been suggested as a possible trigger for multiple sclerosis, several direct and indirect evidences support this hypothesis, but conclusive evidence is still missing [13]. Other pathological conditions that have been associated with HHV-6 include Hashimoto's autoimmune thyroiditis (HHV-6A) [14, 15], myocarditis and chronic cardiomyopathy (both species), mesial temporal lobe epilepsy (HHV-6B) [16], and mononucleosis [17].

An interesting feature of HHV-6 is that approximately 1% of the human population carry the complete chromosomally integrated HHV-6 DNA (ciHHV-6) in their genome in all the host cells, both somatic and germinal, and transmit it vertically to the offspring as a Mendelian character [18]. Both HHV-6 species can integrate, ciHHV-6 can reactivate, and its pathogenic relevance is still to be determined. However, in such cases, high HHV-6 DNA loads normally associated with ciHHV-6 could be misinterpreted as an active infection.

Natural Killer Cells

Natural killer (NK) cells derive from the lymphoid lineage and constitute an innate immune mechanism important in the control of viral infections [19]. Their protective role has been shown against a large number of viral species, including human immunodeficiency virus, papillomaviruses, flaviviruses, paramyxoviruses, and herpesviruses. They have direct and indirect protective effects against viral infections. Direct antiviral effect is represented by cytolytic killing of virus-infected target cells. Indirect effects include early production of proinflammatory cytokines, including interferon gamma. Increasing evidence shows that the NK cells have an important regulatory effect on stimulation of adaptive immunity [20]. NK cell cytotoxic activity is controlled by several activating and inhibitory receptors present on the NK cell membrane, belonging to different functional and genetic groups. The complex balance stemming from the engagement of activating and inhibitory receptors determines whether NK cells are activated or not. In general, inhibitory receptors bind self-molecules, such as major histocompatibility complex-1 (MHC-1) [21]. When target cells express MHC-1 molecules, inhibitory receptors are engaged and NK cells are normally non-activated. Often, virus infection alters the expression of MHC-1 molecules. In addition, activating NK cell receptors can bind non-self peptides and stress-induced molecules. Therefore, during viral infection, there is a lower inhibition of NK cells, due to the altered levels of MHC-1, and a significant activation of their functions, due to the engagements of activating receptors.

HHV-6 Infection and NK Cells

HHV-6 has the ability to infect NK cells [22]. Perhaps, it is even more relevant for the observation that HHV-6 infection of target non-NK cells has significant effects on NK cell functions. In fact, NK cells are cytotoxic for HHV-6-infected autologous cells, and NK activity on HHV-6 is affected by still undetermined polymorphic elements on the target cell that restricts NK cell recognition [23]. HHV-6 infection of peripheral blood mononuclear cells results in the upregulation of NK cell cytotoxicity during the first 48 h of infection, and this effect was mediated by secretion of IL-15 [24].

NK cell activity significantly increases during the acute phase of primary infection, in conjunction with the rash appearance, and decreases in the convalescent phase, suggesting that NK cell activity plays a central role in recovery [25]. Kumagai et al. [26] showed that NK cells play a major role in resolving acute phase infection, while specific lymphocyte activity develops later. Interestingly, veterans affected by Gulf War syndrome had high antibody levels to HHV-6 (as well as to all other HHVs) concomitant with a significant lower activity of NK cells [27], indirectly confirming the important role of NK cells in controlling HHV-6 (and other HHV) infection. Interestingly, patients with Hashimoto's thyroiditis, a condition associated with HHV-6A, show increased levels of a specific subset of NK cells (CD56bright), as well as cytotoxic activity specific for HHV-6 [15], suggesting that these patients might have an altered response to HHV-6. Analysis of endometrial tissue from idiopathic infertile women tissue positive of HHV-6A shows a reduction of CD56bright-resident endometrial NK cells as well as increased activation and cytotoxicity toward HHV-6-infected cells [7].

Conclusions

Taken together, these observations show the importance of NK cells in controlling HHV-6 infection, and that HHV-6 can modify the function of NK cells. In particular, HHV-6 infection is also able to modify NK cell miRNAs and transcription factors expression [28], suggesting an impact on the effector functions of NK cells.

Results previously published by our group with different herpesvirus, herpes simplex type 1 and HHV-8, show that the expression of a specific inhibitory NK cell receptor (KIR2DL2) might be associated with the inability of NK cells to control infection [29–33]. We are currently testing the hypothesis that expression of the inhibitory KIR2DL2 receptor is indeed associated with increased susceptibility to HHV-6 infection.

Acknowledgements

This work has been supported by the HHV-6 Foundation, FISM (Fondazione Italia Sclerosi Multipla) grant 2015/R/20, Ricerca Finalizzata GR-2011-02346947, and Merck research grant.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Salahuddin, S. Z., Ablashi, D. V., Markham, P. D., Josephs, S. F., Sturzenegger, S., Kaplan, M., Halligan, G., Biberfeld, P., Wong-Staal, F., Kramarsky, B.: Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Sciences **234**, 596–601 (1986).
2. Di Luca, D., Mirandola, P., Ravaioli, T., Bigoni, B., Cassai, E.: Distribution of HHV-6 variants in human tissues. Infect Agents Dis **5**, 203–214 (1996).
3. Ablashi, D., Agut, H., Alvarez-Lafuente, R., Clark, D. A., Dewhurst, S., Di Luca, D., Flamand, L., Frenkel, N., Gallo, R., Gompels, U. A., Höllsberg, P., Jacobson, S., Luppi, M., Lusso, P., Malnati, M., Medveczky, P., Mori, Y., Pellett, P. E., Pritchett, J. C., Yamanishi, K., Yoshikawa, T.: Classification of HHV-6A and HHV-6B as distinct viruses. Arch Virol **159**, 863–870 (2014).
4. Caselli, E., Di Luca, D.: Molecular biology and clinical associations of Roseoloviruses human herpesvirus 6 and human herpesvirus 7. New Microbiol **30**, 173–187 (2007).
5. Di Luca, D., Mirandola, P., Ravaioli, T., Dolcetti, R., Frigatti, A., Bovenzi, P., Sighinolfi, L., Monini, P., Cassai, E.: Human herpesviruses 6 and 7 in salivary glands and shedding in saliva of healthy and human immunodeficiency virus positive individuals. J Med Virol **45**, 462–468 (1995).
6. Leach, C. T., Newton, E. R., McParlin, S., Jenson, H. B.: Human herpesvirus 6 infection of the female genital tract. J Infect Dis **169**, 1281–1283 (1994).
7. Marci, R., Gentili, V., Bortolotti, D., Lo Monte, G., Caselli, E., Bolzani, S., Rotola, A., Di Luca, D., Rizzo, R.: Presence of HHV-6A in endometrial epithelial cells from women with primary unexplained infertility. PLoS One **11**, e0158304 (2016).
8. Eliassen, E., Di Luca, D., Rizzo, R., Barao, I.: The interplay between natural killer cells and human herpesvirus-6. Viruses **9**, E367 (2017).
9. Santoro, F., Kennedy, P. E., Locatelli, G., Malnati, M. S., Berger, E. A., Lusso, P.: CD46 is a cellular receptor for human herpesvirus 6. Cell **99**, 817–827 (1999).
10. Tang, H., Serada, S., Kawabata, A., Ota, M., Hayashi, E., Naka, T., Yamanishi, K., Mori, Y.: CD134 is a cellular receptor specific for human herpesvirus-6B entry. Proc Natl Acad Sci U S A **110**, 9096–9099 (2013).
11. Rotola, A., Ravaioli, T., Gonelli, A., Dewhurst, S., Cassai, E., Di Luca, D.: U94 of human herpesvirus 6 is expressed in latently infected peripheral blood mononuclear cells

and blocks viral gene expression in transformed lymphocytes in culture. Proc Natl Acad Sci U S A **95**, 13911–13916 (1998).
12. Ichiche, M., Kiesch, N., De Bels, D.: DRESS syndrome associated with HHV-6 reactivation. Eur J Intern Med **14**, 498–500 (2003).
13. Leibovitch, E. C., Jacobson, S.: Evidence linking HHV-6 with multiple sclerosis: An update. Curr Opin Virol **9**, 127–133 (2014).
14. Caselli, E., Zatelli, M. C., Rizzo, R., Benedetti, S., Martorelli, D., Trasforini, G., Cassai, E., degli Uberti, E. C., Di Luca, D., Dolcetti, R.: Virologic and immunologic evidence supporting an association between HHV-6 and Hashimoto's thyroiditis. PLoS Pathog **8**, e1002951 (2012).
15. Rizzo, R., Zatelli, M. C., Rotola, A., Cassai, E., Degli Uberti, E., Di Luca, D., Caselli, E.: Increase in peripheral CD3-CD56brightCD16- natural killer cells in Hashimoto's thyroiditis associated with HHV-6 infection. Adv Exp Med Biol **897**, 113–120 (2016).
16. Donati, D., Akhyani, N., Fogdell-Hahn, A., Cermelli, C., Cassiani-Ingoni, R., Vortmeyer, A., Heiss, J. D., Cogen, P., Gaillard, W. D., Sato, S., Theodore, W. H., Jacobson, S.: Detection of human herpesvirus-6 in mesial temporal lobe epilepsy surgical brain resections. Neurology **61**, 1405–1411 (2003).
17. Naito, T., Kudo, N., Inui, A., Matsumoto, N., Takeda, N., Isonuma, H., Dambara, T., Hayashida, Y.: Causes of infectious mononucleosis-like syndrome in adult patients. Intern Med **45**, 833–834 (2006).
18. Pellett, P. E., Ablashi, D. V., Ambros, P. F., Agut, H., Caserta, M. T., Descamps, V., Flamand, L., Gautheret-Dejean, A., Hall, C. B., Kamble, R. T., Kuehl, U., Lassner, D., Lautenschlager, I., Loomis, K. S., Luppi, M., Lusso, P., Medveczky, P. G., Montoya, J. G., Mori, Y., Ogata, M., Pritchett, J. C., Rogez, S., Seto, E., Ward, K. N., Yoshikawa, T., Razonable, R. R.: Chromosomally integrated human herpesvirus 6: Questions and answers. Rev Med Virol **22**, 144–155 (2012).
19. Waggoner, S. N., Reighard, S. D., Gyurova, I. E., Cranert, S. A., Mahl, S. E., Karmele, E. P., McNally, J. P., Moran, M. T., Brooks, T. R., Yaqoob, F., Rydyznski, C. E.: Roles of natural killer cells in antiviral immunity. Curr Opin Virol **16**, 15–23 (2016).
20. Krebs, P., Barnes, M. J., Lampe, K., Whitley, K., Bahjat, K. S., Beutler, B., Janssen, E., Hoebe, K.: NK-cell-mediated killing of target cells triggers robust antigen-specific T-cell-mediated and humoral responses. Blood **113**, 6593–6602 (2009).
21. Parham, P.: MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol **5**, 201–214 (2005).
22. Lusso, P., Malnati, M. S., Garzino-Demo, A., Crowley, R. W., Long, E. O., Gallo, R. C.: Infection of natural killer cells by human herpesvirus 6. Nature **362**, 458–462 (1993).
23. Malnati, M. S., Lusso, P., Ciccone, E., Moretta, A., Moretta, L., Long, E. O.: Recognition of virus-infected cells by natural killer cell clones is controlled by polymorphic target cell elements. J Exp Med **178**, 961–969 (1993).
24. Flamand, L., Stefanescu, I., Menezes, J.: Human herpesvirus-6 enhances natural killer cell cytotoxicity via IL-15. J Clin Invest **97**, 1373–1381 (1996).
25. Takahashi, K., Segal, E., Kondo, T., Mukai, T., Moriyama, M., Takahashi, M., Yamanishi, K.: Interferon and natural killer cell activity in patients with exanthem subitum. Pediatr Infect Dis J **11**, 369–373 (1992).
26. Kumagai, T., Yoshikawa, T., Yoshida, M., Okui, T., Ihira, M., Nagata, N., Yano, S., Shiraki, K., Yamada, M., Ichihara, K., Asano, Y.: Time course characteristics of human

herpesvirus 6 specific cellular immune response and natural killer cell activity in patients with exanthema subitum. J Med Virol **78**, 792–799 (2006).
27. Vojdani, A., Thrasher, J. D.: Cellular and humoral immune abnormalities in Gulf War veterans. Environ Health Perspect **112**, 840–846 (2004).
28. Rizzo, R., Soffritti, I., D'Accolti, M., Bortolotti, D., Di Luca, D., Caselli, E.: HHV-6A/6B infection of NK cells modulates the expression of miRNAs and transcription factors potentially associated to impaired NK activity. Front Microbiol **8**, 2143 (2017).
29. Rizzo, R., Gentili, V., Casetta, I., Caselli, E., De Gennaro, R., Granieri, E., Cassai, E., Di Luca, D., Rotola, A.: Altered natural killer cells' response to herpes virus infection in multiple sclerosis involves KIR2DL2 expression. J Neuroimmunol **251**, 55–64 (2012).
30. Caselli, E., Rizzo, R., Ingianni, A., Contini, P., Pompei, R., Di Luca, D.: High prevalence of HHV8 infection and specific killer cell immunoglobulin-like receptors allotypes in Sardinian patients with type 2 diabetes mellitus. J Med Virol **86**, 1745–1751 (2014).
31. Ben Fredj, N., Rizzo, R., Bortolotti, D., Nefzi, F., Chebel, S., Rotola, A., Frih-Ayed, M., Di Luca, D., Aouni, M.: Evaluation of the implication of KIR2DL2 receptor in multiple sclerosis and herpesvirus susceptibility. J Neuroimmunol **271**, 30–35 (2014).
32. Borghi, A., D'Accolti, M., Rizzo, R., Virgili, A., Di Luca, D., Corazza, M., Caselli, E.: High prevalence of specific KIR types in patients with HHV-8 positive cutaneous vascular lesions: A possible predisposing factor? Arch Dermatol Res **308**, 373–377 (2016).
33. Rizzo, R., Bortolotti, D., Fainardi, E., Gentili, V., Bolzani, S., Baldi, E., Casetta, I., Granieri, E., Rotola, A., Furlan, R., Di Luca, D.: KIR2DL2 inhibitory pathway enhances Th17 cytokine secretion by NK cells in response to herpesvirus infection in multiple sclerosis patients. J Neuroimmunol **294**, 1–5 (2016).