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Diagnosis-independent loss of T-cell costimulatory molecules in individuals with cytomegalovirus infection

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Abstract

Major depressive disorder (MDD) is associated with physiological changes commonly observed with increasing age, such as inflammation and impaired immune function. Age-related impaired adaptive immunity is characterized by the loss of naive T-cells and the reciprocal accumulation of memory T-cells together with the loss of T-cell co-stimulatory molecules. Additionally, the presence and activity of cytomegalovirus (CMV) alters the architecture of the T-cell compartment in a manner consistent with premature aging. Because CMV is also thought to reactivate with psychological stress, this study tested whether MDD influences age-related phenotypes of T-cell populations in the context of CMV infection in young and middle-aged adults. Morning blood samples from volunteers with a DSM-IV diagnosis of MDD (n = 98, mean age(SD) = 36(10)

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Ethics Statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.03.013.

years, 74.5% female, 57.1% CMV^+) and comparison controls (n = 98, mean age(SD) = 34(10) years, 68.4% female, 51.0% CMV^+) were evaluated for CMV IgG antibody status and the distribution of late differentiated ($CD27^-CD28^-$) cells within $CD4^+$ and $CD8^+$ T-cell subsets, i.e. naive ($CCR7^+CD45RA^+$), effector memory (EM, $CCR7^-CD45RA^-$), central memory (CM, $CCR7^+CD45RA^-$) and effector memory cells re-expressing CD45RA (EMRA, $CCR7^-CD45RA^+$). Mixed linear regression models controlling for age, sex, ethnicity and flow cytometry batch showed that CMV seropositivity was associated with a reduction in naive T-cells, expansion of EMRA T-cells, and a greater percent distribution of $CD27^-CD28^-$ cells within $CD4^+$ and $CD8^+$ memory T-cell subsets (p's < 0.004), but there was no significant effect of MDD, nor any significant interaction between CMV and diagnosis. Unexpectedly, depressed men were less likely to be CMV^+ and depressed women were more likely to be CMV^+ than sex-matched controls suggesting a possible interaction between sex and MDD on CMV susceptibility, but this three-way interaction did not significantly affect the T-cell subtypes. Our findings suggest that depression in young and middle-aged adults does not prematurely advance aging of the T-cell compartment independently of CMV, but there may be significant sex-specific effects on adaptive immunity that warrant further investigation.

Keywords

Major depressive disorder; Cytomegalovirus; T-cells; Immunosenescence; Biological aging; Depression; Sex differences

1. Background

Lymphoproliferative response to mitogen, natural killer cell cytotoxicity, and virus-specific T-cell response are suppressed in a subset of depressed patients (Irwin and Miller, 2007; Zorrilla et al., 2001). Relevant to these findings is the link between depression and poor control of chronic viral infections (Evans et al., 2002; Phillips et al., 2008) and attenuated response to immunization or accelerated loss of vaccine-induced immunogenicity over time (Afsar et al., 2009; Ford et al., 2019b; Irwin et al., 2013, 2011). These observations mirror those seen in immunosenescence, the general decline in immune function that occurs during aging (Nikolich-Žugich, 2018). This has led to the suggestion that accelerated biological aging at the cellular level may be an important aspect of depression-associated pathology (Wolkowitz et al., 2011). While studies of leukocyte telomere length and epigenetic aging support this hypothesis (Han et al., 2018; Verhoeven et al., 2014), immunosenescence-associated cell surface phenotypes have not been fully characterized in a depressed cohort. Here, we examine cell surface markers indicative of premature biological aging on T-cells from adult volunteers (aged 18–57) with major depressive disorder (MDD) and comparison controls, and evaluate the potential interactive effects of a major driver of T-cell differentiation, cytomegalovirus (CMV) (Pawelec et al., 2009).

Repeated pathogenic encounters result in the accumulation of oligoclonal, late differentiated T-cells, identified by the loss of co-stimulatory cell surface markers, CD27 and CD28 that is associated with immunosenescence (Appay et al., 2002; Koch et al., 2008). CMV, a common herpes virus that establishes latent infections, influences the progression of T-cell

immunosenescence to a greater degree than other subclinical, chronic infections (Derhovanessian et al., 2011; Khan et al., 2004; Olsson et al., 2000). CMV seropositivity is associated with enrichment of oligoclonal, late-differentiated ($CD27^{-}CD28^{-}$) $CD4^{+}$ and $CD8^{+}$ T-cells that may overcrowd the memory T-cell compartment and drive out memory clones for other pathogens (Turner et al. 2014; Weinberger et al. 2007; Burton et al. 2019; Almanzar et al. 2005; Khan et al. 2004). Importantly, neuro-immune stress pathways may promote reactivation of latent CMV infection (Prösch et al. 2000). Consistent with this finding, CMV reactivation can occur during periods of psychological stress. For example, saliva titers of IgG and IgA antibodies against CMV were higher during exam periods in nursing and medical students (Glaser et al., 1985; Sarid et al., 2004). Female caregivers to disabled children over the age of 45 had higher serum titers for CMV IgG antibodies and lower $CD4^{+}$ to $CD8^{+}$ T-cell ratios than age-matched controls (Pariante et al., 1997). Similarly, CMV viral DNA shedding was higher in astronauts directly before and after space travel and higher shedding was associated with longer space missions (Mehta et al., 2000; Rooney et al., 2019).

Depression shares risk factors with CMV including low socioeconomic status (SES) and early life adversity, and may also contribute to poor CMV viral control. In a large occupational sample, low SES increased the odds of CMV seropositivity, and depressive symptoms were associated with higher CMV IgG titers (Rector et al., 2014). In the National Health and Nutrition Examination Survey (NHANES), poverty was associated with higher antibody levels in CMV^{+} children aged 6 to 16 (Dowd et al., 2012). Adverse conditions during childhood, while also predicting depression, may be a particularly important factor in determining susceptibility to CMV (Janicki-Deverts et al., 2014). Self-reported childhood trauma, including physical and sexual abuse, was shown to increase the odds of CMV seropositivity in two independent samples of adults diagnosed with MDD (Ford et al., 2019a). Similarly, in breast cancer survivors, CMV titers were positively correlated with the number of self-reported adverse life events (Fagundes et al., 2013).

The potential interactive effects of CMV infection and MDD on the adaptive immune system has, however, been understudied. One small study provided some evidence that their combined influence amplifies the effects of aging on the immune system: elderly depressed individuals had higher CMV IgG antibody titers and a higher percentage of $CD28^{-}CD57^{+}$ lymphocytes compared to age and sex-matched non-depressed controls (Trzonkowski et al., 2004). This could indicate that CMV and depression act as a “double hit” to the immune system, increasing the rate of immunosenescence. The goal of this investigation was to examine the hypothesis that there is an interaction between CMV and depression such that those individuals with depression who are also CMV^{+} show the greatest evidence of T-cell aging. The primary endpoint for this study was alterations in the percent distribution of naive and memory T-cell subsets and the accumulation of late differentiated ($CD27^{-}CD28^{-}$) T-cells within each subset. In addition, we examined the CMV^{+} subgroups to determine if possible effects of CMV IgG titer on T-cell phenotypes were altered by a diagnosis of MDD. Support for this hypothesis would provide evidence that CMV status moderates the risk for altered immunity as a consequence of depression. If true, CMV antiviral therapy and/or vaccination could mitigate premature aging occurring in the context of MDD.

2. Methods

2.1. Study participants

The study was approved by the Western Institutional Review Board and was conducted according to the principles expressed in the Declaration of Helsinki. Volunteers gave written consent to participate in a study involving neuroimaging and immunophenotyping. One hundred participants with a diagnosis of MDD and 100 comparison controls with no personal history of a psychiatric disorder were included in this sub-study. The groups were matched for age and sex. Diagnoses were determined according to the Diagnostic and Statistical Manual for Mood Disorders, Fourth Edition, Text Revision (DSM-IV-TR) using the Structured Clinical Interview for DSM-IV-TR (SCID) administered by a trained clinical interviewer and an unstructured interview with a psychiatrist. Exclusion criteria were as follows: clinically-significant suicidal ideation or behavior; current medical conditions or concomitant medications likely to influence central nervous system or immunological function including significant cardiovascular, respiratory, endocrine, and neurological diseases; a history of drug or alcohol abuse within six months or a history of drug or alcohol dependence within one year (DSM-IV-TR criteria). Depression severity was measured with the Montgomery-Asberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979). Early life stress was evaluated with the Early Trauma Inventory Self Report (ETI-SR, n = 164) (Bremner et al., 2007) and the Childhood Trauma Questionnaire (CTQ, n = 180) (Bernstein et al., 2003).

2.2. Biological assays

Morning blood samples were collected via venipuncture into Becton-Dickinson (BD) Vacutainer tubes containing EDTA for plasma collection and BD CPT Vacutainer tubes for peripheral blood mononuclear cell (PBMC) isolation. Samples were processed using standard laboratory procedures. PBMCs were suspended in fetal bovine serum with 10% DMSO and stored at −190 °C in liquid nitrogen. Plasma samples were frozen at −80 °C. Thawed plasma samples were tested, blind to diagnosis, for IgG antibodies against CMV using a commercially available immunosorbent assay (IBL America, www.ibl-america.com). A sample was considered seropositive if it had an optical density value of > 0.5, which is equivalent to approximately ten international units of antibody. CMV IgG titers were quantified as a plate-adjusted z-scores with a mean value for each plate of two and a standard deviation of one.

A ten-color flow cytometry panel was developed to identify and quantify T-cell subsets including those with cell surface phenotypes indicative of late differentiation. Isolated PBMCs were stained with fluorochrome-conjugated antibodies purchased from Biolegend (www.biolegend.com) following standard laboratory procedures. In brief, PBMCs were thawed and sequentially incubated on ice with IgG Fc blocker, antibody cocktail, then Zombie Violet viability dye (www.biolegend.com). Supplementary Table 1 details each of the cell surface markers and the fluorescent labels used for its identification. Following the staining procedure, data were collected on a BD LSRII flow cytometer and analyzed using Flowjo software version 10.5.0. Samples were gated on time to eliminate possible instability in the cytometer measurement. Doublets, dead or dying cells, and cells expressing CD14

(monocytes), CD16 (macrophages), CD19 (B-cells) or CD56 (natural killer cells) were excluded, and cells expressing CD3 (T-cells) were positively selected. T-helper cells ($CD4^+$) and cytotoxic T-cells ($CD8^+$) were gated within the T-cells. Quadrant gating on CCR7 vs CD45RA plots was used to define subsets of the $CD4^+$ and $CD8^+$ populations as follows: naive were $CCR7^+CD45RA^+$, central memory (CM) were $CCR7^+CD45RA^-$, effector memory (EM) were $CCR7^-CD45RA^-$ and effector memory re-expressing CD45RA (EMRA) were $CCR7^-CD45RA^+$. The proportion of each subset that was $CD27^-CD28^-$ was considered to be late differentiated. The panel also included an anti-CD62L antibody, but this metric was excluded from the main analysis upon finding that CD62L detection is highly susceptible to loss during freezing (Weinberg et al., 2009). A sample gating strategy for the quantification of CD27 and CD28 expression on $CD8^+$ EM cells is shown in Supplemental Fig. 1.

2.3. Statistical analysis

For each T-cell subtype, the proportion of the parent population was arcsine transformed (2*(asin(sqrt[x])) to stabilize variance. CMV serostatus and diagnosis were tested against each cell population using mixed linear regression models controlling for age, sex, ethnicity (Caucasian vs non-Caucasian) and flow cytometry batch. Secondary analyses included the interactive term for CMV and diagnosis. Further exploratory analyses included the interactive term for CMV, diagnosis, and sex, and CMV IgG titers (calculated as plate dependent z-scores) in the CMV^+ participants, with and without the interactive term with diagnosis. Alpha was adjusted for multiple comparisons using the effective number of tests (M_{eff}) method for non-independent outcome measures (Cheverud, 2001; Nyholt, 2004). Based on the correlation matrix of the sixteen outcome variables (Supplementary Fig. 2), M_{eff} was 14.16 and alpha was set to 0.004. Effect sizes for significant variables were calculated as Cohen's f^2 statistic following the procedure described by Selya et al (2012). Guidelines for interpretation suggest $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively (Cohen, 2013). Statistical analysis was performed using the nlme package in R version 3.5.1 using RStudio version 1.1.463 (Pinheiro et al., 2018; R Core Team, 2018; RStudio Team, 2016). To estimate the strength of evidence in favor of the null hypothesis in the case of MDD and the interactive effect, Bayes factors were calculated for models containing only CMV, MDD or the interactive effect plus the cofactors (age, sex, ethnicity and flow cytometry batch) compared to a model with only the cofactors using the Bayes-Factor R package version 0.9.12–4.2 (Morey and Rouder 2018).

3. Results

Of the 200 samples initially selected for analysis, three samples were excluded because of non-specific binding of the CD45RA-PE-Cy7 antibody and one was excluded due to lack of remaining plasma for CMV antibody testing. Within the MDD group there was no difference between CMV^+ and CMV^- subjects in MADRS scores or in recent history of psychotropic medication use other than sleep aids (p's > 0.10). ETI-SR and CTQ scores were higher in MDD compared to controls (p's < 0.001) and in CMV^+ compared to CMV^- (ETI-SR p = 0.030; CTQ p = 0.030). CMV seropositivity did not differ significantly between the MDD

and control groups (MDD = 57.1%, Ctrl = 51.0%, $\chi^2 = 0.74$, df = 1, p = 0.390, Table 1). With respect to the T-cell phenotypes, a positive CMV serology was associated with a greater proportion of total $CD3^+$ T-cells, (percent of total live cells, p = 0.003, $f^2 = 0.04$, Fig. 1) increased $CD8^+$ T-cells (percent of total $CD3^+$ cells, p < 0.001, $f^2 = 0.07$, Fig. 1), and decreased $CD4^+$ to $CD8^+$ ratio (p = 0.003, $f^2 = 0.04$, Fig. 1). CMV seropositivity was also associated with T-cell profiles intrinsic to advanced aging, including a lower proportion of $CD4^+$ and $CD8^+$ naive T-cells (p's < 0.001, $f^2 = 0.15$ and 0.19, respectively, Fig. 2), a higher proportion of $CD4^+$ and $CD8^+$ EMRA cells (p's < 0.001, $f^2 = 0.29$ and 0.30, respectively, Fig. 2), and a higher proportion of $CD4^+$ and $CD8^+$ late differentiated ($CD27^-CD28^-$) EM (p's < 0.001, $f^2 = 0.34$ and 0.14, respectively, Fig. 3) and EMRA (p's < 0.001, $f^2 = 0.94$ and 0.97, respectively, Fig. 3) T-cells. In contrast, there was no main effect of diagnosis and a secondary analysis showed no significant interactions between diagnosis and CMV for any of the cell populations. Cell populations are reported in Table 2 and regression results for CMV and diagnosis are shown in Table 3 (covariate results are shown in Supplementary Table 2). The evidential strength for the null hypothesis that MDD does not affect the T-cell phenotypes was moderate (Bayes factors > 0.10 and < 0.33) for each cell type except $CD3^+$ and $CD4^+$ CM (Supplementary Table 3). Bayes factors for the interactive term suggest moderate evidence for the null for all $CD8^+$ subtypes except $CD8^+$ EMRA, plus $CD4^+$ EM, $CD4^+$ N and $CD3^+$ T-cells. In this analysis, there was moderate evidence (Bayes factor = 3.40) for an interactive effect on the $CD4^+$:$CD8^+$ ratio, but this effect did not survive correction for multiple testing in the primary analysis.

Within the CMV^+ subset, IgG titer did not significantly differ between diagnostic groups (two-tailed *t* test, p = 0.667). Titer was positively associated with the proportion of $CD4^+$ EMRA cells ($\beta = 0.07$, SE = 0.02, p = 0.005, $f^2 = 0.072$), but the effect size was small and the significance did not survive adjustment for multiple comparisons ($\alpha = 0.004$). There were no significant associations with IgG titer and any of the other T-cell populations, nor was there any significant interactive effect of titer and MDD on any of the T-cell populations.

There was a significant three-way relationship between CMV status, diagnosis, and sex, such that MDD females were *more* likely to be CMV^+ than control females and MDD males were *less* likely to be CMV^+ than control males ($\chi^2 = 24.1$, df = 4, p < 0.001, Fig. 4). This interaction did not significantly affect any of the T-cell populations after controlling for multiple comparisons.

4. Discussion

This investigation tested the hypothesis that there is an interaction between CMV and depression such that young and middle-aged adults with MDD who are also CMV^+ would have greater premature accumulation of age-related T-cell phenotypes than non-depressed CMV^+ controls. There were two main results. The principal finding was that no such interaction was evident. Although CMV infection was associated with an age-related T-cell phenotype, this relationship was independent of MDD diagnosis. Namely, in the CMV^+ groups, there was a greater expansion of $CD4^+$ and $CD8^+$ EMRA T-cell subsets and a decrease in naive T-cells. Further, within the EM and EMRA T-cell subsets, there was a

concurrent accumulation of $CD27^{-}CD28^{-}$ late differentiated cells. Second, surprisingly, CMV serostatus together with MDD status was moderated by sex such that MDD females were *more* likely to be CMV^{+}, whereas MDD males were *less* likely to be CMV^{+}. Taken together, these results suggest that (1) CMV, but not MDD status by itself, may be a driver of immunosenescence, and (2) there may be significant sex-specific effects on adaptive immunity, which will need to be further explored.

Our results confirm the findings of others that indicate that CMV infection is associated with advanced aging of the T-cell compartment (Almanzar et al., 2005; Burton et al., 2019; Turner et al., 2014; Weinberger et al., 2007). Other studies have found that late differentiated T-cells have shortened telomeres and limited proliferative capacity, but retain cytotoxic functionality and are resistant to apoptosis (Moro-García et al., 2013; Strioga et al., 2011). Reduction of the naive to memory T-cell ratio and expansion of late differentiated T-cell populations are associated with immunosenescence in old age and are potentially responsible for increased susceptibility to infection in the elderly (Nikolich-Žugich, 2018).

Because chronic stress is causally linked to depression (Tafet and Bernardini, 2003; Willner, 2017) and is thought to reactivate latent CMV (Glaser et al., 1985; Mehta et al., 2000; Pariante et al., 1997; Rooney et al., 2019; Sarid et al., 2004), we hypothesized that reduced control of CMV in depressed subjects would lead to a more advanced age-related phenotype in CMV^{+} participants with MDD compared to CMV^{+} controls. However, diagnosis did not have a significant effect on any of the T-cell populations studied, nor any interaction with CMV serostatus.

One potential reason for the absence of a significant diagnosis by CMV interaction is that our MDD sample may not have had a significant history of chronic adult stress and/or impaired adaptive immune function that would lead to deficient control of CMV infection. This possibility is supported by the fact that CMV IgG titers did not significantly differ between the seropositive MDD and control groups, possibly indicating similar levels of viral control amongst our participants, at least at the time of sampling. A second possibility is that the proportion of late differentiated T-cells may not rise linearly with the number of lifetime reactivations. Third, our cohort may have been too young (age 18–57 years) to detect an effect of MDD. Two previous studies that detected an interactive effect of CMV and depression (Trzonkowski et al., 2004) or stress (Reed et al., 2019) on immune cell profiles were performed in older adults (age 58+). The cumulative effect of multiple CMV reactivations may be imperceptible until old age when homeostatic mechanisms start to break down. Therefore, if depression were accelerating T-cell differentiation through CMV reactivation, it might only be evident in the elderly.

This work adds to the growing number of recent publications that have explored the relationship of CMV and psychosocial factors on the T-cell compartment. Two recent studies found that adults who were separated from their birth parents at an early age and subsequently adopted had higher frequencies of late differentiated T-cells ($CD57^{+}$) and reduced $CD4^{+}$ to $CD8^{+}$ ratios, indicating that early life adversity (ELA) could have a lasting effect on the biological aging of the immune system (Elwenspoek et al., 2017; Reid et al., 2019). In both of these studies, CMV was a major contributor, either partially or completely

mediating the effects of ELA (Elwenspoek et al., 2017; Reid et al., 2019). It is possible that institutional care is amplifying the effect of CMV in two ways. First, by increasing the exposure rate, as in daycare settings where toddlers shed large amounts of active virus in their saliva (Grosjean et al., 2014). Second, by disrupting the immune system through activation of stress pathways during development, potentially increasing CMV's ability to establish infection upon exposure. This notion is consistent with our previous work showing that childhood trauma increases the odds of CMV seropositivity (Ford et al., 2019a).

On the other hand, our findings are partially inconsistent with two studies that have examined the effects of CMV and stress on T-cells. Prather and colleagues found that young parental caregivers to children with autism had increased effector to naive T-cell ratios in both the $CD4^+$ and $CD8^+$ compartments when compared to non-caregiver comparison controls, independent of CMV serostatus (Prather et al., 2018). The lack of association with CMV in their study may be due to the use of CD45RA and CD62L to define the T-cell subtypes since this approach could potentially combine the naive and EMRA subsets that both express CD45RA. CMV specific $CD8^+$ T-cells have been reported as mostly being $CD62L^-$ with an equal mix of $CD45RA^+$ and $CD45RA^-$ (Crough et al., 2005). Furthermore, our findings indicate that CMV *decreases* the proportion of naive T-cells while *increasing* the proportion of EMRA cells. Therefore, the effect of CMV could be washed-out in a combined sample of the two cellular subsets. Regardless, the CMV infection rates in their experimental groups were not significantly different and did not contribute to the effects of caregiving stress on the reported T-cell profiles (Prather et al., 2018). Another recent study to link stress and CMV to cellular aging examined late differentiated T-cell ($CD28^-$ and $CD57^+$ subsets) accumulation in older adults (Reed et al., 2019). Among CMV^+ participants, the combination of low perceived stress and low IgG titers was associated with lower accumulation of late differentiated T-cells compared to either high perceived stress, high CMV titers or a combination of the two (Reed et al., 2019). As discussed below, a limitation of our study was the absence of a reliable measure of lifetime or current stress and for this reason, we were unable to disentangle the effects of stress and depression. Conceivably, chronic stress in the context of depression is a key driver of CMV reactivation and immunosenescence.

The second major unexpected finding was a strong three-way interaction between CMV, diagnosis, and sex. MDD females were *more* likely to be CMV^+ than control females (age-adjusted OR = 2.03, 95% CI: 1.00–4.18, p = 0.050), while MDD males were *less* likely to be CMV^+ than control males (age-adjusted OR = 0.23, 95%CI: 0.06–0.77, p = 0.024, Fig. 4). This result is consistent with a large cohort study of adults 15–39 years of age from the population based National Health and Nutrition Examination Survey (NHANES) III cohort that found that CMV seropositivity in males was associated with a lower risk of depression and that high CMV IgG titers in seropositive females nominally increased the risk of major depression, dysthymia or bipolar disorder I (Simanek et al., 2018). Nevertheless, in a *post hoc* analysis, we found no interactive effect of sex, CMV and MDD on any of the cell populations after controlling for age and adjusting for multiple comparisons. Additionally, the results were not different in men and women when examined separately.

An important implication of our results is that an imbalance in CMV infection rates in case-control studies could have significant effects on immune-related outcomes, particularly those involving T-cell function or phenotype. CMV serostatus or antibody levels have been associated with depression or mood disorders in some, but not all samples (Maes et al., 1991; Prossin et al., 2015; Simanek et al., 2018, 2014; Sølvsten Burgdorf et al., 2019). Unrecognized differences in CMV infection rates could lead investigators to falsely conclude that the effects of CMV were a result of depression in their sample. Thus, controlling for CMV status is advisable in studies that examine lymphocyte distributions and function in the context of depression. This recommendation receives support from the literature. For instance, lymphoproliferative response to concanavalin A (ConA) stimulation *in vitro* was reduced following cases of CMV mononucleosis and this was shown to be related to the expansion of $CD8^+$ T-cells (Carney et al., 1983). The loss of CD28 on these cells may contribute to this finding. Stimulation of CD28 enhances T-cell proliferation in response to ConA as well as phorbol 12-myristate 13-acetate (PMA) plus ionomycin and anti-CD3 mAb stimulation (Ando et al., 2014). Depression, in turn, has also been associated with decreased lymphoproliferative response to ConA and other TCR-independent mitogens (Zorrilla et al., 2001). It is conceivable that CMV was a contributing factor in these studies given the shared risk factors with major depressive disorder, namely low SES and childhood trauma (Dowd et al., 2009; Ford et al., 2019a; Janicki-Deverts et al., 2014).

There were several limitations to this study. As discussed above, the participants were young and middle-aged adults. The hypothesized combined effect of depression and CMV may only be evident in elderly populations, as observed by Trzonkowski et al. (2004). A future study could include a wider range of ages to test this hypothesis. Second, only one of the MDD participants met DSM-IV criteria for severe depression. The rest were classified as moderate (75.5%), mild (17.3%), or unspecified (6.1%). Therefore, we cannot rule out an effect of depression severity on the results. Third, our study was underpowered to test the effects of recurrent versus single episode depression since only 7% of the MDD cohort had single-episode depression. However, analysis results were not significantly affected by the removal of these single-episode subjects. Fourth, we did not have a measure of lifetime stress exposure, and thus may have been underpowered to detect a depression effect driven by a subset of the sample who experienced a greater number of CMV reactivations due to chronic psychological stress or a more severe course of illness. Nevertheless, a subset of subjects did complete measures of early-life adversity, i.e. the ETI-SR (n = 164) (Bremner et al., 2007) and the CTQ (n = 180) (Bernstein et al., 2003). As predicted by our previous findings, these measures were higher in the CMV^+ participants across the entire sample (Table 1) (Ford et al., 2019a). (Note that there was a significant overlap between the participants in this study and in Ford et al., 2019a), so this result cannot be considered an independent replication.) Early life stress was greater in the MDD sample than the HC sample but there was not a significantly higher rate of CMV seropositivity in the MDD sample. This may be due to sampling variability or the effect size since seropositivity was marginally higher in the MDD group (57% vs 51%). Additionally, the CTQ and ETI-SR were not related to any of the T-cell outcome measures independent of CMV status and did not interact with CMV serostatus.

There were two limitations regarding the flow cytometry panel. First, frozen rather than fresh PBMCs were used. Freeze-thaw cycles can diminish the integrity of cell-surface molecules and hinder binding of fluorescent antibodies for detection by flow cytometry. Specifically, this prevented the detection of CD62L, the loss of which is another marker of late differentiated T-cells. Second, we did not identify CMV-specific T-cells. Quantification of CMV-specific cells would have provided stronger evidence that CMV is the cause of the expansion of the late differentiated T-cells. A future study could identify the CMV-specific cells and test their functionality to *in vitro* stimulation with CMV peptides, and non-CMV pathogenic challenge to determine if this cell expansion is likely to dampen the immune response to non-CMV pathogens.

In conclusion, we found large and significant effects of CMV seropositivity on age-related $CD4^+$ and $CD8^+$ T-cell surface profiles in young and middle-aged adults. Although we hypothesized that MDD in CMV^+ individuals would increase the accumulation of late differentiated T-cells compared to non-depressed CMV^+ controls, we found no significant effect of diagnosis or interaction with CMV. We conclude that CMV should be considered an important cofactor in comparative studies of T-cells, given its strong effect on the makeup of the T-cell compartment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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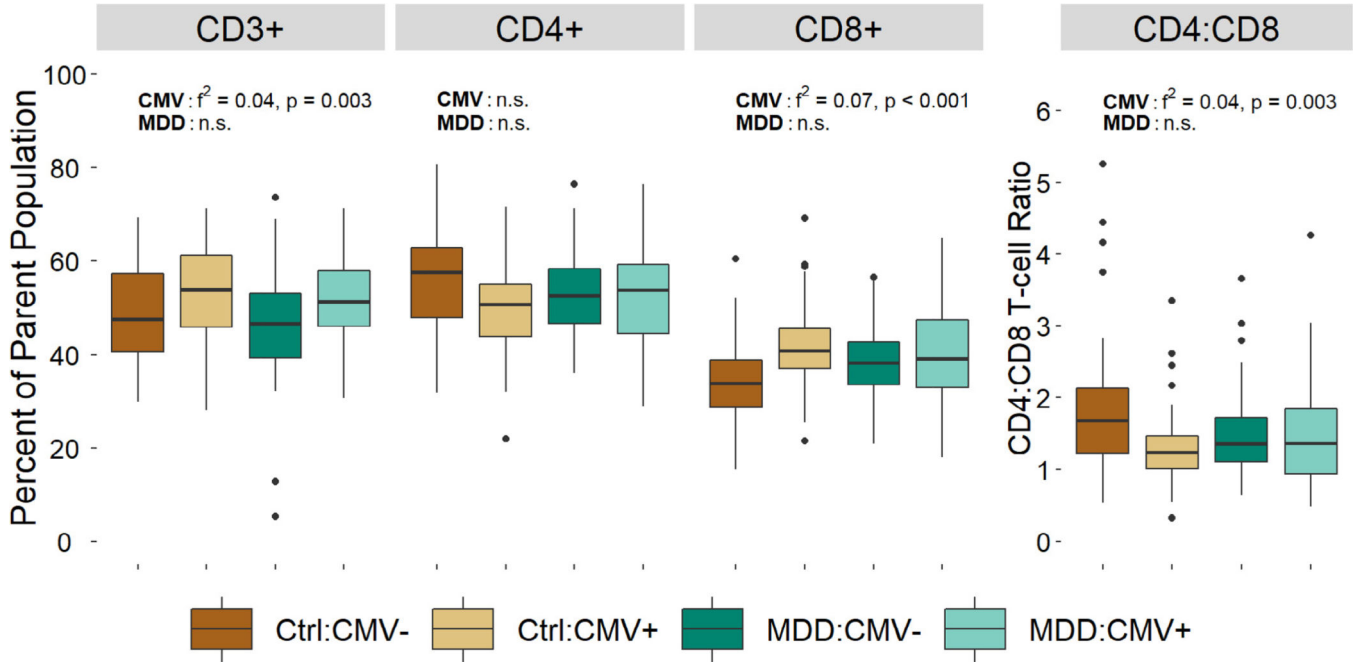

Fig. 1.

T-cell Population Distribution. Distribution (% of the parent population) by CMV serostatus and diagnostic category for total T-cells ($CD3^+$), T-helper cells ($CD4^+$) and cytotoxic T-cells ($CD8^+$), plus the $CD4^+$:$CD8^+$ ratio. Results are shown for the CMV and MDD terms in the linear mixed model controlling for age, sex, ethnicity and analysis batch. Alpha was adjusted to 0.004 using the effective number of tests method. Effect sizes for significant variables were calculated as Cohen's f^2 statistic following the procedure described by Selya, et al. (2012). Guidelines for interpretation suggest $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively (Cohen, 2013). Box plots represent the upper, median and lower quartiles. Whiskers are drawn at 1.5 times the interquartile range; CMV = cytomegalovirus; MDD = major depressive disorder.

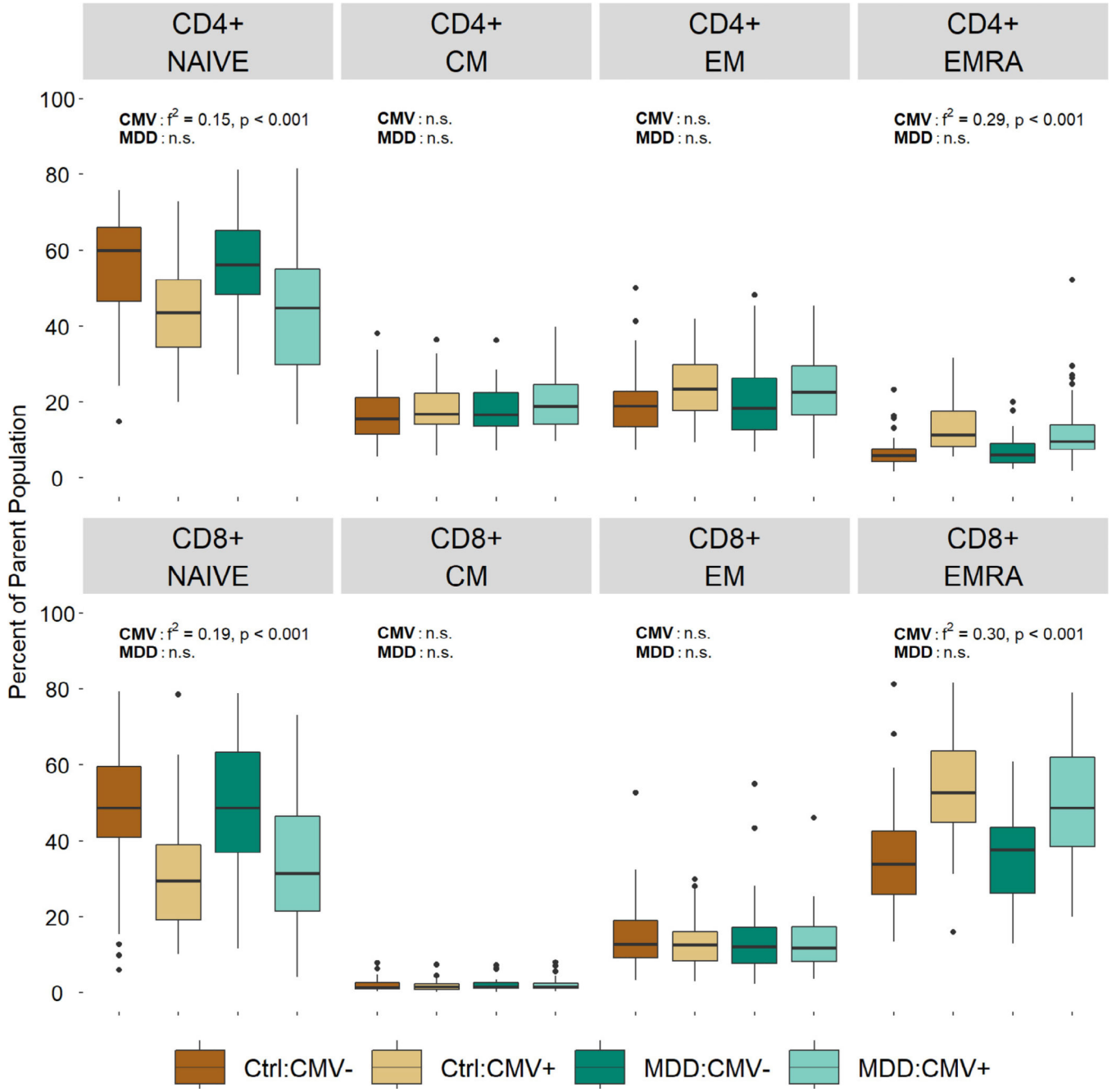

Fig. 2.

Subsets of T-cell Population Distribution. Distribution (% of the parent population) by CMV serostatus and diagnostic category for T-cell subsets. Results are shown for the CMV and MDD terms in the linear mixed model controlling for age, sex, ethnicity and analysis batch. Alpha was adjusted to 0.004 using the effective number of tests method. Effect sizes for significant variables were calculated as Cohen's f^2 statistic following the procedure described by Selya, et al. (2012). Guidelines for interpretation suggest $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively (Cohen, 2013). Box plots represent the upper, median and lower quartiles. Whiskers are drawn at 1.5 times

the interquartile range; CMV = cytomegalovirus; MDD = major depressive disorder, Naive = $CCR7^{+}CD45RA^{+}$, CM = central memory $CCR7^{+}CD45RA^{-}$, EM = effector memory $CCR7^{-}CD45RA^{-}$, EMRA = effector memory re-expressing CD45RA $CCR7^{-}CD45RA^{+}$.

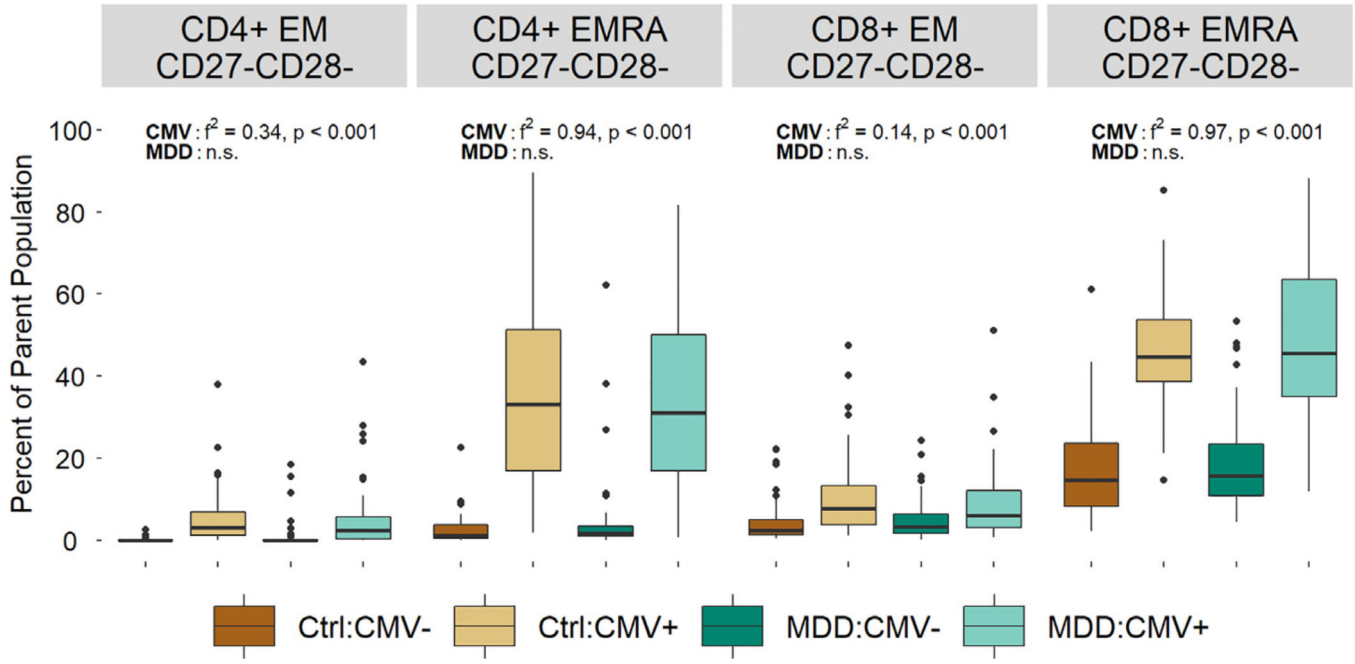

Fig. 3.

Late Differentiated Memory T-cell Subsets. Distribution (% of the parent population) by CMV serostatus and diagnostic category for late differentiated ($CD27^{-}CD28^{-}$) memory T-cell subsets. Results are shown for the CMV and MDD terms in the linear mixed model controlling for age, sex, ethnicity and analysis batch. Alpha was adjusted to 0.004 using the effective number of tests method. Effect sizes for significant variables were calculated as Cohen's f^2 statistic following the procedure described by Selya et al. (2012). Guidelines for interpretation suggest $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively (Cohen, 2013). Box plots represent the upper, median and lower quartiles. Whiskers are drawn at 1.5 times the interquartile range; CMV = cytomegalovirus; MDD = major depressive disorder, EM = effector memory $CCR7^{-}CD45RA^{-}$, EMRA = effector memory re-expressing CD45RA $CCR7^{-}CD45RA^{+}$.

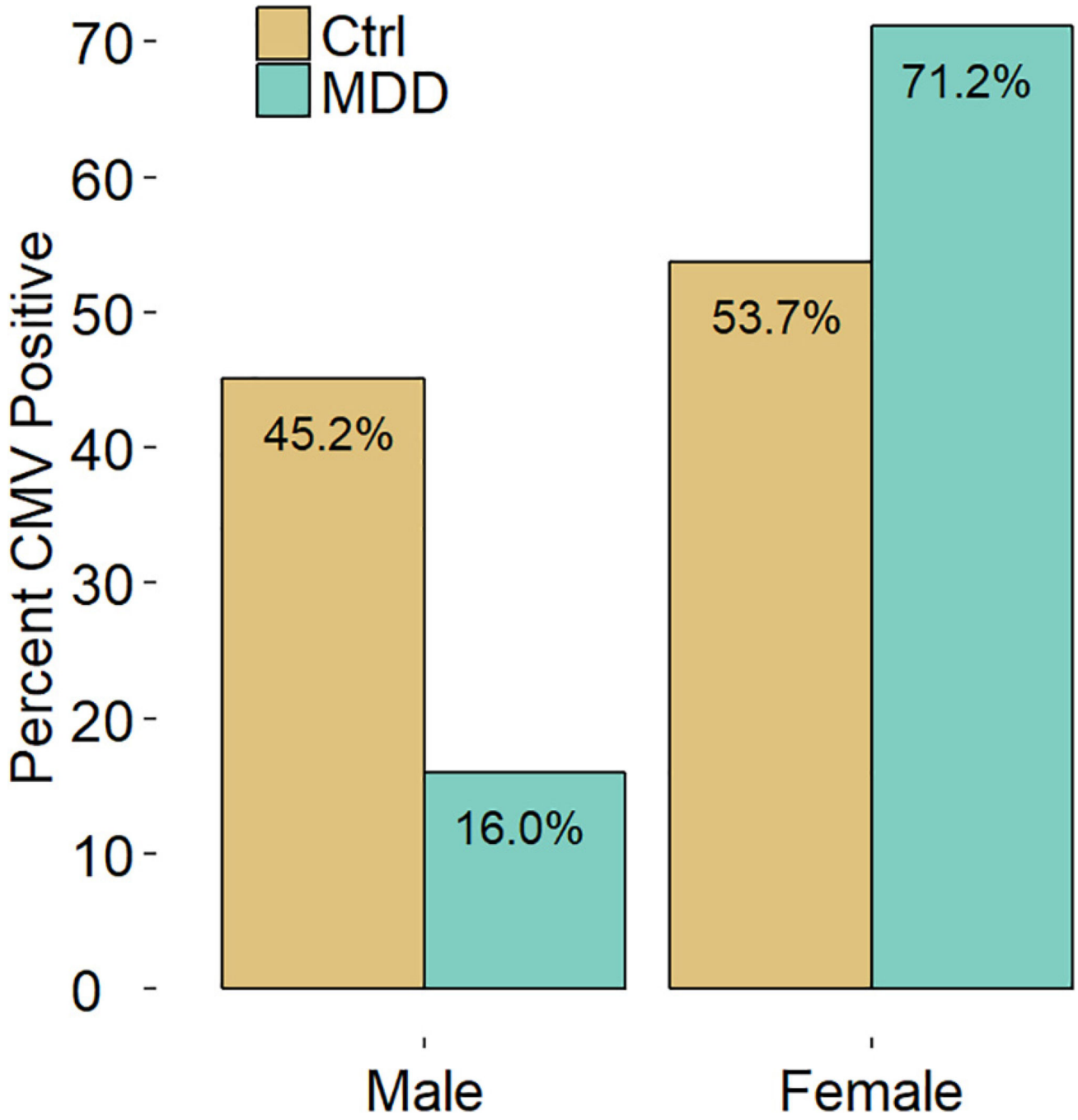

Fig. 4.

Sex differences in the relationship between MDD and CMV Serostatus. Percentage of females and males that were CMV^+ by diagnostic group. CMV = cytomegalovirus; MDD = major depressive disorder.

Table 1

Sample characteristics.[a]

	Controls		MDD		
	CMV$^-$	CMV$^+$	CMV$^-$	CMV$^+$	p-Value[b]
n	48	50	42	56	
CMV IgG Titer[c]	1.26 (0.15)	2.95 (0.88)	1.21 (0.19)	3.02 (0.81)	0.666[d]
Age[a] (SD) (years)	33.0 (12.1)	35.5 (9.0)	34.4 (9.6)	37.5 (10.5)	0.164
Males n (%)	17 (35.4)	14 (28.0)	21 (50.0)	4 (7.1)	< 0.001
Ethnicity: Caucasian n (%)	42 (87.5)	43 (86.0)	36 (85.7)	43 (76.8)	0.426
BMI[a] (SD)	25.6 (5.2)	29.0 (5.8)	28.8 (6.3)	29.6 (7.4)	0.007
Medicated[e]	0 (0.0)	0 (0.0)	11 (39.3)	12 (28.6)	0.350[f]
MADRS	1.16 (1.81)	3.81 (2.38)	10.13 (4.68)	10.23 (3.97)	< 0.001
ETI-SR	1.50 (1.32)	2.13 (1.63)	3.12 (2.48)	3.76 (2.25)	< 0.001
CTQ	31.5 (6.9)	36.6 (17.2)	48.9 (24.3)	55.8 (21.7)	< 0.001

MDD = major depressive disorder, CMV = cytomegalovirus, BMI = body mass index, MADRS = Montgomery-Asberg Depression Rating Scale total score, ETI-SR = Early Trauma Inventory-Self Report total score, CTQ = Childhood Trauma Questionnaire total score.

[a] Presented as mean (SD), where SD is the standard deviation of the mean.

[b] p-Values representing differences between subject groups were derived from chi-square analyses for dichotomous and categorical variables and one-way ANOVA for continuous variables.

[c] Reported as plate based z-scores.

[d] p-value for two-tailed *t* test comparing CMV positive participants by diagnostic group.

[e] Use of psychotropic drugs other than sleep aids within 3 weeks (8 for fluoxetine) prior to visit.

[f] p-value for chi-square test comparing MDD participants by CMV serostatus.

Table 2

T-cell populations by diagnosis and CMV serostatus.

Variable	Controls				MDD			
	CMV⁻		CMV⁺		CMV⁻		CMV⁺	
	Percent of Live Cells[a]	Percent of Parent Population[b]	Percent of Live Cells[a]	Percent of Parent Population[b]	Percent of Live Cells[a]	Percent of Parent Population[b]	Percent of Live Cells[a]	Percent of Parent Population[b]
$CD3^+$	48.26 (10.12)	48.26 (10.12)	53.29 (10.72)	53.29 (10.72)	46.07 (12.87)	46.07 (12.87)	51.42 (8.75)	51.42 (8.75)
├ $CD4^+$	27.10 (7.95)	56.20 (11.44)	26.52 (6.96)	49.89 (9.09)	24.50 (8.65)	52.51 (9.70)	26.68 (6.79)	52.31 (11.36)
├ $CD4^+$ NAIVE	15.56 (6.97)	56.00 (13.90)	11.80 (5.06)	44.15 (12.88)	13.69 (6.76)	54.45 (13.59)	11.56 (5.26)	43.43 (14.90)
├ $CD4^+$ CM	4.61 (2.16)	17.32 (7.54)	5.02 (2.77)	18.38 (6.97)	4.51 (2.54)	18.03 (6.31)	5.46 (2.76)	20.25 (7.64)
├ CD4+ EM	5.16 (2.37)	19.98 (9.01)	6.20 (2.57)	23.90 (8.52)	4.64 (2.25)	20.47 (10.26)	6.39 (3.17)	24.11 (9.82)
└ $CD4^+$ EM $CD27^-CD28^-$	0.01 (0.02)	0.20 (0.43)	0.37 (0.55)	5.42 (6.91)	0.06 (0.21)	1.39 (4.03)	0.35 (0.69)	5.24 (8.15)
└ $CD4^+$ EMRA	1.77 (1.17)	6.70 (4.02)	3.49 (1.85)	13.58 (6.84)	1.67 (1.08)	7.06 (4.12)	3.27 (2.77)	12.20 (8.36)
└ $CD4^+$ EMRA $CD27^-CD28^-$	0.03 (0.05)	2.66 (3.87)	1.52 (1.76)	35.07 (23.81)	0.09 (0.26)	5.37 (11.41)	1.34 (1.98)	33.73 (22.23)
└ $CD8^+$	16.67 (5.38)	34.61 (9.43)	22.47 (6.86)	42.05 (9.42)	17.49 (5.55)	38.25 (7.89)	20.59 (7.13)	39.69 (10.56)
├ $CD8^+$ NAIVE	7.91 (3.92)	47.59 (17.63)	6.86 (3.75)	31.49 (15.54)	8.22 (4.25)	47.58 (18.38)	6.73 (3.61)	34.08 (16.63)
├ $CD8^+$ CM	0.32 (0.21)	2.02 (1.51)	0.40 (0.31)	1.83 (1.30)	0.37 (0.35)	2.08 (1.70)	0.43 (0.36)	2.11 (1.53)
├ $CD8^+$ EM	2.32 (1.57)	14.31 (9.00)	2.86 (1.43)	13.14 (6.56)	2.51 (1.95)	14.40 (10.20)	2.70 (1.60)	13.51 (7.21)
└ $CD8^+$ EM $CD27^-CD28^-$	0.13 (0.22)	4.83 (5.58)	0.31 (0.36)	10.35 (9.93)	0.18 (0.38)	5.38 (5.67)	0.27 (0.45)	9.05 (8.90)
└ $CD8^+$ EMRA	6.12 (4.11)	36.08 (13.98)	12.35 (6.03)	53.56 (13.52)	6.39 (3.15)	35.94 (13.33)	10.73 (5.88)	50.29 (15.27)
└ $CD8^+$ EMRA $CD27^-CD28^-$	1.35 (2.33)	17.74 (12.32)	6.18 (4.62)	46.67 (14.98)	1.38 (1.30)	20.02 (13.04)	5.72 (4.72)	48.22 (18.89)
$CD4^+$:$CD8^+$ Ratio	1.85 (0.97)		1.29 (0.54)		1.49 (0.63)		1.50 (0.77)	

[a] Mean (SD) percent of total live cells collected for each T-cell population.

[b] Mean (SD) percent of parent population for each T-cell population. Parent/child relationships of T-cell populations are denoted by tree structure in the Variable column.

CMV = cytomegalovirus, MDD = major depressive disorder, SD = standard deviation, Naive = $CCR7^{+}CD45RA^{+}$, CM = central memory $CCR7^{+}CD45RA^{-}$, EM = effector memory $CCR7^{-}CD45RA^{-}$, EMRA = effector memory re-expressing CD45RA $CCR7^{-}$ $CD45RA^{+}$.

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Table 3

Primary analysis results.

Variable	CMV				MDD		
	Fixed Effect Coefficient	SE	p-value	Cohen's f^2	Fixed Effect Coefficient	SE	p-value
$CD3^+$	0.10	0.032	0.003	0.04	−0.05	0.030	0.137
├ $CD4^+$	−0.09	0.032	0.005	0.03	−0.02	0.031	0.544
│ ├ $CD4^+$ NAIVE	−0.21	0.040	<0.001	0.15	−0.01	0.037	0.841
│ ├ $CD4^+$ CM	0.03	0.024	0.244	0.00	0.03	0.022	0.171
│ ├ CD4+ EM	0.08	0.028	0.004	0.04	0.00	0.026	0.957
│ │ └ $CD4^+$ EM $CD27^-CD28^-$	0.28	0.035	<0.001	0.34	0.00	0.033	0.882
│ └ $CD4^+$ EMRA	0.21	0.027	<0.001	0.29	−.03	0.026	0.336
│ └ $CD4^+$ EMRA $CD27^-CD28^-$	0.91	0.067	<0.001	0.94	0.03	0.063	0.636
└ $CD8^+$	0.11	0.030	<0.001	0.07	0.02	0.029	0.543
├ $CD8^+$ NAIVE	−0.30	0.049	<0.001	0.19	0.05	0.046	0.287
├ $CD8^+$ CM	−0.01	0.012	0.406	0.00	0.01	0.011	0.521
├ $CD8^+$ EM	−0.03	0.031	0.306	0.00	0.00	0.029	0.866
│ └ $CD8^+$ EM $CD27^-CD28^-$	0.19	0.038	<0.001	0.14	−0.01	0.035	0.770
└ $CD8^+$ EMRA	0.34	0.042	<0.001	0.30	−0.05	0.040	0.248
└ $CD8^+$ EMRA $CD27^-CD28^-$	0.68	0.050	<0.001	0.97	0.04	0.047	0.352
$CD4^+$:$CD8^+$ Ratio	−0.34	0.112	0.003	0.04	−0.10	0.107	0.376

For each T-cell subtype, the proportion of the parent population was arcsine transformed (2*(asin(sqrt[x])) to stabilize variance. CMV serostatus and diagnosis were tested against each cell population using mixed linear regression models controlling for age, sex, ethnicity (Caucasian vs non-Caucasian) and flow cytometry batch. Alpha was adjusted to 0.004 using the effective number of tests method. Effect sizes for significant variables were calculated as Cohen's f^2 statistic following the procedure described by Selya et al. (2012). Guidelines for interpretation suggest $f^2 \geq 0.02$, $f^2 \geq 0.15$, and $f^2 \geq 0.35$ represent small, medium, and large effect sizes, respectively (Cohen, 2013).

CMV = cytomegalovirus, MDD = major depressive disorder, SE = standard error of the coefficient, Naive = $CCR7^+CD45RA^+$, CM = central memory $CCR7^+CD45RA^-$, EM = effector memory $CCR7^-CD45RA^-$, EMRA = effector memory re-expressing CD45RA $CCR7^-CD45RA^+$.