Expression of human cytomegalovirus components in the brain tissues of patients with Rasmussen’s encephalitis

Yao Zhang1#, Yisong Wang2#, Sichang Chen1, Shuai Chen1, Yuguang Guan1, Changqing Liu1, Tianfu Li3, Guoming Luan1,2,3*, Jing An2,4*

1. Department of Neurosurgery, Sanbo Brain Hospital, Capital Medical University, Beijing100093, China
2. Department of Microbiology, School of Basic Medical Science, Capital Medical University, Beijing 100069, China
3. Beijing Key Laboratory of Epilepsy, Beijing100093,China
4. Center of Epilepsy, Beijing Institute for Brain Disorders, Beijing100093, China

Rasmussen’s encephalitis (RE) is a rare and severe progressive epileptic syndrome with unknown etiology. Infection by viruses, including human cytomegalovirus (HCMV), has been speculated to be a potential trigger for RE. However, no viral antigens have been detected in the brains of patients with RE; thus, a possible clinical linkage between viral infections and RE has not been firmly established. In this study, we evaluated the expression of HCMV pp65 antigen in brain sections from 26 patients with RE and 20 non-RE patients by immunohistochemistry and in situ hybridization, and assessed the associations between HCMV infection and clinical parameters. Elevated expression of HCMV pp65 protein and DNA was observed in 88.5% (23/26) and 69.2% (18/26) of RE cases, respectively. In the non-RE group, HCMV pp65 antigen was detected only in two cases (10%), both of which were negative for DNA staining. Additionally, the intensity of HCMV pp65 staining was correlated with a shorter duration of the prodromal stage, younger age of seizure onset, and more severe unilateral cortical atrophy. Elevated expression of HCMV pp65 was observed in RE brain tissue and was correlated with the clinical features of RE disease. In summary, our results suggested that HCMV infection may be involved in the occurrence and progression of RE disease. Thus, further studies are needed to determine whether early treatment with anti-HCMV antibodies could modulate the course of RE.

KEYWORDS Rasmussen’s encephalitis; human cytomegalovirus (HCMV); pp65; epilepsy

INTRODUCTION

Rasmussen’s encephalitis (RE) is a rare, chronic, progressive neurological syndrome that often occurs in childhood (Varadkar et al., 2014). The major clinical characteristics of RE consist of focal epilepsy, progressive hemiparesis, and the decline of cognitive function (Bien et al., 2002b). Without surgical intervention, patients with RE eventually develop to a persistent status of epilepsy, with more severe deterioration of neurological function and cognitive impairment (Bien et al., 2002b). Currently, hemispherectomy is the only effective treatment for controlling seizures and cognitive deterioration (Guan et al., 2014).

The etiology and pathogenesis of RE are unclear (Varadkar et al., 2014). Several factors are thought to be involved in the occurrence of this syndrome, including
Cytomegalovirus in RE

In the present study, we examined the expression of HCMV pp65 protein and DNA in the brain tissues of 26 patients with RE and 20 control patients without RE. We then analyzed the relationships between HCMV pp65 expression levels and clinical parameters. Our results will provide important insights for further clarifying the role of HCMV in the occurrence and progression of RE and regarding the feasibility of anti-HCMV treatment in patients with RE.

MATERIALS AND METHODS

In situ hybridization (ISH)
For the detection of HCMV pp65 DNA in brain sections, a digoxin-labeled DNA probe specific for pp65 (5’-CGTCTGGTTTTTTCGATATCGACTTGTTGCTGCAG CG-3’), digoxin-labeled Alu DNA probe (positive control), and insect genomic DNA probe (negative control) were purchased from Life Technologies (Eugene, USA). For the ISH procedure, after pretreatment with proteinase K (100 μg/mL) for 25 min at 37 °C, tissue sections were hybridized with the probes diluted using DIG Easy Hyb (11603558001; Roche Diagnostics, Indianapolis, USA) at 95 °C for 20 min, followed by incubation for 16 h at 37 °C in a hybridization oven (UVP HL-2000).
Hybrilinker). After incubation with mouse anti-digoxin IgG, the reaction was visualized by the addition of HRP-conjugated goat anti-mouse IgG and DAB, and images were acquired.

**Scoring methodology of IHC and ISH**
The IHC and ISH results were evaluated by following a previously described scoring methodology (Luan and Gao et al., 2013). Cells showing yellow or brown particles in the cytoplasm or nucleus were considered positive and were counted and analyzed using image analysis software (Image-Pro Plus 6.0; Media Cybernetics Inc., Bethesda, USA). The semiquantitative results were expressed as the percentage of positive cells combined with a subjective assessment of staining intensity, which was scored as 0 (colorless), 1 (light yellow), 2 (yellow or brown), or 3 (dark brown); the percentages of positive cells were denoted as 0 (<5%), 1(5%–25%), 2 (26%–50%), 3 (51%–75%), or 4 (>75%). The product of the two scores was used to evaluate the immunostaining results, as follows: overall scores of ≤1, 2–3, 4–5, and > 6 were defined as negative, weakly positive, moderately positive, and strongly positive, respectively.

**Statistical analysis**
Statistical analysis was performed using SPSS 17.0 software. Chi square tests were performed to determine the differences between the expression of HCMV components in patients with or without RE. Student's t tests and check-board analyses were performed to determine the differences in clinical features in subgroups of patients with RE, according to the pp65 IHC intensity. Differences with $P$ values of less than 0.05 were considered statistically significant.

**RESULTS**

**Clinical characteristics of patients with and without RE**
Twenty-six patients with RE were enrolled in this study, including 16 girls (61.5%) and 10 boys (38.5%). Their mean ages were 5.8 years (range: 1.2–18 years) and 7.5 years (range: 2.4–18.5 years) at seizure onset and at time of surgery, respectively. History of infection and/or viral vaccination prior to the seizure onset was observed in 14/26 (53.8%) patients. Eleven patients (42.3%) underwent left-side surgery, including functional hemispherectomy (FH), anatomic hemispherectomy (AH), and hemisphere disconnection. The etiologies for the control group consisted of ganglioglioma ($n = 5$), hippocampal sclerosis ($n = 8$), and focal cortical dysplasia ($n = 7$). The baseline comparison between RE and control groups is shown in Table 1.

| Gender (female) | RE (n = 26) | Non-RE (n = 20) |
|----------------|-------------|-----------------|
| Preceding infection | 14 (53.8%) | 3 (6%) |
| Mean age at seizure onset (years) | 5.8 | 8.6 |
| Mean age at surgery (years) | 7.5 | 14.8 |
| Side of resection (left) | 11 (42.3%) | 13 (65%) |
| Surgical method | | |
| FH | 15 (46.2%) | 0 |
| AH | 2 (15.4%) | 0 |
| Disconnection | 9 (26.9%) | 0 |
| Lobectomy | 0 | 20 (100%) |

Note: FH: functional hemispherectomy; AH: anatomic hemispherectomy

**Magnetic resonance imaging (MRI) and pathological findings in the brain tissues of patients with RE**
Mild to severe progressive unilateral multifocal cortical atrophy around the sylvian fissure and diffuse subcortical hypersignal confined within the involved hemisphere in the FLAIR sequence were observed in all patients (Figure 1A, 1B). The pathological changes, including pyknosis, loss of neurons, diffuse lymphocyte infiltration, perivascular lymphocyte cuffing, and glial cell proliferation (Figure 1C, 1D), were observed in RE brain tissues. However, no obvious morphological features of viral inclusion were observed.

**Expression of HCMV components in the brain tissues of patients with and without RE**
Among the 26 patients with RE, 23 (88.5%) showed positive immunostaining for the HCMV pp65 protein. Among these 23 cases, nine (34.6%) were strongly positive, nine were moderately positive, and five (19.2%) were weakly positive (Figure 2, Table 2). Similarly, HCMV pp65 DNA was detected in 69.2% (18/26) of RE samples; among them, eight (30.8%), six (23.1%), and four (15.4%) were strongly, moderately, and weakly positive, respectively (Figure 3, Table 2). HCMV pp65 protein was predominantly expressed in the cytoplasm of neuron-like cells (Figure 2). In the control group, only two cases (2/20, 10%) were weakly positive for pp65 antigen, and all cases were negative for pp65 DNA (Figure 2, Table 2; $P < 0.05$ for both). Notably, all of the 18 HCMV pp65 DNA-positive RE samples were positive for HCMV pp65 protein (Table 3). In other words, five IHC-positive patients with RE showed negative ISH staining, in-
indicating that combined application of the two methods may be necessary for enhancement of the detection rate.

**Association between HCMV pp65 antigen expression and the clinical parameters of patients with RE**

Based on the IHC intensity of HCMV pp65 antigen, patients with RE were divided into two groups: group A with negative or weakly positive pp65 staining, and group B with moderately or strongly positive pp65 staining. As shown in Figure 4, patients in group B showed a shorter mean duration of the prodromal stage (17.9 ± 5.1 versus 4.6 ± 0.56 months, respectively; Figure 4A; \( P < 0.05 \)) and a younger age at seizure onset as compared with group A patients (4.5 ± 0.5 versus 7.4 ± 0.9 years, respectively; Figure 4A; \( P < 0.01 \)). Additionally, patients with RE with more intense HCMV pp65 staining showed a more severe hemisphere cortical atrophy (Figure 4B; \( P = 0.037 \)). There were no correlations between the HCMV pp65 staining intensity and other clinical parameters.

**DISCUSSION**

RE is now confirmed as an inflammatory autoimmune neurological syndrome that can be triggered by exogenous antigens and somehow mediated by T lymphocyte cytotoxicity as well as autoantibodies (Li et al., 1997; Gahring et al., 2001; Bauer et al., 2002; Bien et al., 2002a; Schwab et al., 2009). However, virus infection, as an important potential initiating factor (Merkler et al., 2006; Chen et al., 2016), has not been carefully studied. In this study, we found significantly elevated pp65 expression in patients with RE in comparison with those in the control group. Additionally, patients with RE showing stronger pp65 staining exhibited a younger age of disease onset, more rapid clinical course, and more severe hemisphere atrophy compared with those having weakly positive or negative pp65 staining. In combination with the frequent history of HCMV infection before...
RE onset, these results suggested a correlation between the occurrence and development of RE and HCMV infection. Previously, several groups have detected nucleic acids of EBV or HCMV in brain samples from patients with RE by ISH and/or PCR (Walter and Renella, 1989), implying the involvement of virus infections in RE development and progression. However, there has been little evidence of the presence of viral antigens in the brain tissues of patients with RE, and no reports have demonstrated the significant associations between the presence of viral components and clinical parameters. In this study, we detected, for the first time, not only a significantly elevation of HCMV pp65 antigen and DNA in patients with RE compared with that in controls, but also the association between HCMV pp65 expression level in the brain tissue and disease parameters in patients with RE. High levels of HCMV pp65 antigen in patients with RE was closely related to a short duration of the prodromal stage, more severe hemisphere atrophy, and younger age at seizure onset. Therefore, our results, along with other reports, have further supported the hypothesis that there is a potential link between HCMV infections and disease progression of RE. However, additional studies are needed to test this hypothesis. A previous study reported no detection of HCMV, EBV, or HSV infections in seven RE cases using ISH (Atkins et al., 1995). According to reports and our experience, we believe that the reasons for these discrepancies may be related to differences in epidemiology, study populations, sample preparation, and detection techniques as well as the small sample sizes of the studies.

Table 2. Summary of IHC and ISH data for brain tissues of patients with and without RE

|          | IHC RE (n = 26) | IHC Non-RE (n = 20) | ISH RE (n = 26) | ISH Non-RE (n = 20) |
|----------|----------------|---------------------|----------------|---------------------|
| Positive | 23 (88.5%)     | 2 (10%)             | 18 (69.2%)     | 0                   |
| Strong   | 9 (34.6%)      | 0                   | 8 (30.8%)      | 0                   |
| Moderate | 9 (34.6%)      | 0                   | 6 (23.1%)      | 0                   |
| Weak     | 5 (19.2%)      | 2 (10%)             | 4 (15.4%)      | 0                   |
| Negative | 3 (11.5%)      | 18 (90%)            | 8 (30.8%)      | 20 (100%)           |

Figure 3. Expression of HCMV pp65 DNA in patients with RE. HCMV pp65 DNA was detected by ISH. Representative images are shown at low (200×, upper panels) or high (400×, lower panels) magnification. Positive staining of pp65 DNA was predominantly observed in the cytoplasm and nucleus of cells (arrows).

Table 3. Comparison of IHC versus ISH results in this study

| Cases      | IHC (positive)  | IHC (negative) | Total |
|------------|-----------------|----------------|-------|
| ISH (positive) | 18              | 0              | 18    |
| ISH (negative)   | 5               | 3              | 8     |
| Total               | 23              | 3              | 26    |

www.virosin.org
The mechanism by which HCMV is involved in the occurrence of RE is not well understood. HCMV and other viruses detected in RE brain tissues are highly prevalent in the general population (Britt, 2015; Looker et al., 2015). Since the average age of seizure onset in patients with RE was 5.8 years, we speculated that congenital and/or neonatal HCMV infections occurred in patients with RE. HCMV pp65 is an abundant structural protein of the virion and is often used as an indicator of the infection. Generally, during primary infection, viral antigens would be recognized by T cells, and a pool of memory T cells would be formed. The responses from memory T cells may damage neurons when being triggered by a secondary virus infection after birth and thus, could be linked to RE occurrence and progression. However, more mechanistic studies are required to further clarify how virus infection triggers the occurrence of RE.

In conclusion, elevated expression of antigen and nucleic acid of HCMV pp65 was detected in the brain tissues of patients with RE, and an abundance of HCMV antigen was found to be correlated with the duration of the prodromal stage, age of seizure onset, and hemisphere atrophy. These results suggested that HCMV may act as a trigger for RE and thereby participate in the occurrence and progression of RE. Additionally, antiviral therapy may be a potential strategy for the prevention and treatment of RE.

ACKNOWLEDGMENTS

This work was supported by the following funds: the National Natural Science Foundation of China (81571275), the Beijing Municipal Natural Science Foundation (7144217), the Capital Applied Clinic Research Programs of Science and Technology (Z131107002213171), the Beijing Rising-star Plan of Science and Technology (Z141107001814042), the Open Research Fund of the Beijing Key Laboratory of Epilepsy Research (No. 2014DXBL02), Capital Medical University (15JL08), Scientific Research Common Program of Beijing Municipal Commission of Education (KM201610025001), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2014 1685).

COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This study was approved by the Ethics Committee of Sanbo Brain Hospital, Capital Medical University (2013 061801), and written informed consent was obtained from all participants or their guardians prior to the study.

AUTHOR CONTRIBUTIONS

JA, GML, YSW conceived and designed the experi-

The mechanism by which HCMV is involved in the occurrence of RE is not well understood. HCMV and other viruses detected in RE brain tissues are highly prevalent in the general population (Britt, 2015; Looker et al., 2015). Since the average age of seizure onset in patients with RE was 5.8 years, we speculated that congenital and/or neonatal HCMV infections occurred in patients with RE. HCMV pp65 is an abundant structural protein of the virion and is often used as an indicator of the infection. Generally, during primary infection, viral antigens would be recognized by T cells, and a pool of memory T cells would be formed. The responses from memory T cells may damage neurons when being triggered by a secondary virus infection after birth and thus, could be linked to RE occurrence and progression. However, more mechanistic studies are required to further clarify how virus infection triggers the occurrence of RE.

In conclusion, elevated expression of antigen and nucleic acid of HCMV pp65 was detected in the brain tissues of patients with RE, and an abundance of HCMV antigen was found to be correlated with the duration of the prodromal stage, age of seizure onset, and hemisphere atrophy. These results suggested that HCMV may act as a trigger for RE and thereby participate in the occurrence and progression of RE. Additionally, antiviral therapy may be a potential strategy for the prevention and treatment of RE.

ACKNOWLEDGMENTS

This work was supported by the following funds: the National Natural Science Foundation of China (81571275), the Beijing Municipal Natural Science Foundation (7144217), the Capital Applied Clinic Research Programs of Science and Technology (Z131107002213171), the Beijing Rising-star Plan of Science and Technology (Z141107001814042), the Open Research Fund of the Beijing Key Laboratory of Epilepsy Research (No. 2014DXBL02), Capital Medical University (15JL08), Scientific Research Common Program of Beijing Municipal Commission of Education (KM201610025001), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2014 1685).

COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This study was approved by the Ethics Committee of Sanbo Brain Hospital, Capital Medical University (2013 061801), and written informed consent was obtained from all participants or their guardians prior to the study.

AUTHOR CONTRIBUTIONS

JA, GML, YSW conceived and designed the experi-

The mechanism by which HCMV is involved in the occurrence of RE is not well understood. HCMV and other viruses detected in RE brain tissues are highly prevalent in the general population (Britt, 2015; Looker et al., 2015). Since the average age of seizure onset in patients with RE was 5.8 years, we speculated that congenital and/or neonatal HCMV infections occurred in patients with RE. HCMV pp65 is an abundant structural protein of the virion and is often used as an indicator of the infection. Generally, during primary infection, viral antigens would be recognized by T cells, and a pool of memory T cells would be formed. The responses from memory T cells may damage neurons when being triggered by a secondary virus infection after birth and thus, could be linked to RE occurrence and progression. However, more mechanistic studies are required to further clarify how virus infection triggers the occurrence of RE.

In conclusion, elevated expression of antigen and nucleic acid of HCMV pp65 was detected in the brain tissues of patients with RE, and an abundance of HCMV antigen was found to be correlated with the duration of the prodromal stage, age of seizure onset, and hemisphere atrophy. These results suggested that HCMV may act as a trigger for RE and thereby participate in the occurrence and progression of RE. Additionally, antiviral therapy may be a potential strategy for the prevention and treatment of RE.

ACKNOWLEDGMENTS

This work was supported by the following funds: the National Natural Science Foundation of China (81571275), the Beijing Municipal Natural Science Foundation (7144217), the Capital Applied Clinic Research Programs of Science and Technology (Z131107002213171), the Beijing Rising-star Plan of Science and Technology (Z141107001814042), the Open Research Fund of the Beijing Key Laboratory of Epilepsy Research (No. 2014DXBL02), Capital Medical University (15JL08), Scientific Research Common Program of Beijing Municipal Commission of Education (KM201610025001), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2014 1685).

COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This study was approved by the Ethics Committee of Sanbo Brain Hospital, Capital Medical University (2013 061801), and written informed consent was obtained from all participants or their guardians prior to the study.

AUTHOR CONTRIBUTIONS

JA, GML, YSW conceived and designed the experi-

The mechanism by which HCMV is involved in the occurrence of RE is not well understood. HCMV and other viruses detected in RE brain tissues are highly prevalent in the general population (Britt, 2015; Looker et al., 2015). Since the average age of seizure onset in patients with RE was 5.8 years, we speculated that congenital and/or neonatal HCMV infections occurred in patients with RE. HCMV pp65 is an abundant structural protein of the virion and is often used as an indicator of the infection. Generally, during primary infection, viral antigens would be recognized by T cells, and a pool of memory T cells would be formed. The responses from memory T cells may damage neurons when being triggered by a secondary virus infection after birth and thus, could be linked to RE occurrence and progression. However, more mechanistic studies are required to further clarify how virus infection triggers the occurrence of RE.

In conclusion, elevated expression of antigen and nucleic acid of HCMV pp65 was detected in the brain tissues of patients with RE, and an abundance of HCMV antigen was found to be correlated with the duration of the prodromal stage, age of seizure onset, and hemisphere atrophy. These results suggested that HCMV may act as a trigger for RE and thereby participate in the occurrence and progression of RE. Additionally, antiviral therapy may be a potential strategy for the prevention and treatment of RE.

ACKNOWLEDGMENTS

This work was supported by the following funds: the National Natural Science Foundation of China (81571275), the Beijing Municipal Natural Science Foundation (7144217), the Capital Applied Clinic Research Programs of Science and Technology (Z131107002213171), the Beijing Rising-star Plan of Science and Technology (Z141107001814042), the Open Research Fund of the Beijing Key Laboratory of Epilepsy Research (No. 2014DXBL02), Capital Medical University (15JL08), Scientific Research Common Program of Beijing Municipal Commission of Education (KM201610025001), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2014 1685).

COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This study was approved by the Ethics Committee of Sanbo Brain Hospital, Capital Medical University (2013 061801), and written informed consent was obtained from all participants or their guardians prior to the study.

AUTHOR CONTRIBUTIONS

JA, GML, YSW conceived and designed the experi-

The mechanism by which HCMV is involved in the occurrence of RE is not well understood. HCMV and other viruses detected in RE brain tissues are highly prevalent in the general population (Britt, 2015; Looker et al., 2015). Since the average age of seizure onset in patients with RE was 5.8 years, we speculated that congenital and/or neonatal HCMV infections occurred in patients with RE. HCMV pp65 is an abundant structural protein of the virion and is often used as an indicator of the infection. Generally, during primary infection, viral antigens would be recognized by T cells, and a pool of memory T cells would be formed. The responses from memory T cells may damage neurons when being triggered by a secondary virus infection after birth and thus, could be linked to RE occurrence and progression. However, more mechanistic studies are required to further clarify how virus infection triggers the occurrence of RE.

In conclusion, elevated expression of antigen and nucleic acid of HCMV pp65 was detected in the brain tissues of patients with RE, and an abundance of HCMV antigen was found to be correlated with the duration of the prodromal stage, age of seizure onset, and hemisphere atrophy. These results suggested that HCMV may act as a trigger for RE and thereby participate in the occurrence and progression of RE. Additionally, antiviral therapy may be a potential strategy for the prevention and treatment of RE.

ACKNOWLEDGMENTS

This work was supported by the following funds: the National Natural Science Foundation of China (81571275), the Beijing Municipal Natural Science Foundation (7144217), the Capital Applied Clinic Research Programs of Science and Technology (Z131107002213171), the Beijing Rising-star Plan of Science and Technology (Z141107001814042), the Open Research Fund of the Beijing Key Laboratory of Epilepsy Research (No. 2014DXBL02), Capital Medical University (15JL08), Scientific Research Common Program of Beijing Municipal Commission of Education (KM201610025001), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (2014 1685).

COMPLIANCE WITH ETHICS GUIDELINES

The authors declare that they have no conflict of interest. This study was approved by the Ethics Committee of Sanbo Brain Hospital, Capital Medical University (2013 061801), and written informed consent was obtained from all participants or their guardians prior to the study.

AUTHOR CONTRIBUTIONS

JA, GML, YSW conceived and designed the experi-
ments. YZ, YSW, SCC performed the experiments. YSW, SCC analyzed the data. YGG, CQL, TFL contributed reagents/materials/analysis tools. YZ, YSW, SCC wrote the manuscript. YSW, SCC prepared the figures and/or tables. JA, GML checked and finalized the manuscript. All authors read and approved the final manuscript.

OPEN ACCESS

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

REFERENCES

Atkins MR, Terrell W, Hulette CM. 1995. Rasmussen's syndrome: a study of potential viral etiology. Clin Neuropathol, 14: 7–12.
Bauer J, Bien CG, Lassmann H. 2002. Rasmussen's encephalitis: a role for autoimmune cytotoxic T lymphocytes. Curr Opin Neurol, 15: 197–200.
Bien CG, Bauer J, Deckwerth TL, Wiendl H, Deckert M, Wiestler OD, Schramm J, Elger CE, Lassmann H. 2002a. Destruction of neurons by cytotoxic T cells: a new pathogenic mechanism in Rasmussen's encephalitis. Ann Neurol, 51: 311–318.
Bien CG, Granata T, Antozzi C, Cross JH, Dulac O, Kurthen M, Lassmann H, Mantegazza R, Villemure JG, Spreefico R, Elger CE. 2005. Pathogenesis, diagnosis and treatment of Rasmussen encephalitis: a European consensus statement. Brain, 128: 454–471.
Bien CG, Widman G, Urbach H, Sassen R, Kuczaty S, Wiestler OD, Schramm J, Elger CE. 2002b. The natural history of Rasmussen's encephalitis. Brain, 125: 1751–1759.
Britt W. 2015. Controversies in the natural history of congenital human cytomegalovirus infection: the paradox of infection and disease in offspring of women with immunity prior to pregnancy. Med Microbiol Rev, 204: 263–271.
Chen S, Chen S, Guan Y, Zhang Y, Qi X, An J, Wang Y, Luan G. 2016. Elevated expression of human papillomavirus antigen in brain tissue of patients with Rasmussen's encephalitis. Epilepsy Res, 126: 119–125.
Farrell MA, Cheng L, Cornford ME, Grody WW, Vinters HV. 1991. Cytomegalovirus and Rasmussen's encephalitis. Lancet, 337: 1551–1552.
Gehring L, Carlson NG, Meyer EL, Rogers SW. 2001. Granzyme B proteolysis of a neuronal glutamate receptor generates an autoantigen and is modulated by glycosylation. J Immunol, 166: 1433–1438.
Guan Y, Zhou J, Luan G, Liu X. 2014. Surgical treatment of patients with Rasmussen encephalitis. Stereotact Funct Neurosurg, 92: 86–93.
Jay V, Becker LE, Otsubo H, Cortez M, Hwang P, Hoffman HJ, Zielenska M. 1995. Chronic encephalitis and epilepsy (Rasmussen's encephalitis): detection of cytomegalovirus and herpes simplex virus 1 by the polymerase chain reaction and in situ hybridization. Neurology, 45: 108–117.
Li Y, Uccelli A, Laxer KD, Jeong MC, Vinters HV, Tourtellotte WW, Hauser SL, Okenberg JR. 1997. Local-clonal expansion of infiltrating T lymphocytes in chronic encephalitis of Rasmussen. J Immunol, 158: 1428–1437.
Looker KJ, Magaret AS, Turner KM, Vickerman P, Gottlieb SL, Newman LM. 2015. Global estimates of prevalent and incident herpes simplex virus type 2 infections in 2012. PLoS One, 10: e114989.
Luan G, Gao Q, Guan Y, Zhai F, Zhou J, Liu C, Chen Y, Yao K, Qi X, Li T. 2013. Upregulation of adenosine kinase in Rasmussen encephalitis. J Neuropathol Exp Neurol, 72: 1000–1008.
Luan G, Gao Q, Zhai F, Chen Y, Li T. 2016. Upregulation of HMGB1, toll-like receptor and RAGE in human Rasmussen's encephalitis. Epilepsia Res, 123: 36–49.
Manicklal S, Emery VC, Lazzarotto T, Boppana SB, Gupta RK. 2013. The “silent” global burden of congenital cytomegalovirus. Clin Microbiol Rev, 26: 86–102.
Merkler D, Horvath E, Bruck W, Zinkernagel RM, Del la Torre JC, Pinschwer DD. 2006. "Viral deja vu" elicits organ-specific immune disease independent of reactivity to self. J Clin Invest, 116: 1254–1263.
Pardo CA, Vining EP, Guo L, Skolasly RK, Carson BS, Freeman JM. 2004. The pathology of Rasmussen syndrome: stages of cortical involvement and neuropathological studies in 45 hemispherectomies. Epilepsia, 45: 516–526.
Power C, Poland SD, Blume WT, Girvin JP, Rice GP. 1990. Cytomegalovirus and Rasmussen's encephalitis. Lancet, 336: 1282–1284.
Rasmussen T, Olszewski J, Lloydsmith D. 1958. Focal seizures due to chronic localized encephalitis. Neurology, 8: 435–445.
Rogers SW, Andrews PI, Gehring LC, Whisenand T, Cauley K, Crain B, Hughes TE, Heinemann SF, McNamara JO. 1994. Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis. Science, 265: 648–651.
Schwab N, Bien CG, Waschbisch A, Becker A, Vince GH, Dornmair K, Wiendl H. 2009. CD8+ T-cell clones dominate brain infiltrates in Rasmussen encephalitis and persist in the periphery. Brain, 132: 1236–1246.
Sakahashi Y, Matsuda K, Kubota Y, Shimomura J, Yamasaki E, Kudo T, Fukushima K, Osaka H, Akasaka N, Inamura A, Yamada S, Kondo N, Fujiwara T. 2006. Vaccination and infection as causative factors in Japanese patients with Rasmussen syndrome: molecular mimicry and HLA class I. Clin Dev Immunol, 13: 381–387.
Tandon R, Mocarski ES. 2012. Viral and host control of cytomegalovirus maturation. Trends Microbiol, 20: 392–401.
Varadkar S, Bien CG, Kruse CA, Jensen FE, Bauer J, Pardo CA, Vincent A, Mathern GW, Cross JH. 2014. Rasmussen's encephalitis: clinical features, pathobiology, and treatment advances. Lancet Neurol, 13: 195–205.
Walter GF, Renella RR. 1989. Epstein-Barr virus in brain and Rasmussen's encephalitis. Lancet, 1: 279–280.