Cytomegalovirus antibodies are associated with mood disorders, suicide, markers of neuroinflammation, and microglia activation in postmortem brain samples

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Article

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Abstract

Cytomegalovirus (CMV) is a common, neurotrophic herpesvirus that can be reactivated by inflammation and cause neurological disease. CMV replication can in turn worsen inflammation, raising the possibility that poorly controlled CMV infections may contribute to the neuroinflammation underlying some psychiatric disorders. We investigated whether the presence of anti-CMV antibodies in blood were associated with mental illness, suicide, neuroinflammation, and microglial density in the dorsolateral prefrontal cortex in postmortem samples. Data (n = 114 with schizophrenia; n = 78 with bipolar disorder; n = 87 with depression; n = 85 controls) were obtained from the Stanley Medical Research Institute. Gene expression data from a subset of 82 cases were categorized into “high” (n = 30), and “low” (n = 52) inflammation groups based on a recursive two-step cluster analysis using expression data for four inflammation-related genes. Measurements of the density of non-ramified and ramified microglia were available for an independent subset of 51 samples. All analyses controlled for age, sex, ethnicity, postmortem interval, and pH. CMV seropositivity significantly increased the odds of a mood disorder diagnosis (bipolar disorder: OR = 2.49; major depression: OR = 2.62) and among the psychiatric cases, of suicide (OR = 2.21). Samples in the upper tercile of anti-CMV antibody titers were more likely to be members of the “high” inflammation group (OR = 4.41). CMV positive cases also showed an increased ratio of non-ramified to ramified microglia in layer I of the dorsolateral prefrontal cortex (Cohen’s d = 0.81). The results raise the possibility that the reactivation of CMV contributes to the neuroinflammation that underlies some cases of psychiatric disorders.

Introduction

Cytomegalovirus (CMV) is a common herpesvirus that establishes lifelong latent infections, undergoes periodic reactivation, and may cause disease in vulnerable populations including neonates and the immunocompromised. CMV has attracted the attention of the psychiatric field because CMV (a) is neurotrophic and may cause neurological disease, (b) it can be reactivated by psychological stress which is linked with the onset and exacerbation of many psychiatric disorders, and (c) it can be reactivated by inflammation which is linked with multiple psychiatric disorders including mood disorders and schizophrenia. Importantly, lytic CMV replication can in turn worsen inflammation, raising the possibility that poorly-controlled CMV infections may be one source of the systemic or neuroinflammation underlying the development of some cases of mental illness.

Most of the evidence linking CMV infections to psychiatric disorders is epidemiological. At least 20 studies have reported either a higher frequency of seropositivity to CMV in depressed cases versus controls or a positive correlation between anti-CMV IgG levels and depressive symptoms. In addition, two prospective studies found that CMV seropositivity was associated with an increased risk of subsequent depression. Similarly, a large Swedish cohort study showed that children hospitalized with a CMV infection were 16.6 times more likely to develop a non-affective psychosis in the future and several studies have linked schizophrenia with CMV infection.
We recently published some of the first work linking CMV seropositivity to neuroimaging abnormalities in the context of major depressive disorder (MDD). In up to three independent samples, we demonstrated that compared to CMV seronegative individuals with MDD, CMV positive individuals with MDD had widespread reductions in gray matter volume, decreased white matter integrity in a tract connecting the frontal and occipital lobes (inferior frontal occipital fasciculus), and reduced functional connectivity between hubs of the sensorimotor and salience networks. Leboyer and colleagues had previously reported that CMV antibody levels were inversely associated with hippocampal volume in individuals with bipolar disorder (BD) and schizophrenia. Similarly, Agartz and colleagues recently found that CMV positive patients with bipolar or schizophrenia spectrum disorders had smaller dentate gyri and reduced total cortical area as compared to CMV negative patients. However, whether CMV is playing a causal role in these neuroimaging abnormalities and if so, whether these findings relate to a heightened inflammatory process, is still unclear. At least *in vitro*, herpesvirus infections trigger the production of a range of cytokines and chemokines by glial cells, but to our knowledge the link between CMV infection and inflammation in the central nervous system (CNS) has never been tested in people with psychiatric disorders.

Here, we investigated in a postmortem sample whether CMV serostatus and serum antibody levels to CMV were associated with the odds of: 1) having a psychiatric disorder, 2) dying by suicide, 3) having higher neuroinflammation (based on a previously performed clustering analysis of immune-related gene expression in the dorsolateral prefrontal cortex), and 4) showing increased microglia activation in the dorsolateral prefrontal cortex (i.e., an increased ratio of non-ramified to ramified microglia). We hypothesized that CMV seropositivity and/or higher CMV antibody levels would be associated with increased odds of 1) diagnosis with a psychiatric disorder, 2), suicide, 3) assignment to the “high” neuroinflammation group, and 4) an increased ratio of non-ramified to ramified microglia.

**Methods**

**Postmortem samples**

The Stanley Medical Research Institute's (SMRI) brain tissue collection is described in detail elsewhere. In short, a standard neuropathological examination was performed by a pathologist with screening for cardiovascular disease, hemorrhage, trauma, tumors, dementia, hypoxia, or other pathology. All medical and psychiatric records were obtained and reviewed by two senior psychiatrists. A psychiatrist also contacted one or more family members by telephone before making a final DSM-IV diagnosis. For the controls, a psychiatrist conducted a structured telephone interview with first-degree family members to obtain all pertinent psychiatric and medical history. Of the 377 postmortem cases obtained from the SMRI’s brain collection, we excluded one case with a primary diagnosis of alcohol dependence, one case without diagnostic information, and one case with indeterminate CMV serology results. Therefore 374 cases (n = 114 with schizophrenia; n = 78 with BD; n = 87 with a depressive disorder; n = 85 controls) were included in the statistical analyses. A total number of 119 cases died by suicide (n = 34 with...
schizophrenia; n = 37 with BD; n = 48 with depressive disorder). Demographic details and clinical variables are shown in Table 1.

### Table 1
Demographic and clinical details of postmortem cases.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Schizophrenia</th>
<th>Bipolar Disorder</th>
<th>Depressive Disorder</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>85</td>
<td>114</td>
<td>78</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>45.54 (11.16)</td>
<td>44.24 (11.41)</td>
<td>45.23 (12.31)</td>
<td>41.78 (11.83)</td>
<td>0.142</td>
</tr>
<tr>
<td>Sex</td>
<td>21 (24.7)</td>
<td>34 (29.8)</td>
<td>37 (47.4)</td>
<td>31 (35.6)</td>
<td>0.014</td>
</tr>
<tr>
<td>Post-mortem interval</td>
<td>28.04 (13.15)</td>
<td>38.61 (24.22)</td>
<td>34.33 (14.61)</td>
<td>38.25 (25.14)</td>
<td>0.002</td>
</tr>
<tr>
<td>Brain pH</td>
<td>6.53 (0.29)</td>
<td>6.42 (0.28)</td>
<td>6.44 (0.30)</td>
<td>6.49 (0.27)</td>
<td>0.027</td>
</tr>
<tr>
<td>CMV serostatus</td>
<td>27 (31.8)</td>
<td>43 (37.7)</td>
<td>42 (53.8)</td>
<td>48 (55.2)</td>
<td>0.002</td>
</tr>
<tr>
<td>CMV antibody levels¹</td>
<td>1.03 (0.76)</td>
<td>0.77 (0.56)</td>
<td>1.24 (0.62)</td>
<td>1.02 (0.55)</td>
<td>0.006</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>80 (95.1)</td>
<td>103 (90.4)</td>
<td>73 (93.6)</td>
<td>86 (98.9)</td>
<td>0.098</td>
</tr>
<tr>
<td>Manner of death</td>
<td>0 (0.0)</td>
<td>34 (29.8)</td>
<td>37 (48.1)</td>
<td>48 (55.8)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

¹. Only CMV seropositive cases.

**CMV serostatus and IgG antibody levels**

IgG antibodies against CMV were measured from serum using an enzyme-linked immunosorbent assay (ELISA, IBL America). A sample was generally considered CMV seropositive if it had an optical density (OD) value equivalent to approximately 20% over the supplied cutoff standard or a cutoff based on the distribution of the values.
To normalize data across batches, the OD values were quantified as plate-adjusted z-scores with a mean value of zero and a standard deviation of one. We further divided the IgG antibody levels into terciles based on IgG antibody percentile among the CMV seropositive cases (i.e., bottom: 0-33rd percentile, middle: 34-66th percentile, and upper: above 66th percentile).

**Gene Expression**

We previously identified several inflammation markers in the brains of people with schizophrenia using RNA-seq analysis \(^{10}\). We confirmed gene expression changes via quantitative real-time polymerase chain reaction (qPCR) with an ABI Prism 7900HT Fast Real Time PCR system with a 384-well format for both targets and housekeeper normalizing controls (Applied Biosystems, Foster City, CA, USA) as previously reported \(^{10,30}\). Inflammatory status was determined by a two-step recursive cluster analysis using expression data for seven inflammation-related genes that identified four transcripts as contributing significantly to the model used to define the two groups (SERPINA3, IL-6, IL-8 and IL-1β). Pre-designed Taqman gene expression assays (Applied Biosystems, Foster City, CA, USA) were used to measure the following transcripts: SERPINA3 (Hs003153674_m1), IL-6 (Hs00174131_m1), IL-8 (HS00174103_m1), IL-1B (Hs01555410_m1), ACTB (Hs99999903_m1), GAPDH (Hs99999905_m1), TBP (Hs00427621_m1), and UBC (Hs00824723_m1). For the current study, we clustered a subset of the postmortem cases with both CMV antibody data and gene expression data (n = 82; 30 schizophrenia, 23 BD, and 29 controls) into “high” (n = 30, 13 schizophrenia, 8 BD, and 9 controls) or “low” inflammation groups (n = 52, 17 schizophrenia, 15 BD, and 20 controls) using the methodology described above.

**Microglial cells**

Microglia were quantified in a subset of 60 cases (20 schizophrenia, 20 BD, and 20 controls). For the current study, we excluded 9 participants (out of 60) without CMV antibody data. Briefly, tissue blocks were dissected from the dorsolateral prefrontal cortex gray matter. Staining for ionized calcium-binding adaptor molecule-1 (IBA-1) was performed on three 6µm thick coronal paraffin-embedded sections, as previously described \(^{31}\). For each stained section, two counting frames were placed to encompass cortical layers I-VI. IBA-1 positive cells were categorized based on morphology and location into ramified, non-ramified or vascular subtypes. Ramified cells displayed small, round soma with abundant thin, branched processes. Non-ramified cells displayed enlarged cell bodies with thickened processes that were fewer in number, or absent. Non-ramified cells included primed, reactive and ameboid morphologies previously identified in human brain tissue \(^{32}\). The ratio of non-ramified to ramified microglia was used as a surrogate marker of microglial activation.

**Statistical analysis**

All statistical analysis were performed using R Statistical Software (version 4.1.3; R Core Team 2021). The Shapiro-Wilk's test was performed to confirm the normality of continuous variables. We first calculated the prevalence of CMV seropositivity among control and psychiatric disorder groups. Second, a multivariable logistic regression model was used to test whether CMV serostatus and CMV antibody IgG levels were associated with the likelihood of having a psychiatric disorder or having committed
suicide. The odds ratios (ORs) and 95% confidence intervals (95%CIs) were estimated from the multivariable logistic regression model. A Chi square test was performed post-hoc to assess which specific psychiatric disorder accounted for the observed results. The possible confounders, age, sex, ethnicity, post-mortem interval (PMI), and brain acidity (pH) were included as covariates in the models. Third, a multivariable logistic regression model was used to test whether CMV seropositivity and CMV antibody IgG levels increased the odds of being assigned to the “high” vs. “low” inflammation groups. In addition to age, sex, PMI, and pH, diagnosis was also controlled for in this model as psychiatric disorders were previously linked to differences in inflammation-related gene expression. Fourth, multivariable linear regression models were used to investigate the association between CMV serostatus and microglia activation (ratio of non-ramified to ramified microglia) controlling for age, sex, PMI, pH, and diagnosis. Finally, we conducted sensitivity analyses using the aforementioned models but without controlling for brain pH. Decreased brain pH has been linked with psychiatric disorders and neuroinflammation, and therefore including it in statistical models where psychiatric status or neuroinflammation are outcomes may regress out some of the inflammatory effect. The variance inflation factor was computed to assess model multicollinearity in all linear regression models.

Results

CMV seropositivity rates across the diagnostic groups

As summarized in Table 1, the prevalence of CMV seropositivity was higher in cases with a psychiatric disorder (133/279, 47.7%) than controls (27/85, 31.8%). Among the samples with a psychiatric disorder, cases with a depressive disorder showed the highest CMV prevalence (48/87, 55.2%), followed by BD (44/78, 53.8%), and schizophrenia (43/114, 37.7%).

CMV serostatus, IgG antibody levels and psychiatric disorder

Relative to CMV seronegative cases, CMV seropositive cases were more than twice as likely to have a psychiatric disorder (OR = 2.13, 95%CI = 1.21–3.82, p = 0.009) after controlling for confounders (Fig. 1A). Post-hoc analysis revealed that CMV seropositivity did not significantly increase the odds of having schizophrenia (OR = 1.30, 95%CI = 0.72–2.37, p = 0.39). However, CMV seropositivity significantly increased the odds of having a mood disorder (BD: OR = 2.49, 95%CI = 1.32–4.76, p = 0.004; depressive disorder: OR = 2.62, 95%CI: 1.41–4.95, p = 0.002).

To test for an association between CMV IgG antibody levels and the risk of having a psychiatric disorder, we grouped antibody levels into bottom, middle, and upper terciles. Relative to CMV seronegative cases, cases in the bottom tercile had 45% increased odds of having a psychiatric disorder which was not statistically significant (OR = 1.45, 95%CI = 0.66–3.35, p = 0.366). Cases in the middle and upper terciles were more than twice as likely to have a psychiatric disorder (middle: OR = 2.48, 95%CI = 1.04–6.68, p =
0.053; upper: OR = 2.50, 95%CI = 1.14–5.98, p = 0.029) after controlling for confounders (Fig. 1B). The variance inflation factor for each covariate ranged from 1.01 to 1.15 suggesting that multicollinearity was not a concern in the models.

**CMV serostatus, IgG antibody levels, and suicide**

Among those individuals with a psychiatric disorder, CMV seropositive cases were more than twice as likely to have committed suicide (OR = 2.21, 95%CI = 1.33–3.73, p = 0.003) relative to CMV seronegative cases, after controlling for potential confounders (Fig. 2A).

We also tested for an association between CMV IgG antibody levels and suicide within the psychiatric disorder group. Relative to CMV seronegative cases, cases in the bottom tercile were more likely to have committed suicide (OR = 2.38, 95%CI = 1.12–5.05, p = 0.024) but CMV seropositive cases in the middle tercile group were not significantly different in terms of suicide status compared to CMV seronegative cases (OR = 1.82, 95%CI = 0.87–3.77, p = 0.107). Cases in the upper tercile were almost three times more likely to have committed suicide (OR = 2.81, 95%CI = 1.40–5.66, p = 0.004) as compared to CMV seronegative cases after controlling for potential confounders (Fig. 2B). The variance inflation factor for each covariate ranged from 1.01 to 1.17 suggesting that multicollinearity was not a concern in the models.

**CMV serostatus, IgG antibody levels, and neuroinflammation**

There was no significant relationship between CMV serostatus and inflammation-related gene expression levels (Supplementary Fig. 1 and Supplementary Fig. 2). However, relative to CMV seronegative cases, cases with anti-CMV antibody levels in the upper tercile were more than four times more likely to have high inflammation-related gene expression (OR = 4.41, 95%CI = 0.93 ~ 23.64, p = 0.068, Supplementary Fig. 2). Due to the smaller sample size and wide confidence intervals, this association only trended significant (although it was statistically significant when brain pH was omitted from the model – see Table 2). The variance inflation factor for each covariate ranged from 1.08 to 1.78 suggesting that multicollinearity was not a concern in the models.
### Table 2
Summary of sensitivity analyses results

<table>
<thead>
<tr>
<th>Associations with diagnosis</th>
<th>OR</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV seropositivity</td>
<td>2.14</td>
<td>1.23–3.82</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>CMV antibody levels low</td>
<td>1.58</td>
<td>0.74–3.60</td>
<td>0.252</td>
</tr>
<tr>
<td>CMV antibody levels medium</td>
<td>2.52</td>
<td>1.07–6.74</td>
<td><strong>0.047</strong></td>
</tr>
<tr>
<td>CMV antibody levels high</td>
<td>2.27</td>
<td>1.04–5.40</td>
<td><strong>0.049</strong></td>
</tr>
<tr>
<td>Associations with suicide status</td>
<td>OR</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>CMV seropositivity</td>
<td>2.06</td>
<td>1.25–3.46</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>CMV antibody levels low</td>
<td>2.06</td>
<td>0.98–4.31</td>
<td>0.055</td>
</tr>
<tr>
<td>CMV antibody levels medium</td>
<td>1.71</td>
<td>0.82–3.52</td>
<td>0.146</td>
</tr>
<tr>
<td>CMV antibody levels high</td>
<td>2.83</td>
<td>1.42–5.65</td>
<td><strong>0.003</strong></td>
</tr>
<tr>
<td>Associations with neuroinflammation status</td>
<td>OR</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>CMV seropositivity</td>
<td>2.10</td>
<td>0.74–6.12</td>
<td>0.163</td>
</tr>
<tr>
<td>CMV antibody levels low</td>
<td>0.39</td>
<td>0.02–2.61</td>
<td>0.405</td>
</tr>
<tr>
<td>CMV antibody levels medium</td>
<td>2.94</td>
<td>0.55–16.72</td>
<td>0.203</td>
</tr>
<tr>
<td>CMV antibody levels high</td>
<td>4.20</td>
<td>1.09–18.24</td>
<td><strong>0.042</strong></td>
</tr>
<tr>
<td>Associations with microglia activation</td>
<td>Cohen's d</td>
<td>Std.E</td>
<td>p</td>
</tr>
<tr>
<td>dIPFC Layer I</td>
<td>0.83</td>
<td>0.34</td>
<td><strong>0.019</strong></td>
</tr>
<tr>
<td>dIPFC Layer II</td>
<td>0.56</td>
<td>0.36</td>
<td>0.126</td>
</tr>
<tr>
<td>dIPFC Layer III</td>
<td>0.40</td>
<td>0.37</td>
<td>0.281</td>
</tr>
<tr>
<td>dIPFC Layer IV</td>
<td>0.48</td>
<td>0.36</td>
<td>0.194</td>
</tr>
<tr>
<td>dIPFC Layer V</td>
<td>0.56</td>
<td>0.36</td>
<td>0.133</td>
</tr>
<tr>
<td>dIPFC Layer VI</td>
<td>0.29</td>
<td>0.37</td>
<td>0.443</td>
</tr>
</tbody>
</table>

Note: The ratio of non-ramified to ramified microglia was used as a surrogate marker of microglial activation. OR, odds ratio; 95% CI, 95% confidence interval; Std.E, standard error. All results obtained using similar statistical models as reported in the main article but without controlling for brain pH.

## CMV serostatus and microglia activation

Relative to CMV seronegative cases, CMV seropositive cases showed an increase in the ratio of non-ramified to ramified microglia in all six layers of dorsolateral prefrontal cortex although the effect only reached statistical significance in layer I (Cohen's d = 0.81, standard error = 0.34, p = 0.023, Fig. 3,
Supplementary Table S1). To avoid generating heavily biased results using extremely small samples (i.e., 3 cases in bottom tercile group, 6 cases in middle tercile group, and 6 cases in the upper tercile group), we did not run the analyses to test for associations between CMV antibody levels and microglia activation.

**Sensitivity analyses**

Sensitivity analyses using similar statistical models but without controlling for brain pH showed consistent results with similar effect-size (Table 2) supporting the robustness of the findings reported above.

**Discussion**

To our knowledge, this is the first study to examine the correlates of CMV infection at the time of death within the context of psychiatric illness. There were three main results. First, samples from individuals with CMV seropositivity were significantly more likely to have BD (OR = 2.49) or a unipolar depressive disorder (OR = 2.62) than individuals who were CMV seronegative. The effect appeared to be driven by cases with antibody levels in the middle and upper terciles. Second, among the cases with a psychiatric disorder, those individuals who tested CMV seropositive were significantly more likely to commit suicide than the individuals who tested seronegative for CMV (OR = 2.21). The effect appeared to be driven by cases in the upper antibody tercile who were approximately three times more likely to be suicides than CMV negative cases. Third, the CMV seropositive group displayed a greater ratio of non-ramified to ramified microglia in layer I of the dorsolateral prefrontal cortex, and consistent with this putative indicator of inflammation, individuals in the upper antibody tercile were more than four times more likely than CMV negative cases to be members of the “high” inflammation group, which was previously shown to have increased numbers of reactive astrocytes and elevated cytokine concentrations compared to the “low” inflammation group 10, 11.

The association between CMV seropositivity and mood disorders is broadly consistent with the epidemiological literature. At least 14 observational studies have linked CMV infection with unipolar depression 17, 18, 35–46. Less work has been done on BD, but one case-control study reported higher CMV seropositivity rates in 1,200 participants with BD relative to 745 healthy controls 47. Also notable are two large prospective studies which found that CMV seropositivity was associated with an increased risk of future depression 17 or mood disorders, more generally 18. Other studies have reported a link between CMV antibody titers and mood disorders. Anti-CMV titers are a surrogate marker of viral reactivation, with higher antibody levels generally indicative of an active infection 48. For instance, the Detroit Neighborhood Health Study showed that for every one unit increase in CMV IgG antibody titer, the odds of incident depression increased by 26% so that individuals with IgG antibody titers in the highest quartile had four times greater odds of depression compared to participants in the lower three quartiles 41. In the case of schizophrenia, results have been more equivocal 14 which may explain the negative findings of this study. Although some early papers reported a higher frequency of CMV seropositivity in
schizophrenia\textsuperscript{49–51}, these findings proved challenging to replicate and several meta-analyses or systematic reviews have failed to detect a statistical association between CMV serostatus and schizophrenia\textsuperscript{52–54}. However, since heightened neuroinflammation is observed in a subset of people with schizophrenia, a more stratified approach may be required to test the association between schizophrenia and CMV IgG antibody titers in the future.

Several published studies support the link between CMV infection and suicide. A case-control study including over 80,000 Danish blood donors found that CMV seropositivity was associated with an increased risk of attempting or committing suicide\textsuperscript{18}. Similarly, Dickerson and colleagues followed over 1,000 individuals with schizophrenia, BD, and major depressive disorder over 8 years and found that increasing levels of CMV antibodies were associated with increasing hazard ratios for suicide\textsuperscript{42}. Another group reported higher IgG anti-CMV antibody levels in depressed patients with at least two suicide attempts compared with depressed participants with no history of suicide attempts\textsuperscript{45}. Nevertheless, it should be noted that not all studies support the link between CMV and suicide\textsuperscript{55, 56} while another paper detected an association between suicide and anti-CMV IgM titers but not IgG titers\textsuperscript{40}. The results of the current paper provide support to those epidemiological studies that have reported a positive association between CMV and suicide.

At “rest”, microglia display a ramified morphology whereas upon activation they display a graded series of morphological changes leading up to a non-ramified, ameboid-like phenotype\textsuperscript{57, 58}. Here, we used the ratio of non-ramified to ramified microglia as a surrogate marker of microglia activation. The increased ratio of non-ramified to ramified microglia in the CMV positive versus the CMV negative samples in layer I of dorsolateral prefrontal cortex is consistent with preclinical studies demonstrating that microglia play an important role in protecting the brain against CMV infection\textsuperscript{59}. Similarly, murine models of congenital CMV infection are characterized by elevated levels of microglia-derived chemokines, infiltration of leukocytes, and activation of microglia in the brain\textsuperscript{60–62}. In addition, in humans, CMV has been shown to cause microglial nodular encephalitis in the context of HIV infection\textsuperscript{63, 64}. Although the difference in the ratio of non-ramified to ramified microglia density between CMV positive and CMV negative samples was only statistically significant in the most superficial cortical layer, we found this ratio to be greater in the CMV positive group in all six cortical layers (Fig. 3). Thus, we suggest that the microglial activation is likely to be anatomically generalized but larger sample sizes are required to confirm this hypothesis.

The microglia result together with the finding that samples in the upper tercile of CMV antibody levels were more than four times more likely than CMV negative samples to be assigned to the “high” neuroinflammation group (which in previously published work showed higher cytokines, increased astrogliosis, and greater HLA-DR\textsuperscript{+} cell density) is suggestive of at least two possibilities. First, that reactivation of CMV is a cause of neuroinflammation or second, that neuroinflammation triggers CMV reactivation. The first possibility is consistent with work in other fields showing that CMV infection may trigger graft rejection and reduce the survival of transplant recipients\textsuperscript{65} as well as contribute to medical morbidity in the context of HIV\textsuperscript{13}, sepsis\textsuperscript{66}, and COVID-19\textsuperscript{67, 68}. Further, treatment of HIV positive
patients with the anti-CMV medication, valganciclovir, was demonstrated to reduce CD8+ (CD38+ HLA-DR+) cell immune activation as well as plasma concentrations of sTNFR2, sCD163, and sCD14 compared to placebo\textsuperscript{13,69}. CMV also increases the risk of developing coronary artery disease (CAD) after heart transplantation potentially as a result of increased inflammation\textsuperscript{70,71}. Thus, a clinical trial was conducted comparing four weeks of treatment with ganciclovir to placebo immediately after transplantation\textsuperscript{72}. CAD was assessed 4.7 years later and was reported to be reduced in the ganciclovir group not treated with calcium blockers compared to the placebo group\textsuperscript{72}. On the other hand, it is well established that inflammatory mediators such as tumor necrosis factor (TNF) and interleukin 6 (IL-6) promote CMV reactivation via AP-1 or NF-kB-induced transcription of the immediate early CMV promoter\textsuperscript{6,73}. Experimental designs are required to disambiguate the relative contributions of these opposing processes. However, our hypothesis is that both phenomena are likely at play. That is, inflammation reactivates CMV, and lytic viral replication in turn exacerbates the underlying inflammation.

This study has several limitations. First, serum was not available for all cases in the SMRI brain bank and thus CMV serostatus could not be determined for all samples. As a result, the gene expression and histological analyses may have been underpowered, especially as we elected to control for up to six potential confounders in the statistical models, an approach that is not always taken in the postmortem literature because of sample size limitations. Second, there are several other neurotrophic viruses that may play a role in psychiatric illness. While our primary focus has been on CMV because of its association with neurological disease\textsuperscript{1} and its significant impact on the immune system\textsuperscript{74}, future studies should examine the potential effects of other viral agents. Third, samples were labeled as showing “high” or “low” inflammation based on the expression of four different genes (two cytokines, a chemokine, and a serine protease inhibitor found in reactive astrocytes\textsuperscript{75}) that provided a snapshot of immune system activity rather than capturing the full extent of inflammatory signaling. Fourth, the distinction between non-ramified and ramified microglia density should be viewed as heuristic since microglia are thought to display a continuum of activation rather than a simple binary “on” or “off” phenotype.

In sum, the current study raises the possibility that CMV is a risk factor for mood disorders and suicide through its possible neuroinflammatory effects. Further research is necessary to follow-up on these initial results to determine whether CMV is playing a causal role in psychiatric illness. If CMV does indeed contribute to neuroinflammation in the context of mental illness, then this may open-up a novel avenue of treatment given the existence of approved medications for the treatment of CMV\textsuperscript{76–78}.

**Declarations**

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data Availability Statement

Data used in current study are available at https://stanleyresearch.org. The statistical analysis R scripts used for current study are available upon request to the corresponding author.

Author Contributions

Conceptualization, H.Z., M.J.W., M.P.P., R.H.Y., and J.S; data collection, M.J.W., C.S.W., C.L.B., and R.H.Y; methodology and data analysis, H.Z., M.J.W., C.S.W., C.L.B., and R.H.Y; manuscript writing—original draft preparation, H.Z., and J.S; manuscript writing—review and editing, H.Z., M.J.W., C.S.W., C.L.B., M.P.P., R.H.Y., and J.S; All authors have read and agreed to the published version of the manuscript.

Supplementary information is available at MP's website

References


Figures
Figure 1

Associations between CMV infection and psychiatric disorders.

A, CMV seropositivity is associated with increased odds of having a psychiatric disorder. B, Higher CMV antibody IgG levels are associated with increased odds of having a psychiatric disorder.
Figure 2

Associations between CMV infection and suicide status.

A, CMV seropositivity is associated with an increased likelihood of committing suicide. B, Higher CMV antibody IgG levels are associated with an increased likelihood of committing suicide.
Figure 3

CMV seropositivity is associated with an increased ratio of non-ramified to ramified microglia. Note that the figure illustrates the ratio after regressing out the effects of age, PMI, sex, brain pH, and diagnosis.

Supplementary Files
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