Psychoneuroendocrinology 144 (2022) 105867

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Salivary immune markers are not associated with self-reported childhood maltreatment or psychopathology in adults

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A R T I C L E I N F O

Keywords:
Immune markers
Inflammation
Saliva
Stress
Maltreatment
Psychopathology

A B S T R A C T

Background: Psychological stress has repeatedly been found to be associated with pro-inflammatory markers in blood, and neuro-inflammation may play a role in the development of psychopathology after early life stress. Salivary immune testing is a novel method to non-invasively assess immune functioning. We examined a large range of salivary immune markers in relation to self-reported childhood maltreatment and psychopathology in an adult sample.
Methods: Participants (N = 118, 51% female, mean age = 46.6 yrs, range 22–64) were drawn from a cross-sectional three-generation study, and supplied 2 ml of saliva via passive drool. They reported on childhood maltreatment experiences and on psychopathological symptoms in the last 6 months. Hair cortisol was additionally assessed in a subsample ($n = 68$). Levels of IL1ß, IL6, IL8, IFNγ, TNFα, tIgE, sIgA, FLCƛ, and FLCƙ were assessed.
Results: Linear mixed model analyses showed that several salivary immune markers were associated with age (sIgA and IgE), BMI (sIgA, IL1ß, and IL6), sex (FLCs and IgE), and bad health (IL6, IL8, TNFα). No associations with (anti-inflammatory) medication use or oral health problems were found. Notably, no associations between the immune markers and self-reported childhood maltreatment, psychopathology, or hair cortisol were found.
Conclusions: Salivary immune measures were found to be sensitive to individual differences in age, sex, health and BMI. However. in the current sample there was no indication of inflammation in relation to chronic psychological stress. Larger studies, including participants with higher stress levels, are needed to further examine associations between salivary immune markers and psychological stress.

1. Introduction

Chronic psychological stress has been associated with elevated levels of mental health problems such as depression and anxiety (Jaffee, 2017), but also with somatic health problems such as cardiovascular and autoimmune diseases, diabetes and dementia (Wolkowitz et al., 2011; Danese and McEwen, 2012). It is thought that the increase in disease proneness after chronic stress is due to disbalances in the

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https://doi.org/10.1016/j.psyneuen.2022.105867
Received 27 April 2022; Received in revised form 5 July 2022; Accepted 9 July 2022
Available online 13 July 2022

hypothalamus-pituitary-adrenal (HPA) axis and the immune system, resulting in chronic low-grade systemic inflammation (Acabchuk et al., 2017; Dantzer, 2018). A major source of chronic stress is childhood maltreatment, which encompasses (often repetitive) experiences of threat or deprivation due to abuse or neglect early in life. Immune disbalances due to early life stress may contribute to the development of immune-related health problems in later life and possibly even in the development of psychopathology (Slavich and Irwin, 2014; Danese and Lewis, 2017). Both cross-sectional and longitudinal studies have shown associations between early life stress and inflammation (for reviews see, Baumeister et al., 2016; Gill et al., 2020; Lacey et al., 2020; Kerr et al., 2021), as well as between mental health problems and inflammation (Liu et al., 2012; Goldsmith et al., 2016; Jonker et al., 2017; Speer et al., 2018). However, findings are not always consistent and seem dependent on type of population (e.g., healthy versus clinical samples), age, sex, type of stress or adversity, and methodology (e.g., type of immune markers assessed). In this study we will therefore examine associations of both early life stress as well as mental health problems with a large range of immune markers in a mixed-sex sample spanning a large age range.

Non-invasive techniques are welcomed to examine immune system disbalances in human studies. So far, studies have mostly relied on blood sampling. Recently, however, the use of saliva as a non-invasive method to study immune system markers has been on the rise (Lim et al., 2016; Szabo and Slavish, 2021). Several studies indicate that immune markers in saliva are elevated in people who experienced acute or early life stress (Tyrka et al., 2015; Szabo et al., 2020). However, not all studies confirm these associations (Byrne et al., 2013), and discussions are ongoing whether saliva is only reflective of oral or also of more systemic inflammatory levels (Riis et al., 2014; Lim et al., 2016). Hence, it is not yet clear to what extent salivary assessments are sensitive to stress-related immune dysregulations in the body or brain. To this end, we set out to study a broad range of immune markers in saliva in relation to psychological stress, including both commonly studied cytokines (i.e. the Interleukins [IL]1ß, 6, and 8, Interferon gamma [IFNγ], and Tumor Necrosis Factor alpha [TNFα]), as well as less commonly studied immune markers (i.e. Immunoglobulin [Ig] E and secretory IgA [sIgA], and Free light Chains lambda and kappa [FLC ƛ and ƙ]).

Cytokines are small signaling proteins secreted in response to infection or injury. While cytokines can be produced locally in the mouth, they can also filter into saliva from different origins (e.g. the gingival fold and mucous from the nasal cavity (Desai and Mathews, 2014)). Correlations have been found between salivary and blood-based IL6 (r between 0.07 to 0.71), with small correlations for salivary and blood-based IL1β ($r = 0.01$–0.11) and TNFα ($r = -0.15$ to 0.32) (Szabo and Slavish, 2021). Salivary sIgA is considered as the main adaptive immune mechanism in the oral cavity and concentrations depend on antigenic stimulation as well as on alterations in neuroendocrine functioning, e.g. due to stress (Teeuw et al., 2004). IgE is a diagnostic parameter for allergy, usually measured in blood. However, IgE can also be locally produced in saliva and correlates with inflammatory symptoms such as allergic rhinitis (Mimura et al., 2010). As allergies are associated with psychosocial stress (Harter et al., 2019), this salivary marker may be of interest for stress studies as well. Lastly, immunoglobulin free light chains (FLC) are a component of antibodies, but are also observed in the circulation in its free form. While FLCs were long considered as irrelevant spillover during antibody assembly, recent studies indicate that FLCs may also serve as signaling effectors or anti-inflammatory molecules (Nakano et al., 2011). Interestingly, salivary FLC secretion rates were found to be significantly lower in response to recent life stress, correlating with a lower secretion rate of salivary IgA (Irshad et al., 2020). The impact of more chronic or early life stress on salivary FLC secretion is yet unknown.

In the current study we examined whether this broad range of salivary immune markers would be associated with early life stress and mental health problems in an adult sample drawn from a cross-sectional three-generation family study (Buisman et al., 2020). We investigated (self-reported) experiences of childhood maltreatment as indicators of early life stress, and (self-reported) symptoms of psychopathology in the last 6 months as an indicator of recent life stress. To include a more objective measure of recent life stress, in a subsample of our population we also measured the stress hormone cortisol. Cortisol has been found to be involved in immune system disbalances (Dantzer, 2018) and is affected by both early life and acute stress (Russell and Lightman, 2019). Cortisol was derived from 3 cm of hair close to the scalp, which gives an average estimator of cortisol levels in the past 3 months and is found to be associated with stress exposure (Staufenbiel et al., 2013).

We expected that the immune markers, especially the cytokines, would be heightened in participants with higher levels of childhood maltreatment or more current psychological problems, reflecting low-grade inflammation. Levels of sIgA and FLCs might also be suppressed in response to stress (Vermeer et al., 2012; Irshad et al., 2020). Furthermore, associations of the immune markers with cortisol were explored (Baldwin et al., 2018). As we previously found that abuse and neglect may differently predict biological outcomes (Buisman et al., 2019; Pittner et al., 2020), we performed exploratory follow-up analyses for abuse versus neglect experiences. Lastly, it has been suggested that inflammation may be an underlying mechanism by which childhood maltreatment may lead to psychopathology (e.g., see Slavich and Irwin, 2014, and Danese and Lewis, 2017). Hence, in case we would find immune markers that are associated with both childhood maltreatment and psychopathology we additionally test whether these immune markers mediate the association between childhood maltreatment and psychopathology.

2. Material and methods

2.1. Participants

The current sample was drawn from the 3-Generation (3G) parenting study on the intergenerational transmission of parenting styles, stress and emotion regulation (Buisman et al., 2020), in which three generations of family members from 63 families were included. Assessments took place between 2013 and 2018, i.e., before the Covid-19 pandemic. Next to a range of experimental tasks and interactive paradigms, self-reported childhood maltreatment and psychopathology were assessed. Saliva was sampled as well, and all adult participants from the 3G study for whom enough saliva was collected (i.e., 2 ml) were included in the current study. This led to a total of 120 eligible adults. Due to 1 labeling error, the sample for the immunological analyses included 119 participants (39.7% of total adults in the 3G study). After exclusion of 1 outlier on the immunological data (see below), the final sample for statistical analyses was 118 participants (51% female, age range = 22–64 yrs, $M = 46.6$, SD = 9.94). Supplementary Figure S1 shows a flow chart of the included participants and missing data.

The 118 participants were part of 53 extended families, with a maximum of 5 siblings from 1 nuclear family. All participants gave informed consent before participation and the study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Centre (reference number: P11.134). The study was carried out in accordance with the Code of Ethics of the World Medical Association.

2.2. Immune analyses

Saliva was collected via passive drooling, placed on dry ice for transport to the freezer, and stored the same day at − 80 °C till analyses. Sampling was performed between 9 am and 4 pm, with 82% of samples collected after 12 pm, and always at least 1 h after food intake. Only water was allowed in the hour before assessment. Samples were collected during a lab visit with no physical exercise.

IL1ß, IL6, IL8, IFNγ, TNFα were analyzed using the Bio-Plex Pro Human Cytokine 5-plex Assay (BioRad) according to the manufacturer’s instructions. This method was used instead of ELISA kits for each separate marker as many cytokines are extremely sensitive for freeze-thaw cycles (Szabo and Slavish, 2021). No freeze-thaw cycles are needed when using a multiplex assay, and excellent correlations of multiplex assays with ELISA have been reported (Fu et al., 2010; Richens et al., 2010). Intra- and inter-assay variation was assessed following the EMA guidelines (2011). Intra-assay variation was good (<15%), while inter-assay variation including freeze-thaw cycles was not sufficient (>20%). Sensitivity of the different cytokines was 0.12 pg/ml (IL1ß), 0.59 pg/ml (IL6), 13.34 pg/ml (IL8), 0.45 pg/ml (IFNγ) and 3.41 pg/ml (TNFα). Saliva samples were analyzed undiluted and were not defrosted before use.

Total IgE [tIgE] was analyzed using the ImmunoCAP technology (ThermoFisher), a fully automated system, according the manufacturer's instructions. The human free light chains lambda [FLCƛ] and kappa [FLCƘ] were measured using a commercial ELISA kit (BioVendor) according to the manufacturer's instructions. Saliva samples were measured for FLCƛ in a 2x dilution and for FLCƘ in a 4x dilution. Secretory IgA [sIgA] was measured using an in house ELISA, fully validated for saliva and human milk samples (Dingess et al., 2021). Saliva samples were measured in a 100x and 500x dilution.

Six data points (from 3 participants) were missing due to insufficient saliva (<1%). Values below detection limit were replaced by 0 (15 values, 1.4%) or when possible extrapolated by software from Flexmap 3D to obtain an estimated concentration (38 values, 3.6%). Due to skewness of the data all immune values were log-transformed before analyses. One participant, who reported fever in the days before the salvia sample was given, had the highest scores of the sample on all 5 cytokines, with all values 2.5 SD or more above the mean. This participant furthermore had missing sIgA, IgE and both FLC values. Hence, this participant was excluded from further analyses (see Supplementary Fig. S1). Before analyses, data was standardized and outliers were winsorized to 3.29 standard deviations (SD) from the mean to reduce the impact of outliers in the analyses (12 values in total [1%]).

2.3. Childhood maltreatment

Experiences of maltreatment in childhood inflicted by mother and/or father before the age of 18 were assessed via self-report with an adapted version of the Parent-Child Conflict Tactics Scales (CTS) (Straus et al., 1998), to asses both perpetrated behaviors (towards the child) and experienced behaviors (by parents). It was supplemented with items for emotional neglect from the Childhood Trauma Questionnaire (CTQ) (Thombs et al., 2009), as the CTS has no emotional neglect scale. With the CTS emotional and physical abuse were assessed with the psychological aggression (5 items) and physical assault (13 items) scales, which were combined into an overall abuse score. Neglect was assessed with 5 items of the CTS and combined with 5 emotional neglect items from the CTQ into an overall neglect score. All items were rated on a 5-point scale, ranging from 1 (i.e., "never") to 5 (i.e., "almost always"). These abuse and neglect scales were found to have high internal consistency (see Buisman et al., 2020). The highest scores for father or mother of each scale were used and the abuse and neglect scores were averaged to determine the total maltreatment score.

First, the maltreatment data was log-transformed to reduce skewness. Then after standardization, 1 outlying value for total maltreatment and 1 for the abuse subscale was winsorized to 3.29 SD from the mean. The correlation between the abuse and neglect scale in this sample is .53 ($p < .001$).

2.4. Psychopathology

Symptoms of psychopathology in the past 6 months were assessed with the Adult Self Report (ASR) scale (Achenbach, 2015), which originally consists of 120 items rated on a three-point scale (0 = "not true", 1 = "somewhat true", 2 = "very true"). In the current study only the scales pertaining to the internalizing and externalizing dimensions were included (75 items). Sum scores of the scales Withdrawn (9 items), Somatic Complaints (12 items), and Anxious/Depressed (18 items) are combined into the internalizing dimension of psychopathology (Cronbach's alpha =0.89), and the scales Aggressive (16 items), Rule-Breaking (14 items), and Intrusive (6 items) behaviors are combined into the externalizing dimension (Cronbach's alpha =0.78). The correlation between the internalizing and externalizing scale is .51 ($p <$ .001). The sum of the internalizing and externalizing scales was included as an indicator of general psychopathology in the analyses, with a Cronbach's alpha of .90, showing high internal consistency of the scale. No outliers were present on this scale.

2.5. Cortisol

In a subsample ($n = 68$, 58%) approximately 100 hairs, close to the scalp at the back of the head, were collected during the assessment day. This subsample included those who agreed to provide a hair sample, had sufficient hair growth, and had not used corticosteroids in the previous three months. This subsample was younger and included more females than those not providing hair samples ($ps < 0.05$). The most proximal 3 cm to the scalp were analyzed to measure cortisol and cortisone levels reflective of the past 3 months. Values were log-transformed, standardized and winsorized for analyses (for details on the full methodology see Pittner et al., 2020).

2.6. Covariates

We examined sex, age, sampling time, body mass index [BMI] ($M = 26.6$, $SD = 4.1$, range = 19.1–52.2), the use of anti-inflammatory medication ($n = 19$, 16%), self-reported current health status (i.e., bad health in the last 2 days or fever in the past 3 days [$n = 4$, 3%]), and self-reported oral health problems (e.g., bleeding or infected gums in the past week, cuts in the mouth; scale ranging from 0 to 8 problems [$M = 1.8$, $SD = 1.9$]) as possible covariates in the analyses.

2.7. Analyses

Missing data was completely at random (Little's MCAR test: $p > .05$) and imputed via the Expectation Maximization method (EM; BMI, $n = 4$; ASR, $n = 1$). The remaining 2 missing immune values -after the outlier with 4 missing values was removed- came from 2 different participants (missing, respectively, FLCƛ and IgE) and were also imputed via EM based on the other 8 immune markers, so all participants could be included in the Principal Component (PC) analysis. That is, before the main analyses a PC analysis with varimax rotation was performed to reduce the number of outcome variables. The PCs with an eigenvalue above 1 were calculated and included in the main analyses as the primary outcome measures. Post-hoc analyses for the separate immune markers were only performed if findings were significant for the pertinent PC.

To examine associations between covariates, childhood maltreatment, psychopathology and inflammation, bivariate correlations were followed by linear mixed model analyses in SPSS v27. In our study, participants were part of nuclear families within larger extended families, including partners, cousins and in-laws. Hence, the data is not fully independent, as family members share genes and siblings have shared their childhood environment. As most variance is shared between siblings (i.e., both 50% of their genes and their childhood environment), we nested individual participants ($n = 118$) with their siblings within their nuclear families ($n = 103$). We applied a multilevel model with individual participants at level 1 and family-membership at level 2 and included a random intercept. With this method we can model the relationship between siblings and estimate error terms at both the individual and family level. This allows for computing more accurate standard errors and leads to more appropriate significance tests in the presence of intraclass correlations (Krull, 2007).

Two hierarchical multilevel models were built, one for each inflammatory PC. We first created an empty random intercept model to assess the intraclass correlations coefficients (ICCs). In the next step we examined the covariates, and only significant covariates were kept in the model. In the next step childhood maltreatment was added as a predictor and then, in the third step, psychopathology. Covariates and predictors were all included as fixed factors, and b-values with their 95% confidence intervals are presented. Because BMI and the health-related covariates may be directly related to stress and inflammation (Miller and Lumeng, 2018; Vasiliou et al., 2016), analyses were also run without these covariates. We performed sensitivity analyses to examine the unique effects of abuse versus neglect and exploratively, we examined associations with hair cortisol. Alpha was set at .05, and Bonferroni-corrected for the number of PCs (i.e., to .025).

Power calculations in multilevel models can only be estimated (Atkins, 2005). In case of fully independent sampling (i.e., ICC = 0), a sample size of 118 would allow for the evaluation of small to medium effect sizes (f^2 >0.08) with a power of .80 for alpha at .05 with 2 regression predictors (calculated using GPower 3.1 (Erdfelder et al., 2009)). However, in case of full likeliness between siblings (i.e., ICC = 1), power would be reduced according to the number of level 2 cases (i.e., $n = 103$), and only effect sizes $f^2 > .09$ could be evaluated for alpha at .05 with 2 regression predictors. Hence minimum effect sizes ranging between .08 and .09 could be detected with our sample size, depending on the ICC of the outcome measures.

3. Results

In Table 1 the (raw) descriptive values for the immune markers, psychopathology, childhood maltreatment, and hair cortisol/cortisone values are presented. Table 2 displays the correlations between the predictors, covariates and immune measures. The correlation between maltreatment and total psychopathology was .36 ($p < .001$). Two PCs were found with an eigenvalue over 1 explaining 57% of variance in the immune data (see Supplementary Fig. S2), coinciding with a first factor loading predominantly on the cytokines (IL1ß, IL6, IL8, IFNγ, TNFα), and a second factor loading on the immunoglobulin-related factors, i.e., positively on sIgA and the two FLCs, and negatively on the allergy marker IgE. Component loadings of the 9 immune markers on the two PCs are given in Table 1. The ICC for the first Cytokine-PC was negligible (<0.001), but for the second Immunoglobulin-PC the ICC was .37. For psychopathology ICC = 0.40 and for maltreatment ICC = 0.50, showing significant clustering within families. Despite the low ICC for the Cytokine-PC, we proceeded all analyses with the nested structure.

3.1. Associations between childhood maltreatment, psychopathology and inflammation

Table 3 shows the outcomes of the final mixed models for the 2 immune PCs, including the significant covariates, childhood maltreatment, and psychopathology as predictors. The Cytokine-PC was positively associated with BMI (driven by IL1ß and IL6), but negatively associated with self-reported bad health (driven by IL6, IL8 and TNFα). The Immunoglobulin-PC was associated with higher BMI (driven by sIgA) and older age (driven by sIgA and inversely by IgE). Females showed lower values than males on the Immunoglobulin-PC (driven by the FLCs, and inversely by IgE). The PCs were not associated with oral health problems or (anti-inflammatory) medication use. The covariates explained all random intercept variance, i.e., differences between families, for the Immunoglobulin-PC.

In contrast to our hypotheses, no associations of childhood maltreatment or psychopathology with the inflammatory PCs were found, see Table 3. Results did not differ when removing the covariates from the models. Hence, follow-up analyses for the separate immune markers or mediation analyses for maltreatment and psychopathology were not indicated.

3.2. Sensitivity and explorative analyses

We performed sensitivity analyses by splitting maltreatment into abuse and neglect,. Supplementary Tables S1a-b show that no associations were found with the inflammatory PCs when including these subscales either. Furthermore, hair cortisol and cortisone levels were not associated with the inflammatory PCs, see Supplementary Tables S2a-d. As hair cortisol levels may be impacted by hair color, hair product use, or coloring, we checked whether these covariates would change the results, but they did not impact outcomes (data not shown).

4. Discussion

We set out to study whether a broad range of salivary immune markers would be associated with early life stress, as measured via childhood maltreatment experiences, or to more recent life stress, assessed via mental health problems, in an adult sample. In contrast to our expectations, no significant associations were found between the salivary immune markers and self-reported childhood maltreatment or psychopathology in the current sample. No associations of the immune

Table 1
Descriptive statistics and rotated PC loadings of the immune markers.

	Mean	SD	Min	Max	PC1 (Cytokines)	PC2 (Immunoglobulins)
Immune markers:						
IL1ß (pg/ml)	21.23	26.14	0	163.08	.87 *	.17
IL6 (pg/ml)	3.27	4.78	0	42.13	.80 *	.14
IL8 (pg/ml)	412.89	345.97	0	2136.52	.83 *	-0.12
IFNγ (pg/ml)	1.23	1.17	0	5.90	.74 *	-0.11
TNFα (pg/ml)	22.30	20.17	0	124.13	.95 *	.04
IgE (kU/l)	4.18	3.05	.97	23.79	.12	-0.40 *
sIgA (ug/ml)	972.11	2554.39	59.43	21,488.00	.30	.72 *
FLCλ (ug/ml)	320.97	190.23	71.16	1071.73	.23	.65 *
FLCk (ug/ml)	131.30	85.20	0	529.53	-0.08	.57 *
Stress measures:						
Maltreatment	1.83	.57	1	4.75		
Abuse	1.65	.59	1	4.50		
Neglect	2.02	.70	1	5		
Psychopathology	95.10	12.31	74	122		
Hair Cortisol (pg/mg)	2.57	1.76	.10	10.55		
Hair Cortisone (pg/ng)	8.35	5.95	.10	33.94		

Notes. IL = Interleukin, IFN = Interferon, TNF = Tumor Necrosis Factor, (s)Ig = (secretory) immunoglobin, FLC = Free Light Chain, PC = Principal Component, * PC loadings over.40

Table 2
Bivariate correlations between the covariates, stress measures and immune markers.

Immune markers		IL1ß	Il6	Il8	IFNγ	TNFα	IgE	sIgA	FLCλ	FLCκ	PC1	PC2
Covariates:												
Female sex	*r*	.04	.03	.00	.09	**.19** *	**.19** *	-0.04	**-0.19** *	**-0.19** *	.11	**-0.26** * *
	p-value	*.636*	*.767*	*.987*	*.317*	*.043*	*.043*	*.642*	*.042*	*.040*	*.253*	*.004*
Age	*r*	**.22** *	.18	**.19** *	.14	-0.01	**-0.22** *	**.37** * *	.15	.15	.15	**.36** * *
	p-value	*.015*	*.057*	*.045*	*.140*	*.915*	*.016*	*< 0.001*	*.097*	*.103*	*.107*	*< 0.001*
BMI	*r*	**.32** * *	**.18** *	.09	.15	.16	-0.10	**.35** * *	.14	.15	**.20** *	**.30** * *
	p-value	*< .001*	*.048*	*.352*	*.095*	*.084*	*.285*	*< 0.001*	*.126*	*.112*	*.031*	*.001*
Oral health problems	*r*	.06	.04	.01	-0.02	.02	-0.02	-0.04	-0.04	.10	.02	.01
	p-value	*.523*	*.688*	*.910*	*.870*	*.815*	*.806*	*.669*	*.685*	*.302*	*.872*	*.906*
Bad health	*r*	-0.11	**-0.23** *	**-0.22** *	**-0.21** *	-0.17	.01	-0.12	-0.15	.04	**-0.22** *	-0.06
	p-value	*.260*	*.011*	*.017*	*.021*	*.071*	*.933*	*.215*	*.118*	*.693*	*.015*	*.509*
Medication use	*r*	.05	-0.10	.00	-0.01	-0.05	.05	.16	-0.09	.11	-0.02	.07
	p-value	*.613*	*.302*	*.988*	*.921*	*.612*	*.595*	*.082*	*.311*	*.253*	*.795*	*.440*
Anti-inflammatory medication use	*r*	-0.15	-0.03	-0.15	-0.14	-0.02	.01	-0.02	-0.14	.15	-0.13	.00
	p-value	*.102*	*.768*	*.099*	*.125*	*.875*	*.916*	*.851*	*.138*	*.118*	*.165*	*.964*
Sampling time	*r*	.01	.12	-0.06	**.28**	.06	-0.17	.12	.12	-0.05	.09	.10
	p-value	*.89*	*.22*	*.55*	*.003*	*.53*	*.08*	*.20*	*.20*	*.61*	*.36*	*.27*
Stress Measures:												
Maltreatment	*r*	.08	.02	-0.03	-0.02	-0.02	-0.12	.13	-0.07	.01	-0.002	.08
	p-value	*.393*	*.857*	*.770*	*.812*	*.820*	*.185*	*.151*	*.436*	*.943*	*.986*	*.382*
- Abuse	*r*	.10	.05	.05	-0.02	.03	-0.16	.16	-0.11	.06	.04	.10
	p-value	*.273*	*.565*	*.588*	*.854*	*.776*	*.090*	*.076*	*.227*	*.501*	*.688*	*.263*
- Neglect	*r*	.05	.01	-0.06	-0.02	-0.04	-0.08	.09	-0.02	-0.03	-0.01	.05
	p-value	*.588*	*.921*	*.529*	*.839*	*.662*	*.410*	*.354*	*.843*	*.722*	*.861*	*.559*
Psychopathology	*r*	.14	.02	.15	.02	.02	-0.04	.03	-0.10	.06	.07	.00
	p-value	*.119*	*.810*	*.113*	*.849*	*.865*	*.673*	*.734*	*.297*	*.521*	*.428*	*.998*
Cortisol	*r*	.10	.05	.06	-0.01	-0.24	-0.13	.15	-0.18	.07	-0.03	.10
	p-value	*.422*	*.673*	*.634*	*.914*	*.053*	*.296*	*.226*	*.156*	*.582*	*.800*	*.407*
Cortisone	*r*	.02	-0.09	-0.05	-0.04	-0.19	-0.09	.09	**-0.31** * *	.00	-0.10	-0.03
	p-value	*.887*	*.492*	*.691*	*.763*	*.129*	*.449*	*.452*	*.009*	*.999*	*.439*	*.823*

Notes. * p < .05. ** p < .01, significant associations are listed in bold. PC1 = Cytokine Principal Component, PC2 = Immunoglobulins Principal Component, BMI = Body Mass Index

Table 3
Mixed model outcomes for the 2 inflammatory PCs, showing fixed effects for significant covariates, childhood maltreatment, and psychopathology.

	PC1 (Cytokines)				PC2 (Immunoglobulins)			
	b	*t*(113)	95% CI (b) [min: max]	*p*	b	*t*(112)	95% CI (b) [min:max]	*p*
Covariates								
Female sex					-0.491 **	-2.94	-0.823: −0.159	.004
Age					.287 **	3.28	.114:.461	.001
BMI	.052 **	2.71	.014:.090	.008	.053 **	2.95	.018:.089	.004
Bad health	-1.47 **	-2.95	-2.46: −0.483	.004				
Childhood maltreatment	-0.056	-0.564	-0.251:.140	.574	-0.055	-0.590	-0.240:.130	.556
Psychopathology	.104	1.09	-0.084:.292	.277	.0077	.087	-0.168:.184	.931

Notes. Oral health problems, sampling time, and anti-inflammatory medication are not included in the Table as these variables were not associated with any of the PCs (Principal Component), BMI = body mass index, b = unstandardized beta coefficient, CI = Confidence interval, ***p* < .01.

markers with hair cortisol were found either. These results contrast with previous reports of systemic low-grade inflammation in relation to chronic psychological stress (for reviews see Baumeister et al., 2016; Gill et al., 2020), also using salivary assessments (Tyrka et al., 2015; Szabo et al., 2020; Hori et al., 2022). However, negative and mixed findings have also been reported (Chen and Lacey, 2018; Palmos et al., 2019; Warrier et al., 2021), indicating that these associations may depend on the specific sample, methodology, and covariates used (Horn et al., 2018; Szabo et al., 2020; Kerr et al., 2021).

The absence of associations between our measures of stress and inflammation may indicate that inflammation does not represent a mechanism by which chronic stress leads to stress-related symptomatology. However, several other explanations for these null findings should be discussed. To start, our null findings may also pertain to the possibly limited validity of salivary immune markers as an indicator of systemic inflammation, as was previously suggested by others (Byrne et al., 2013; Riis et al., 2014; Szabo and Slavish, 2021). We did, on the other hand, find several of our salivary immune markers to be associated with higher age (i.e., with higher levels of sIgA and lower levels of IgE), higher BMI (i.e., with higher levels of sIgA, IL1ß, and IL6), and male sex (with higher levels of FLCs and lower levels of IgE). This indicates that these markers are sensitive to physical individual differences and may partly reflect systemic immune functioning. Especially higher BMI has previously been associated with higher levels of systemic inflammation (Jafarzadeh et al., 2010; Palmos et al., 2019). We furthermore found lower levels of IL6, IL8 and TNFα in participants reporting fever or bad health in the past few days, which may reflect a weakened immune system in those individuals.

Other possibilities for our null findings with regards to stress may be that we only assessed a single saliva sample. There are indications of a circadian rhythm in salivary immune markers, and stress effects may become visible only at certain times of the day (Hori et al., 2022; Vermeer et al., 2012). Although we found no association of assessment time with the inflammatory PCs in the current sample, repeated salivary measurements over time or in response to a psychosocial challenge (Schreier et al., 2020) may shed more light on the impact of early and recent life stress on salivary immune markers. Of note though, it is even less clear to what extent salivary or blood-based immune activation reflect neuro-inflammation, even though neuro-inflammation may be most relevant for the development of stress-related disorders (Nettis and Pariante, 2020). Lastly, our null findings could be due to low power given a limited sample size with a nested design, and by the relatively low levels of childhood maltreatment. While this sample was partly selected for a heightened risk of childhood maltreatment (Buisman et al., 2020), on average the maltreatment scores rated 1.82 on a scale from 1 to 5, showing on average relatively low levels of abuse and neglect. However, there was sufficient variation in the data (with scores ranging from 1 to 4.75), and we have previously shown that maltreatment in this sample is related to both behavioral and brain outcomes (e.g., see Buisman et al., 2019; Van den Berg et al., 2018).

A strength of this study is that we included both commonly assessed immune markers (i.e., IL1ß, IL6, IL8, IFNγ, TNFα, and sIgA) as well as less commonly studied immune markers (i.e. IgE, FLCλ, and FLCκ). As to be expected, the cytokines clustered together, as these are all regarded as pro-inflammatory cytokines (Zhang and An, 2007). The FLCs furthermore correlated with sIgA as was previously found (Irshad et al., 2020), and are seen as emerging immune biomarkers (Rapson et al., 2020). IgE was negatively associated with the FLCs and sIgA. This may be explained by the finding that IgE is specifically implicated in allergies and auto-immune responses versus more common inflammatory responses (Karagiannis et al., 2013; Sanjuan et al., 2016). Immunoglobins are part of the adaptive immune system, and future studies should include markers of both the innate as well as the adaptive immune system, as they may be differentially impacted by chronic stress. More knowledge about the impact of both early life stress and more acute stressors on immune functioning may provide new routes to interventions that counteract the impact of psychological stress on both mental and physical wellbeing (Milaneschi et al., 2020).

Strengths of the current study furthermore include the measurement of different types of stress, including childhood maltreatment experiences, current psychopathology, and a more objective measure of stress with hair cortisol. Also, the inclusion of a large number of immune markers and relevant covariates is a strength, but some limitations not yet mentioned may also have impacted our findings. That is, salivary flow rate was not assessed, and several life style factors like diet, sport, and smoking behavior were not included as possible covariates, as these were not available for a large part of this sample. We used self-report scales for both childhood maltreatment as well as psychopathology, which may be subject to recall bias or socially desirable responding. Objective measures of childhood maltreatment and clinical diagnoses could reduce some of this bias, but may at the same time underestimate the amount of stress experienced in undisclosed cases of maltreatment or by subclinical symptoms of psychopathology. Lastly, our sample was not large enough to detect small effect sizes (f^2<0.08–0.09) and a focus on larger and more homogenous samples may offer a clearer picture on the use of salivary immune assessments in relation to chronic stress.

4.1. Conclusions

In sum, in the last decade the potential role of (neuro)inflammation in mental health is being revealed and non-invasive techniques like salivary immune measurement are welcomed. However, while we found associations between a number of salivary immune markers and individual differences in age, sex, and BMI, no associations with either self-reported stress or cortisol levels were found in the current sample. Hence, it is yet unclear to what extend stress and stress-related psychopathology is associated with salivary markers of inflammation. Larger studies, including participants with higher levels of stress and using more sampling occasions, are needed to further examine associations between salivary immune markers and psychological stress.

Funding sources

The study was supported by the Netherlands Organization for Scientific Research (MB-K: VICI grant [no. 453-09-003]; LA: VIDI grant [no. 016.145.360]; MI: NWO SPINOZA prize) and grants of Leiden University to initiate and support the Research Profile Area Health, Prevention and the Human Life Cycle awarded to MI, P. Assendelft, and B. van Hemert. These organizations had no involvement in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Nicole Buurman, Pauline van Dam, and Cleo Arkenaar for their excellent technical assistance. We are grateful to all the families that have invested their time by participating in this study and to the students whose contribution to the data collection was invaluable.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105867.

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