COMMENTARY

Peroxisome proliferator-activated receptor γ (PPAR γ) activation: A key determinant of neuropathogeny during congenital infection by cytomegalovirus

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ABSTRACT

Congenital infection by human cytomegalovirus (HCMV) might result in permanent neurological sequelae, including sensorineural deafness, cerebral palsies or devastating neurodevelopmental abnormalities. We recently disclosed that Peroxisome Proliferator-Activated Receptor gamma (PPAR γ), a transcription factor of the nuclear receptor superfamily, is a key determinant of HCMV pathogenesis in developing brain. Using neural stem cells from human embryonic stem cells, we showed that HCMV infection strongly increases levels and activity of PPAR γ in NSCs. Further in vitro experiments showed that PPAR γ activity inhibits the neuronogenic differentiation of NSCs into neurons. Consistently, increased PPAR γ expression was found in brain section of fetuses infected by HCMV, but not in uninfected controls. In this commentary, we summarize and discuss our findings and the new insights they provide on the neuropathogenesis of HCMV congenital infection.

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The pathogeny of infectious diseases dysregulates a variety of cellular pathways, which identification may shed new light about their role in the healthy cell. In particular, infections by neurotropic viruses in pregnancy may lead to neurodevelopmental abnormalities in the fetus and point out novel mechanisms contributing to neurogenesis. Particularly relevant from this point of view is congenital infection by Human Cytomegalovirus (HCMV), as it is the most frequent infectious cause of permanent neurological sequelae.

HCMV congenital infection

HCMV is a BetaHerpes virus with a worldwide distribution. It is highly prevalent in the general population: seroprevalence ranges between 40 and 90%, with the greatest values among racial/ethnic minorities and persons of lower socio-economic background status.¹ HCMV is transmitted through close non-sexual or sexual contact, breastfeeding, blood transfusions, and organ transplantation.² For the pregnant woman, the most likely source of infection is the contact with the

urine or saliva of young children, including her own children.² No vaccine is available so far. Lifelong latency is established after a primary infection. Though infection of immunocompetent adults is almost always benign, HCMV is responsible for serious illness and death in immunocompromised hosts, and is a major hazard for the fetus after infection during pregnancy. HCMV infection *in utero* is believed to occur through transplacental hematogenous spread.³ It affects 0.5–2 % of live births, whereas approximately 10 % of infected newborns are symptomatic at birth, and up to 15 % of those asymptomatic at birth develop delayed HCMV-related disease manifestations in their early years.⁴ Congenital HCMV disease is associated with a wide range of neurodevelopmental disabilities, including hearing and vision loss and mental retardation, as well as structural brain abnormalities including intracranial calcifications, microcephaly, hydrocephalus, ventriculomegaly, ventriculomegaly, polymicrogyria, porencephaly, and schizencephaly.⁵ Neurological outcomes are more severe when infection occurs during the first trimester.⁵

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HCMV tropism in the developing brain

Deciphering HCMV tropism in the brain was critical to investigate its neuropathy. Founder studies in the mouse revealed that HCMV murine counterpart, namely murine cytomegalovirus (MCMV), infected the developing brain the cerebral ventricular walls, a region known to contain neural progenitors.⁶ Strikingly, infected cells seemed to migrate from the (sub-) ventricular zones to the cortical plate or the hippocampus.⁶ Mouse neurons were also found to be sensitive to infection. Studies in human were limited for obvious reasons, and in vitro studies were performed with primary cultures of human brain cells prepared from deceased, uninfected fetus. Brain microvascular endothelial cells, astrocytes, neuronal cells, oligodendroglial cells, microglia/macrophages, and neural progenitor/stem cells were found to be sensitive to HCMV.⁵ However, no histological data identifying the different cell types actually infected in utero during congenital HCMV infection were available, except histopathological analysis of postmortem brain samples which revealed HCMV inclusion bodies in the brain.⁷ In our study, we explored the expression of the immediate early HCMV antigen (IE), a factor encoded by the HCMV genome and critical for virus replication, in histopathological slides from deceased infected or non-infected fetus.⁸ In agreement with the studies in the mouse model, we observed cells clearly immunoreactive to IE in the ependymal and germinative zones of the brain of infected cases. However, no labeling was found in the white matter, whereas, as expected, brain vessels were positive to IE. These findings disclosed that neural progenitors and ependymal cells were the preferential, if not the only, neural cells targeted by HCMV during fetal brain infection.

Modeling HCMV neuropathogenesis

Based on the assumption that HCMV infection is likely to disturb neural progenitor homeostasis or differentiation, a number of studies investigated the ability of neural progenitors infected in vitro by HCMV to generate neurons or astrocytes. These studies used progenitors which were prepared from brain from deceased, uninfected fetus, and eventually infected in vitro and driven to differentiate. They showed sometimes conflicting results, and, at least, revealed considerable diversity in the phenotype of such progenitors following HCMV infection. Indeed, HCMV infection

of neural progenitors was found to (1) inhibit self-renewal and proliferation, inhibit neuronal differentiation, and induce apoptosis,⁹ or (2) inhibit astrocyte differentiation,¹⁰ or (3) cause premature and abnormal differentiation into an uncharacterized cell type,¹¹ or (4) reduce the number of proliferating CD24-expressing progenitors.¹² Two other studies used neural stem cells generated from human induced pluripotent stem (iPS) cells and reported that HCMV infection impaired neuronal differentiation.^{13,14}

In order to perform molecular investigations on the outcomes of HCMV infection on neural progenitor cells, we used a new model, highly neurogenic neural stem cells from embryonic stem cells (NSCs) (Fig. 1). NSCs were generated through early neuroepithelial differentiation of human ES cells in a monolayer system using 2 SMAD inhibitors (SB431542, Noggin) and the defined medium N2B27.¹⁵ This method allowed for efficient neural commitment and avoided possible instrumental factors as donor variability (including gestation age), use of batch-dependent components, and feeder cell conditioned medium. NSCs showed self-renewal and continuous growth in defined conditions without the need of generating neurospheres. They displayed a cortical phenotype with positive immunostaining and/or high levels of expression of polarized neural stem cells and radial glia markers such as nestin, GFAP, BLBP, SOX2, PAX6, POU3F3, NRCAM and PARD3, in addition to genes involved in corticogenesis (*MEF2C*, *BDNF*, *RTN4*, *ODZ1*, *FLNA* and *PFAH1B1*), in the absence of immunoreactivity to non-cortical markers.¹⁶ This phenotype was particularly relevant with respect to the fact that congenital HCMV infection targets cortical areas of the developing brain as well as radial glia cells.¹⁷ NSCs showed ability to differentiate into neurons positive for the markers HUC/D and β -III tubulin upon growth factor removal. On this basis, we investigated the outcomes of infection on neuronogenic differentiation of NSCs. Consistently with the works cited above, we found that NSCs were permissive to HCMV infection (Fig. 2) and that infection dramatically impaired NSC differentiation into neurons.⁸ In the search for the molecular bases of such inhibition of differentiation, we next investigated the levels and activity of the nuclear receptor PPAR gamma (PPAR γ) in infected NSCs.

Figure 1. Immunofluorescence analysis of neural stem cells from human embryonic stem cells using dapi or antibodies specific to nestin or SOX2. Scale bar: 25 μ m.

Role of PPAR γ in HCMV neuropathy

PPAR γ is a host-encoded transcription factor with pleiotropic roles in metabolism, inflammation, cell differentiation and migration. Notably, brain development abnormalities were reported in PPAR $\gamma^{-/-}$ and PPAR $\gamma^{-/+}$ mice embryos.¹⁸ Various, and sometimes discordant, effects of PPAR γ agonists were also observed in a variety of neural cell models (reviewed in ref. 19). Together those findings suggested that

PPAR γ could play a role in brain development. Incidentally, we had previously showed that PPAR γ activity is mandatory for HCMV replication and is dramatically enhanced in cytotrophoblasts and placentae infected by HCMV.²⁰ Therefore, we investigated the outcomes of HCMV infection on PPAR γ in NSCs.

Uninfected NSCs showed weak expression of PPAR γ as assessed by immunofluorescence analysis,

Figure 2. Immunofluorescence analysis of neural stem cells from human embryonic stem cells 48h after infection by HCMV at a MOI of 10 (HCMV) or uninfected (NI) using dapi or an antibody to HCMV immediate early antigen (IE). Scale bar: 25 μ m.

Figure 3. Immunohistological analysis of brain sections from HCMV-infected (A) or non-infected (B) fetus, showing nuclear PPAR γ in the germinative zone. Gestational ages are 23 weeks. Scale bar: 100 μ m.

western blot, and luciferase reporter assays.⁸ Conversely, HCMV infection strongly increased PPAR γ levels and activity in NSCs, as shown by immunofluorescence analysis, western blot, luciferase reporter assays and chromatin immunoprecipitation.⁸ Notably, we found that infected NSCs exerted a bystander effect on neighboring uninfected cells, which also showed enhanced PPAR γ levels. Levels of 9-hydroxyoctadecadienoic acid (9-HODE), a known PPAR γ agonist, were significantly increased in infected NSC cultures. Likewise, exposure of uninfected NSCs to 9-HODE recapitulated the effect of infection on PPAR γ activity. However, these results did not demonstrate that PPAR γ activation in infected NSCs was a causative factor for impaired differentiation. Hence we designed and performed a number of experiments to determine whether PPAR γ activation was necessary and/or sufficient to impair neurogenesis in vitro. Thus we demonstrated that (1) pharmacological activation of PPAR γ ectopically expressed through lentiviral transduction under the control of the EF1 α minimal promoter was sufficient to induce impaired neurogenesis of uninfected NSCs, (2) treatment of uninfected NSCs with 9-HODE impaired NSC differentiation and (3) treatment of HCMV-infected NSCs with the PPAR γ inhibitor T0070907 restored a normal rate of differentiation.⁸ These findings revealed that PPAR γ activation was necessary and sufficient to impair NSC differentiation into neurons. Last, we explored the relevance of these findings in vivo, through immunohistological examination of brain slices from a set of infected (N = 20) or uninfected control (N = 5) fetus of different gestational ages. Strikingly, nuclear PPAR γ was immunodetected in the brain germinative zones of congenitally infected fetuses, but in none of the control samples⁸ (Fig. 3),

what strongly supported the role of PPAR γ in the disease phenotype.

Conclusion

Our findings revealed that PPAR γ is a key determinant of the pathogeny of HCMV congenital infection. Further, they demonstrated that NSCs are a relevant tool which fairly recapitulates disease mechanisms in vitro. Hence, this cell platform should also help investigating the molecular and cellular pathogenic bases of other neurotropic viruses, such Zika virus (ZIKV), especially given that NSCs are sensitive to ZIKV infection in vitro.²¹ Last, our findings reveal a key role for PPAR γ in neurogenesis and in the pathophysiology of HCMV congenital infection. Identification of PPAR γ gene targets in the infected brain is critical to decipher the genetic and molecular events leading to impaired neurogenic differentiation in NSCs with abnormal activation of PPAR γ (should they be infected or not). In the long term, dissecting the PPAR γ signaling pathways will provide new insight on neurogenesis in health and disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

- [1] Cannon MJ. Congenital cytomegalovirus (CMV) epidemiology and awareness. *J Clin Virol* 2009; 46 Suppl 4:S6-10; PMID:19800841; <http://dx.doi.org/10.1016/j.jcv.2009.09.002>
- [2] Cannon M, Davis K. Washing our hands of the congenital cytomegalovirus disease epidemic. *BMC Public Health* 2005; 5:70; PMID:15967030; <http://dx.doi.org/10.1186/1471-2458-5-70>

- [3] Fisher S, Genbacev O, Maidji E, Pereira L. Human cytomegalovirus infection of placental cytotrophoblasts in vitro and in utero: implications for transmission and pathogenesis. *J Virol* 2000; 74:6808-20; PMID:10888620; <http://dx.doi.org/10.1128/JVI.74.15.6808-6820.2000>
- [4] Weisblum Y, Panet A, Haimov-Kochman R, Wolf DG. Models of vertical cytomegalovirus (CMV) transmission and pathogenesis. *Semin. Immunopathol* 2014; 36:615-25; PMID:25291972; <http://dx.doi.org/10.1007/s00281-014-0449-1>
- [5] Cheeran MCJ, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev* 2009; 22:99-126 Table of Contents; PMID:19136436; <http://dx.doi.org/10.1128/CMR.00023-08>
- [6] Tsutsui Y, Kosugi I, Kawasaki H, Arai Y, Han GP, Li L, Kaneta M. Roles of neural stem progenitor cells in cytomegalovirus infection of the brain in mouse models. *Pathol Int* 2008; 58:257-67; PMID:18429823; <http://dx.doi.org/10.1111/j.1440-1827.2008.02221.x>
- [7] Perlman C, Argyle J. Lethal cytomegalovirus infection in preterm infants: clinical, radiological, and neuropathological findings. *Ann Neurol* 1992; 31:64-8; PMID:1311911; <http://dx.doi.org/10.1002/ana.410310112>
- [8] Rolland M, Li X, Sellier Y, Martin H, Perez-Berezo T, Rauwel B, Benchoua A, Bessieres B, Aziza J, Cenac N, et al. PPAR Is Activated during Congenital Cytomegalovirus Infection and Inhibits Neuronogenesis from Human Neural Stem Cells. *PLoS Pathog* 2016; 12: e1005547; PMID:27078877; <http://dx.doi.org/10.1371/journal.ppat.1005547>
- [9] Odeberg J, Wolmer N, Falci S, Westgren M, Seiger A, Soderberg-Nauclr C. Human cytomegalovirus inhibits neuronal differentiation and induces apoptosis in human neural precursor cells. *J Virol* 2006; 80:8929-39; PMID:16940505; <http://dx.doi.org/10.1128/JVI.00676-06>
- [10] Odeberg J, Wolmer N, Falci S, Westgren M, Sundtrm E, Seiger A, Soderberg-Nauclr C. Late human cytomegalovirus (HCMV) proteins inhibit differentiation of human neural precursor cells into astrocytes. *J Neurosci Res* 2007; 85:583-93; PMID:17154414; <http://dx.doi.org/10.1002/jnr.21144>
- [11] Luo MH, Hannemann H, Kulkarni AS, Schwartz PH, O'Dowd JM, Fortunato EA. Human Cytomegalovirus Infection Causes Premature and Abnormal Differentiation of Human Neural Progenitor Cells. *J Virol* 2010; 84:3528-41; PMID:20071566; <http://dx.doi.org/10.1128/JVI.02161-09>
- [12] Mutnal MB, Cheeran MCJ, Hu S, Lokensgard JR. Murine Cytomegalovirus Infection of Neural Stem Cells Alters Neurogenesis in the Developing Brain. *PLoS One* 2011; 6:e16211; PMID:21249143; <http://dx.doi.org/10.1371/journal.pone.0016211>
- [13] D'Aiuto L, Di Maio R, Heath B, Raimondi G, Milosevic J, Watson AM, Bamne M, Parks WT, Yang L, Lin B, et al. Human Induced Pluripotent Stem Cell-Derived Models to Investigate Human Cytomegalovirus Infection in Neural Cells. *PLoS One* 2012; 7:e49700; <http://dx.doi.org/10.1371/journal.pone.0049700>
- [14] Nakamura H, Liao H, Minami K, Toyoda M, Akutsu H, Miyagawa Y, Okita H, Kiyokawa N, Umezawa A, Ima-dome K, et al. Human cytomegalovirus induces apoptosis in neural stem/progenitor cells derived from induced pluripotent stem cells by generating mitochondrial dysfunction and endoplasmic reticulum stress. *Herpesviridae* 2013; 4:2; PMID:24144363; <http://dx.doi.org/10.1186/2042-4280-4-2>
- [15] Boissart C, Nissan X, Giraud-Triboult K, Peschanski M, Benchoua A. miR-125 potentiates early neural specification of human embryonic stem cells. *Development* 2012; 139:1247-57; PMID:22357933; <http://dx.doi.org/10.1242/dev.073627>
- [16] Boissart C, Poulet A, Georges P, Darville H, Julita E, Delorme R, Bourgeron T, Peschanski M, Benchoua A. Differentiation from human pluripotent stem cells of cortical neurons of the superficial layers amenable to psychiatric disease modeling and high-throughput drug screening. *Transl Psychiatry* 2013; 3:e294; PMID:23962924; <http://dx.doi.org/10.1038/tp.2013.71>
- [17] van den Pol AN, Mocarski E, Saederup N, Vieira J, Meier TJ. Cytomegalovirus Cell Tropism, Replication, and Gene Transfer in Brain. *J Neurosci* 1999; 19:10948-65; PMID:10594076
- [18] Wada K, Nakajima A, Katayama K, Kudo C, Shibuya A, Kubota N, Terauchi Y, Tachibana M, Miyoshi H, Kamisaki Y, et al. Peroxisome proliferator-activated receptor gamma-mediated regulation of neural stem cell proliferation and differentiation. *J Biol Chem* 2006; 281:12673-81; PMID:16524877; <http://dx.doi.org/10.1074/jbc.M513786200>
- [19] Stergiopoulos A, Politis PK. The role of nuclear receptors in controlling the fine balance between proliferation and differentiation of neural stem cells. *Arch Biochem Biophys* 2013; 534:27-37; PMID:23044345; <http://dx.doi.org/10.1016/j.abb.2012.09.009>
- [20] Leghmari K, Cenac N, Rolland M, Martin H, Rauwel B, Bertrand-Michel J, Le Faouder P, Bnard M, Casper C, Davrinche C, et al. Cytomegalovirus Infection Triggers the Secretion of the PPARgamma Agonists 15-Hydroxyeicosatetraenoic Acid (15-HETE) and 13-Hydroxyoctadecadienoic Acid (13-HODE) in Human Cytotrophoblasts and Placental Cultures. *PLoS One* 2015; 10:e0132627; PMID:26171612; <http://dx.doi.org/10.1371/journal.pone.0132627>
- [21] Garcez PP, Loiola EC, Madeiro da Costa R, Higa LM, Trindade P, Delvecchio R, Nascimento JM, Brindeiro R, Tanuri A, Rehen SK. Zika virus impairs growth in human neurospheres and brain organoids. *Science* 2016; 352:816-8; PMID:27064148; <http://dx.doi.org/10.1126/science.aaf6116>