An immunogenomic phenotype predicting behavioral treatment response: Toward precision psychiatry for mothers and children with trauma exposure

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ABSTRACT

Inflammatory pathways predict antidepressant treatment non-response among individuals with major depression; yet, this phenomenon may have broader transdiagnostic and transtherapeutic relevance. Among trauma-exposed mothers (M_age = 32 years) and their young children (M_age = 4 years), we tested whether genomic and proteomic biomarkers of pro-inflammatory imbalance prospectively predicted treatment response (PTSD and depression) to an empirically-supported behavioral treatment. Forty-three mother–child dyads without chronic disease completed Child Parent Psychotherapy (CPP) for roughly 9 months. Maternal blood was drawn pre-treatment, CD14+ monocytes isolated, gene expression derived from RNA sequencing (n = 34; Illumina HiSeq 4000; TruSeq cDNA library), and serum assayed (n = 43) for C-Reactive Protein (CRP) and interleukin-1β (IL-1β). Symptoms of PTSD and depression decreased significantly from pre- to post-treatment for both mothers and children (all p < 0.01). Nonetheless, a higher pre-treatment maternal pro-inflammatory imbalance of M1-like versus M2-like macrophage-associated RNA expression (M1/M2) (β = 0.476, p = .004) and IL-1β (β = 0.333, p = .029), but not CRP, predicted lesser improvements in maternal PTSD symptoms, unadjusted and adjusting for maternal age, BMI, ethnicity, antidepressant use, income, education, and US birth. Only higher pre-treatment M1/M2 predicted a clinically-relevant threshold of PTSD non-response among mothers (OR = 3.364, p = .015; ROC-AUC = 0.78). Additionally, higher M1/M2 predicted lesser decline in maternal depressive symptoms (β = 0.556, p = .001), though not independent of PTSD symptoms. For child outcomes, higher maternal IL-1β significantly predicted poorer PTSD and depression symptom trajectories (β’s = 0.318-0.429, p’s < 0.01), while M1/M2 and CRP were marginally associated with poorer PTSD symptom improvement (β’s = 0.295–0.333, p’s < 0.056). Pre-treatment pro-inflammatory imbalance prospectively predicts poorer transdiagnostic symptom response to an empirically-supported behavioral treatment for trauma-exposed women and their young children.

1. Introduction

Over 70% of people in the world will experience a significant trauma in their lifetime (Kessler et al., 2017), increasing the risk for the development of post-traumatic stress disorder (PTSD) and depression. Although PTSD and depression affect several hundreds of millions
people globally (Kessler et al., 2005; Koenen et al., 2017; World Health Organization, 2017), only 50–60% of individuals with PTSD respond to traditional pharmacologic treatments, and less than one-third achieve full remission (Berger et al., 2009). Leading treatments for depression also fail to achieve remission for a sizable percentage of patients (Shim et al., 2011). However, the growing revelation that the immune system may contribute to mental health disorders (Miller and Raison, 2016), particularly symptoms of depression and PTSD, offers a new avenue to improve treatment efficacy. Peripheral inflammation can influence the brain, inducing mental health symptoms via afferent nervous system, humoral, and cellular trafficking pathways (Miller and Raison, 2016). In patients with depression, inflammatory markers predict poor treatment response to antidepressants (Strawbridge et al., 2015) and cognitive behavioral therapy (Lopresti, 2017), while anti-cytokine therapies show promise in individuals with elevated pre-treatment inflammatory markers (Kappelmann et al., 2018). Herein, we test whether markers of inflammatory imbalance may have much broader transdiagnostic relevance in predicting trajectories of behavioral treatment response for PTSD, as well as depressive symptoms, among trauma-exposed individuals. This demonstration would establish the foundation for novel approaches to Precision Psychiatry in the treatment of trauma.

PTSD and depression are independently associated with heightened levels of inflammatory cytokines or proteins (Miller et al., 2009; Passos et al., 2015). In some studies, pro-inflammatory cytokines or genes, particularly interleukin (IL)-1β or IL-6, have predicted future trajectories of PTSD and depressive symptoms (Aschbacher et al., 2012; DellaGoia and Hannestad, 2010; Glatt et al., 2013; Pervanidou et al., 2007; van den Biggelaar et al., 2007). Conversely, remittance of PTSD has been associated with significant reductions in pro-inflammatory cytokines (Gill et al., 2013), although it is unclear whether this association is causal. Lower levels of anti-inflammatory or immunoregulatory factors (e.g., IL-4 and TGF-β1) have also been associated with PTSD (Von Känel et al., 2007) and poorer symptom trajectories (Cohen et al., 2011). IL-1β bears the strongest association with PTSD, trauma, and reactivity to acute psychological stress per the effect sizes reported in multiple reviews (Passos et al., 2015; Steptoe et al., 2007; Tursich et al., 2014). Further, mechanistic research in animal models demonstrates that IL-1β signaling plays an important role in blood brain barrier permeability to circulating monocytes and consequent neuroinflammatory fear-related behaviors (Lisboa et al., 2018; Weber et al., 2017). Nonetheless, no single cytokine marker emerges consistently across studies. Other seemingly contradictory evidence suggests that low levels of inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) in the immediate 24 hours following a trauma may prospectively predict chronic PTSD (Michopoulos et al., 2020). Hence, given that inflammatory responses involve hundreds of genes that orchestrate pro- and anti-inflammatory processes, deeper elucidation of the underlying inflammatory gene expression signatures is warranted. Immunophenotypes may be used to quantify an index that captures the homeostatic regulation of inflammation, involving the balance of pro- and anti-inflammatory factors.

In the initial stages of inflammation, circulating monocytes infiltrate tissues and differentiate into macrophages exhibiting a “classically activated” M1-like phenotype, which evolves dynamically over time across a spectrum of pro-resolving, “alternatively activated” M2-like phenotypes (Murray et al., 2014). Consequently, macrophages are said to exhibit a polarization state, which describes the M1- to M2-like balance within a heterogeneous population that may be identified by distinct gene expression profiles. In general, PTSD and depression are associated with leukocyte gene expression profiles that are more classically activated, or M1-like (Breen et al., 2017; Jansen et al., 2015; Mostafavi et al., 2013; O’Donovan et al., 2011). Monocytes in circulating blood are easier to access, isolate, and assay than such macrophages in tissue; however, the phenotypes of monocytes only partially overlap with macrophages. Nonetheless, animal and human evidence suggests that psychological stress, via sympathetic pathways, alters bone-marrow derived progenitors, which leads to phenotypic alterations impacting downstream myeloid cell populations (Aschbacher et al., 2017; Heidt et al., 2014; McKim et al., 2018; Powell et al., 2013). Indeed, exposure to trauma during sensitive periods of development, including childhood and pregnancy, have been associated with a greater relative expression of M1 versus M2-like genes (Aschbacher et al., 2021). In sum, an immunogenomic phenotype, reflecting the relative balance of M1-like to M2-like expressed genes, may provide a predictive biomarker(s) of PTSD treatment response.

Beyond associations with symptoms, inflammatory biomarkers also prospectively predict treatment response to antidepressants in depression (Miller and Raison, 2016), thereby providing the foundation for Precision Psychiatry interventions (Kappelmann et al., 2018). In addition, three studies report that inflammatory markers predict poorer response to cognitive behavioral therapy (CBT) (Lopresti, 2017), suggesting the impact of inflammation on treatment response may be transtherapeutic. However, those studies included individuals with high pre-treatment pro-inflammatory biomarker C-Reactive Protein (CRP) levels (>10 mg/L), chronic pain, or diabetes; hence, it is unclear whether similar associations would be present among individuals without signs of an underlying inflammatory condition. We sought to investigate whether similar prospective predictions of behavioral treatment response could be observed among trauma-exposed individuals exhibiting symptoms of PTSD and depression, in the absence of diagnosed chronic disease. Prior studies have used levels of CRP (>1 mg/L) to predict treatment resistance to traditional antidepressants among individuals with major depression (Jha et al., 2017; Uher et al., 2014). Although CRP is convenient to measure, as a downstream marker of inflammatory pathways, it offers little mechanistic insight. As an alternative, one study reported that high levels of absolute mRNA for IL-1β and macrophage migration inhibitory factor (MIF) predicted resistance to selective serotonin reuptake inhibitors (SSRIs) in major depression (Cattaneo et al., 2016). Providing further evidence for a causal pathway, the administration of anti-cytokine treatments to patients classified as antidepressant non-responders has successfully reduced depressive symptoms across five randomized controlled trials (RCTs) (Kappelmann et al., 2018). These observations support a mechanistic role for inflammation in antidepressant treatment of major depression. However, it is unclear how broadly this phenomenon might apply, in terms of other disorders, like PTSD, or other forms of treatment, such as evidenced-based behavioral therapies.

There is good reason to suspect that inflammation might impair behavioral treatment response. Inflammatory cytokines, and macrophage polarization specifically, play an important role in neuroplasticity, learning, and shaping behavior (Hu et al., 2015). For behavioral interventions to succeed, participants need to learn new skills, and to unlearn prior maladaptive perceptions and behaviors. For example, animal research demonstrates that peripheral inflammation can impair fear extinction (a critical neurocognitive mechanism of PTSD recovery) (Doenni et al., 2017; Quinones et al., 2016). In humans, induction of peripheral inflammation provokes heightened amygdalar reactivity to social threat, which in turn, predicts feelings of disconnection from others (Inagaki et al., 2012). Inflammation-induced feelings of social disconnection may pose a critical barrier to psychotherapy that relies on interpersonal processes as a primary treatment mechanism (e.g., attachment-oriented frameworks). Furthermore, immune cells are known to traffic into the central nervous system (CNS) via the meninges and to the choroid plexus, even in normal, non-pathologic conditions (Rut and McGavern, 2018). Moreover, in mice, deficits in learning and memory are specifically associated with the presence of meningeal macrophages exhibiting a pro-inflammatory (M1-like) phenotype, while injection of M2 cells significantly reduced these deficits (Derecki et al., 2011). In animal models of repeated social defeat, which is arguably a model for exposure to interpersonal violence (IPV), monocytes and relative asymmetry of M1/M2-like macrophage phenotypes provide crucial signals determining neuroinflammation, neurocognitive...
alterations and reemergence of anxiety symptoms after extinction (Weber et al., 2017; Wohleb et al., 2014). Thus, we hypothesized that a novel biomarker of pro-inflammatory imbalance (the M1/M2 phenotype), and secondarily, elevated levels of pro-inflammatory proteins (CRP, IL-18), would predict poor treatment response to behavioral therapy among IPV-exposed women with PTSD symptoms.

Mothers of young children exposed to IPV constitute an important population to study in a treatment context, given that IPV is disturbingly prevalent, constitutes a potent precipitant of PTSD, and may kindle intergenerational transmission (Yehuda and Lehrner, 2018). One in every three women worldwide will experience IPV (World Health Organization, 2013), which is associated with a 2- to 4-fold greater risk for developing PTSD than exposure to combat or a natural disaster (Blanco et al., 2018). Moreover, the immunologic fingerprint of IPV exposure in women differs from that in men and is distinct from other types of trauma (e.g., combat) (Breen et al., 2017). Mothers’ experiences of IPV adversely impact their young children’s behavior, in part via maternal mental health symptomatology and related changes in responsive behavior (Miller-Graff et al., 2019). Additionally, for these young children, exposure to early life adversity may result in biomarking with long-lasting implications for health (Baumeister et al., 2016). Hence, to help young children and mitigate the long-term societal harm of early life adversity, we must start by treating their mothers.

The leading empirically supported trauma therapy designed to treat trauma-exposed young children and their primary caregivers is Child Parent Psychotherapy (CPP) ( Cicchetti et al., 2006; Cicchetti et al., 2000; Lieberman et al., 2005; Lieberman et al., 1991; Toth et al., 2000; Toth et al., 2002). CPP treats caregivers (or herein, their biological mothers) and children together, targeting caregiving and child attachment behaviors (Lieberman et al., 2015). Five rigorous RCTs have tested the efficacy of CPP against comparison treatments or controls and have found it efficacious ( Cicchetti et al., 2006; Cicchetti et al., 2000; Lieberman et al., 2005; Lieberman et al., 1991; Toth et al., 2006; Toth et al., 2002) in improving child cognitive development ( Cicchetti et al., 2000), emotional and behavioral problems and trauma symptoms (Lieberman et al., 2005), and maternal PTSD symptoms (Lieberman et al., 2005). The beneficial effects of CPP on child mental health are mediated through changes in the parent–child relationship and maternal behaviors ( Cicchetti et al., 2006; Lieberman et al., 1991; Toth et al., 2002). Children’s recovery from trauma depends, in part, on their mother’s ability to recover through therapy (Hagan et al., 2017). Hence, to the extent that maternal inflammation may alter the maternal behavioral mechanisms of intervention efficacy (e.g., social disconnection, heightened threat reactivity, impaired learning and fear extinction) (Dantzer and Kelley, 2007; Inagaki et al., 2012; Lisboa et al., 2018), we hypothesized that maternal biomarkers would predict treatment responses to CPP for both mothers and their children.

We hypothesized that a biomarker reflecting pro-inflammatory imbalance (an a priori, literature-based M1/M2 phenotype (Aschbacher et al., 2021; Labonte et al., 2014; Murray et al., 2014) quantified by RNA-seq in circulating CD14+ monocytes), and secondarily, the serum-derived pro-inflammatory markers CRP and IL-18, would predict treatment response to an empirically supported behavioral therapy for trauma exposure and related symptomatology. Our sample consisted of racially/ethnically diverse, low-income mothers and their biological children seeking treatment due to high levels of interpersonal trauma exposure. Treatment response was assessed as the decrease in symptoms of PTSD (primary outcome) and depression (secondary outcome), among both mothers and children across roughly 9 months of therapy. We also assessed clinically relevant non-response, defined by previously published, validated cut-offs.

### 2. Materials and methods

#### 2.1. Participants

These data come from a larger study, The Child Parent Psychotherapy biomarker study (CPP-HEALTH), based on a previously validated intervention ( Cicchetti et al., 2006; Cicchetti et al., 2000; Lieberman et al., 2005; Lieberman et al., 1991; Toth et al., 2006, Toth et al., 2002), which enrolled a total of 62 women and their children aged 2–6 years. The present analyses included 43 mother–child dyads who completed the pre-treatment assessment, blood draw, and participated in a course of CPP. The sample was ethnically diverse: Hispanic/Latina Caucasian (n = 31), Non-Hispanic/Latina Caucasian (n = 5), Asian (n = 5), African American (n = 2). Additionally, the sample was predominantly of low socioeconomic status (SES): 65% the families (n = 28) met the national criteria for poverty, per the 2016 Census guidelines, which adjust for family size (Semega et al., 2016). Of these 43 mother–child dyads, gene expression for phenotyping was obtained in a convenience subset of 34 (demographics in Table 1).

Women and their children were recruited via phone screenings of families seeking treatment at Child Trauma Research Program at Zuckerberg San Francisco General (ZSFG) for child trauma symptoms. Inclusion criteria for CPP-Health included biological mothers, age 18 and over, with children between 2 and 6 years of age, who had been exposed to interpersonal trauma (63% of children had experienced IPV within the family), and were fluent in English and/or Spanish. Exclusion criteria included: child was a ward of the state, homelessness, current family violence, pregnancy, substance abuse, child developmental disorder, psychosis, and chronic medical conditions in mother or child, per maternal self-report. This research was approved by the Institutional Review Board at ZSFG and the University of California, San Francisco (UCSF). Maternal participants provided written consent and were compensated for their time.

#### Table 1

| Sample Characteristics | Full Cohort (N = 43) | Phenotyped Subcohort (N = 34) |
|------------------------|----------------------|-------------------------------|
| **Sociodemographic, Mental Health, and Medical Factors** | | |
| **Mother’s Age, years** | 32.27 (0.95) | 31.58 (0.88) |
| **Non-Hispanic Caucasian** | 5 (12%) | 2 (6%) |
| **Hispanic/Latina Caucasian** | 31 (72%) | 27 (80%) |
| **African American** | 5 (2%) | 2 (6%) |
| **Asian American** | 5 (12%) | 3 (9%) |
| **High School Education** | 28 (65%) | 19 (56%) |
| **Family Poverty** | 28 (65%) | 23 (66%) |
| **US Born** | 6 (14%) | 6 (18%) |
| **Child’s Age, months** | 50.44 (2.06) | 50.12 (2.35) |
| **Maternal Early Life Adversity** | 0.81 (0.18) | 0.79 (0.20) |
| **Maternal Cumulative Life Adversity** | 11.15 (0.69) | 11.61 (0.75) |
| **Child Exposed to Family Violence** | 27 (65%) | 20 (59%) |
| **Maternal PTSD Severity (PSSI)** | 22.00 (1.75) | 22.00 (1.98) |
| **Maternal Depressive Symptoms (CES-D)** | 25.67 (1.97) | 25.24 (2.11) |
| **Child PTSD Symptoms (TSC)** | 43.28 (2.09) | 42.18 (2.35) |
| **Child Depressive Symptoms (TSC)** | 13.43 (0.62) | 13.45 (0.67) |
| **Body Mass Index** | 28.00 (0.95) | 28.11 (1.07) |
| **Current Antidepressant Use** | 6 (14%) | 4 (12%) |
| **NSAID Use** | 1 (2%) | 1 (3%) |

Note: a = Mean (SEM) from independent t-tests; b = n (%) from Chi-Squared or Fisher’s Exact Test as appropriate. PSSI = PTSD Symptom Scale Interview, total score. CES-D = Center for Epidemiologic Studies Depression Scale, total score. TSC = N = 43 constitutes the full sample with serum cytokine data and N = 34 constitutes the subsample with gene expression data. High school education was coded 0 for participants who attended school for <12 years, and 1 for 12 or more years of attendance. Poverty was calculated using 2016 Census criteria (see methods).
2.2. Child Parent Psychotherapy

Child-Parent Psychotherapy (CPP) (Lieberman et al., 2006) is a well-validated dyadic psychotherapeutic intervention empirically demonstrated to treat trauma-exposed children under the age of 6 and their primary caregiver (here biological mothers). CPP was delivered in weekly one-hour sessions by a CPP-trained master’s or doctoral-level therapist. Intervention targets included parents’ and children’s affect regulation, responses to traumatic reminders, and dyadic interactions and behaviors that interfere with the child’s mental health. Intervention modalities included the co-creation between mother and child of trauma and protective narratives, correcting maladaptive perceptions of danger and safety, addressing trauma triggers, and offering reflective developmental guidance, empathic support, and concrete assistance with living. CPP was accredited in 2011 by the SAMHSA National Registry of Evidence-Based and Promising Practices (NREPP) and in 2005 achieved mental health guidance, empathic support, and concrete assistance with living.

2.3. Maternal PTSD & Depression symptoms

Maternal PTSD symptoms were measured via clinical interview with a therapist, using the 17-item Posttraumatic Stress Scale Interview (Foia et al., 1993) (PSSI); Cronbach’s α = 0.88 in this sample) at pre- and post- treatment to assess trauma symptoms in the past two weeks. Items assess symptom frequency and severity following the PTSD diagnostic criteria in the Diagnostic and Statistical Manual of Mental Disorders-IV across three symptom subscales (American Psychiatric Association, 2000), and clinicians ensured item comprehension and probed if answers were uncertain. Subsequent analyses examined both continuous scores and categorical definitions. We defined the clinically relevant categorization of PTSD treatment non-response using previously published cut-offs, requiring that participants met criteria for at least two hyperarousal, one intrusion, and three avoidance subscale items (Foia and Tolin, 2000). Depressive symptoms in the past week prior to pre- and post-treatment assessments were assessed with the sum of the 20-item Center for Epidemiologic Studies Depression Scale Revised (CESD-R) (Eaton et al., 2004). A clinically relevant treatment non-response was quantified as individuals whose CESD scores were >16 at post-treatment (Lewinsohn et al., 1997).

2.4. Child PTSD & Depression symptoms

Child post-traumatic stress and depression symptoms were assessed as secondary outcomes using the two 10-item Depression and Post-Traumatic Stress Symptom subscales of the Trauma Symptom Checklist for Young Children (TSCYC) (Briere et al., 2001). These scales have demonstrated high reliability and predictive validity in large samples of traumatized children (Briere et al., 2001). Per the TSCYC manual (Briere, 1996) (Briere, 2005), clinically significant post-traumatic stress symptomatology among children was quantified as having a PTSD subscale raw score >40, while clinically relevant depressive symptoms were defined as having a Depression subscale t-score ≥70 (Briere et al., 2001). One data point was missing for the treatment-related change in child depressive symptoms and was dropped from analyses.

2.5. Blood draw procedure

Prior to the blood draw, mothers were asked to fast and consume nothing other than water or coffee in the morning and to reschedule if they felt ill with a cold or fever, or had used any medications, such as antihistamines, that might affect collection in the prior three days. One woman who did not reschedule but did report using NSAIDS in the three days prior was included in a sensitivity analysis to address potential confounds. Blood was collected and serum stored at −80°C.

2.6. Inflammatory protein assays

CRP was measured using a high-sensitivity immunoturbidimetric assay from Randox (Kearneysville WV) and a PolyChem clinical chemistry analyzer (PolymedCo, Cortlandt Manor, NY), and provided an intra-assay coefficient of variation (CV) of 3.6%. The pro-inflammatory cytokine IL-1β was measured in duplicate using a chemiluminescent multiplex assay from Meso Scale Discovery (Rockville, MD), with an intras-assay coefficient of variation (CV) of 11.9%.

2.7. M1/M2 Phenotype

Genes reflecting an M1 and M2-like profile were selected based on the literature (Supplementary Table 1) (Labonte et al., 2014). Genes considered markers of the M1-like phenotype included inducible nitric oxide synthase (iNOS), Toll-like receptors (TLRs) 2 and 4, and IL-1 receptor type 1 (IL1R1), the receptor by which IL-1β initiates a pro-inflammatory signal (Roerink et al., 2017). The M2-like phenotype focused on M2a and M2b-associated genes, which included anti-inflammatory cytokines such as IL-1 receptor antagonist (IL-1RN gene) and IL-10, as well as genes coding for the human leukocyte antigen-DR (HLA-DR) isotype, a Major Histocompatibility Complex (MHC) class II complex receptor, which constitutes a proposed marker of the M2a and M2b subtypes of M2-like polarization, emphasized herein (Labonte et al., 2014). The HLA-DR complex plays a crucial role in monocyte/macrophage communications with T-helper cells, thereby mediating homeostatic regulation of inflammation in the setting of repeated physical trauma (Heftig et al., 2017) as well as influencing organ transplant, autoimmunity, PTSD, and potential trade-offs between innate and adaptive immunity (Katrinli et al., 2019; Taneja and David, 1998).

2.8. CD14 + Monocyte isolation and RNA sequencing, Read alignment and expression modeling

CD14 + cells were isolated from 3x10⁷ PBMC by immunomagnetic positive selection. The resultant CD14 + cells were resuspended in MACS buffer and 0.5x10⁶ to 10 x 10⁶ CD14 + cells were lysed in 600uL of RNAprotect Cell Reagent, and immediately placed in –80°C for storage prior to RNA-seq assays (see Supplementary Methods for details). Demultiplexed reads in FASTQ format were aligned to the reference human transcriptome (HG38) using HISAT2 (Kim et al., 2015; Pertea et al., 2016). Transcript and gene expression was modeled with StringTie (Pertea et al., 2016; Pertea et al., 2015). FASTQ files are available from the NCBI Sequence Read Archive (SRA) under BioProject PRJNA626346. Gene expression values were calculated as transcripts per million (TPM), and log2 transformed prior to downstream analysis. Exploratory data analysis was performed on the RNA-Seq data. A single sample was excluded from downstream analysis given its low RIN number of 3, which was also noted to be an outlier on initial principal component analysis. The remaining 34 samples were used for downstream analysis.

2.9. Data analysis

All variables were inspected for normality, and Blom-transformations were used to improve the distribution of inflammatory cytokines and clinical outcomes. Gene counts were log2-normalized, after which, we computed an aggregate sum for M1-like genes and another for M2b-like genes, and then calculated the final M1/M2 ratio.

Repeated measures ANOVA tests were run to determine whether symptoms of PTSD and depression significantly changed from pre- to post-treatment. To evaluate the hypothesis that maternal immunologic biomarkers at pre-treatment would predict symptom trajectories from pre- to post-treatment (for mothers and their children both), we created...
residualized change scores by regressing post-treatment on pre-treatment, saving the standardized residuals, and Blom-transforming them; this ensured that change was not better accounted for by pretreatment values. Next, linear regression tests were conducted to examine whether pre-treatment immunologic markers significantly predicted residualized change in symptoms, both unadjusted or adjusted for covariates. To assess biomarker predictions of a clinically relevant response, defined through categorical diagnostic thresholds (see methods section 2.4), logistic regression analyses were conducted. Significant associations between the M1/M2 ratio (as a single index) and the outcomes were followed by more fine-grained analyses to better quantify the potential to discriminate between treatment responders versus non-responders, by quantifying the receiver operating characteristic area under the curve (ROC-AUC). Whereas the M1/M2 ratio assumes a 1:1 weighting of M1 genes with M2a and M2b genes, in this follow-up analysis to the significant M1/M2 ratio test, M1, M2a, and M2b were entered as separate aggregated factors, to allow coefficient weights to be learned by the model and achieve a maximal AUC. Covariates for linear regression models were selected a priori, and were run in three stages: 1) unadjusted, 2) adjusted model 1 (maternal health): maternal age, body mass index (BMI), race/ethnicity, and current antidepressant use (AD), and 3) adjusted model 2 (socioeconomic status; SES): family poverty (per national census criteria), maternal high school graduation (yes/no), and whether mother was US born (yes/no). Covariates for model 1 were selected based on being crucial determinants of inflammatory markers (Mora et al., 2006), treatment response, or this sample’s racial/ethnic diversity (Stowe et al., 2010). Covariates for model 2 were chosen to evaluate whether immunologic markers would remain significant predictors of treatment response after adjusting for SES. The number of treatment sessions was not significantly correlated with the changes in symptoms of PTSD or depression, for mothers or children; thus, it was not included as a covariate. Similarly, neither exposure to adversity in early life nor total life adversity were associated with the pre-post treatment changes in PTSD or depressive symptoms, both in mothers and in their children; hence, adversity exposure was not included in models. To visually depict the protein–protein interactions amongst proteins encoded for by genes included in the M1/M2 signature, we computed a gene co-expression network derived from Pearson correlations of this M1/M2 gene set, and overlaid that upon the network derived from the STRING database in Cytoscape v.3.7.2. To elucidate which of the genes comprising the M1/M2 phenotype likely drive the results, we conducted fold change analyses of differential gene expression predicting clinically relevant response, in mothers and in their children.

3. Results

3.1. Participant characteristics

Table 1 provides the sociodemographic, mental health, and medical characteristics of the participants for the full cohort (n = 43) and the portion with gene expression data (n = 34) (see methods and materials for details). The sample ranged in age from 22 to 48 years (M = 32) and was racially/ethnically diverse (80% Hispanic/Latina), predominantly below the national family poverty line, and had a high prevalence of lifetime trauma exposures. The average BMI was overweight (M = 28.60; range: 17.54–49.75, 25th–75th%: 23.65–31.66). Pre-treatment CRP levels, a marker of systemic inflammation, were elevated in this sample: 47% (n = 20) were low-risk (<1 mg/L), 23% (n = 10) were moderate risk (1–3 mg/L), and 30% (n = 13) were high-risk (>3 mg/L)(Myers et al., 2004). The three biomarkers (M1/M2 phenotype, CRP, and IL-18) were not significantly correlated with one another (Pearson r’s: -0.168 to 0.220, all p’s < 0.34, n = 34).

3.2. Maternal outcomes

3.2.1. Maternal symptom changes across treatment

On average, mothers showed significant reductions in total PTSD and depressive symptoms (DEP) from pre- to post-treatment in repeated measures analyses (all p’s < 0.001; Supplementary Table 2), with respective Cohen’s d effect sizes, −0.83 (PTSD) and −0.84 (DEP). Changes in PTSD were strongly significantly correlated (r = 0.69, p < .001) with changes in DEP; nonetheless, the changes in PTSD and DEP during CPP each remained independently statistically significant, even when adjusting for each other.

Additionally, we considered clinically relevant non-response, defined as meeting diagnostic criteria post-treatment. Maternal PTSD and Depression clinical non-response rates were 35% and 32%, respectively, for the subsample with genomics, and 40% each outcome for the larger sample with cytokines. Maternal DEP clinical non-response was moderately correlated with PTSD clinical non-response (r = 0.611, p < .001).

3.2.2. Biomarker predictors of maternal symptom improvement

The M1/M2 Phenotype. As hypothesized, a higher score on the maternal M1/M2 phenotype (measured in mothers) was a significant predictor of poorer maternal symptom response, as indexed by lesser reductions in symptoms of PTSD (p=.004) and DEP (p=.001) in unadjusted linear regression analyses (Table 2, Fig. 1). This pattern of significance held, adjusting for all covariates: including maternal age, BMI, antidepressant use, race/ethnicity, poverty, education, and being US born. Lastly, we confirmed that the M1/M2 phenotype remained a significant predictor of both PTSD and DEP symptom changes (p’s < 0.01) in a sensitivity analysis, which excluded 5 participants with potential medical confounds: NSAID use (n = 1), current breastfeeding (n = 3), and cigarette smoking (n = 1).

Pro-Inflammatory Proteins. Also shown in Table 2, pre-treatment levels of maternal IL-18 significantly predicted poorer treatment response (lesser declines) in maternal PTSD symptoms (p=.029) and a non-significant trend toward lesser reductions in DEP symptoms (p=.089) in unadjusted linear regression analyses, with a similar pattern of significance in adjusted analyses, and when excluding participants for sensitivity analyses. Pre-treatment CRP levels did not significantly predict maternal treatment response.

3.2.3. Biomarker predictors of maternal clinical non-response

Next, we investigated whether the associations demonstrated above constituted clinically relevant non-response, defined as meeting diagnostic criteria post-treatment, using logistic regression analyses. As shown in Table 3 and Fig. 2, a higher M1/M2 ratio (p = .015), but not a higher serum IL-18 (p = .427) or CRP (p = .277) level, significantly predicted the likelihood of PTSD clinical non-response. The association between maternal M1/M2 and maternal PTSD non-response remained significant when controlling for all covariates. No biomarkers significantly predicted maternal DEP clinical non-response (p’s > 0.05).

Finally, we conducted a follow-up analysis to quantify model performance using the receiver operating characteristic (ROC) area under the curve (AUC). As shown in Fig. 3, this logistic regression model, which included the M1/M2 phenotype components entered separately, provided strong model performance per the ROC-AUC in terms of predicting maternal non-response. For prediction of maternal PTSD and DEP non-response, M1/M2 successfully discriminated 78% and 75% of non-responders respectively from responders, whereas, for comparison, an AUC of 50% would reflect chance-level discrimination.

3.3. Child outcomes

3.3.1. Child symptom changes across treatment

Overall, children exhibited significant reductions in total PTSD and DEP symptom severity scores (p’s < 0.01) from pre- to post-treatment,
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and CRP).
The positive standardized regression coefficients shown indicate that phenotypes with relatively greater M1-like polarization were prospectively associated with lesser
degree of freedom (df) are shown first for the M1/M2 phenotype, then for serum protein markers (IL-1

Table 2

Pre-Treatment Maternal Immune Biomarkers Predicting Maternal Symptom Improvement During Child-Parent Psychotherapy (CPP).

| Treatment-Related Change (Δ) | Pre-Treatment Maternal M1/M2-like Phenotype | Pre-Treatment Maternal Interleukin-1β (IL-1β) | Pre-Treatment Maternal C-Reactive Protein (CRP) |
|-----------------------------|--------------------------------------------|--------------------------------------------|-----------------------------------------------|
| β                           | t-test p-value                             | β                                          | t-test p-value |
| Unadjusted (df = 32, 41)    | 0.476 3.060 0.004**                        | 0.333 2.264 0.029*                        | -0.084 -0.573 0.570 |
| Adjusted Model 1 (df = 28, 37) | 0.489 2.685 0.012*                         | 0.355 2.354 0.024*                        | 0.089 0.399 0.692 |
| Adjusted Model 2 (df = 29, 38) | 0.413 2.451 0.021*                         | 0.348 2.392 0.022*                        | 0.035 0.191 0.850 |
| Δ Maternal Depression (CES-D) | β t-test p-value                           | β                                          | t-test p-value |
| Unadjusted (df = 32, 41)    | 0.556 3.780 0.001**                        | 0.262 1.740 0.089†                        | -0.115 -0.743 0.462 |
| Adjusted Model 1 (df = 28, 37) | 0.606 3.497 0.002**                         | 0.268 1.731 0.092‡                        | -0.058 -0.244 0.809 |
| Adjusted Model 2 (df = 29, 38) | 0.567 3.457 0.002**                         | 0.264 1.708 0.096‡                        | -0.095 -0.500 0.620 |

Note: *p < 0.01, †p < 0.05, ‡p < 0.10. PTSD = Post-Traumatic Stress Disorder; DEP = Depression; TPSS = Total score; CES-D = Center for Epidemiologic Studies Depression Scale; df = degrees of freedom; Adj. = adjusted for covariates; B = β weight; Δ = change score. Separate linear regression models were fitted, each specifying the given biomarker (measured pre-treatment) as the independent variable and a mental health symptom change score as the outcome (change scores were computed as change pre-post CPP treatment, residualized to adjust for baseline and blom-transformed for normality). The positive standardized regression coefficients shown indicate that phenotypes with relatively greater M1-like polarization were prospectively associated with lesser reductions in maternal symptoms of PTSD and depression over the subsequent year of treatment with CPP. Adjusted model 1 includes the covariates: median age, BMI, and non-white Hispanic race/ethnicity and current antidepressant use. Adjusted model 2 includes socioeconomic status factors including: high school education, US born, and meeting the census criteria for family poverty. Degrees of freedom (df) are shown first for the M1/M2 phenotype, then for serum protein markers (IL-1β and CRP).

Fig. 1. Biomarkers of Inflammatory Balance Predicting Behavioral Treatment Response in Mothers. Note: *p < 0.01, †p < 0.05. The scatterplots depict the relationships between the pre-treatment levels of the maternal M1/M2 phenotype as a prospective predictor of changes (Δ) in maternal symptoms of PTSD (left) and depression (DEP, right) during behavioral treatment for trauma. The unadjusted linear regression coefficients and p-values are displayed above. Higher scores on the y-axis indicate poorer treatment response, as indexed per lesser decreases in symptoms over time. Symptom change indices are standardized residualized change scores, which adjust for pre-treatment symptom levels. The colors display concomitant levels of pre-treatment IL-1β protein (blom-transformed) in mothers (see Table 2 for full analyses).

per repeated measures ANOVA (Supplementary Table 2), with the respective effect sizes: -0.51 (PTSD) and -0.53 (depression). Changes in both PTSD and DEP remained significant, independent of one another. Further supporting the rationale for examining maternal biomarker predictors of child clinical outcomes and replicating prior findings (Hagan et al., 2017), this study observed that improvements in child symptoms of PTSD and DEP over treatment were significant predictors of improvements in symptoms of PTSD and DEP over treatment (p’s < 0.05, Supplementary Fig. 1).

Clinically relevant non-response in children, defined as meeting diagnostic criteria post-treatment, were 21% (genomics subsample) and 28% (serum protein sample) for child PTSD (see Table 5). Because the treatment was highly successful in terms of child depression, only two children (5%) were DEP non-responders, resulting in insufficient variance for analysis of this outcome.

3.3.2. Biomarker predictors of child symptom improvement

The M1/M2 Phenotype. The maternal M1/M2 phenotype exhibited a marginally significant trend toward predicting lesser reductions in child PTSD symptoms in unadjusted analyses (p=.054; Table 4; Fig. 4). This effect became significant after adjusting for maternal health covariates (p=.039), but was again marginal when adjusting for SES covariates in model 2 (p=.082). Maternal M1/M2 did not significantly predict treatment-related changes in children’s DEP symptoms (p’s > 0.10).

Pro-Inflammatory Proteins. As shown in Table 4, higher pre-treatment maternal IL-1β significantly predicted lesser reductions in child symptoms of PTSD (p=.004; see also Fig. 4) and DEP (p=.040), in the unadjusted analyses. The overall pattern of effects sizes and significance was similar in adjusted analyses. In contrast, higher maternal CRP levels at pre-treatment was marginally associated with less improvement in child symptoms of PTSD and DEP (p < .10) in some models; however, CRP was not consistently associated with either outcome in all unadjusted and adjusted models.

3.3.3. Biomarker predictors of child clinical non-response

To determine whether these biomarker predictions of child outcomes reached clinical significance, we conducted logistic regression analyses. As shown in Table 5, maternal serum IL-1β cytokine levels showed a non-significant trend toward predicting the child’s clinical non-response (p = 0.91). Although the M1/M2 phenotype was not significantly associated with child PTSD clinical response, to parallel the maternal models, we conducted a follow-up analysis to quantify model performance using the ROC-AUC. In line with what was found for maternal PTSD clinical response, pre-treatment maternal M1/M2 gene expression successfully discriminated their children’s likelihood of clinically significant PTSD response versus non-response (not meeting criteria for a diagnosis of PTSD) with an AUC of 74%, almost as high as the predictive value for mothers’ own responder status (AUC = 78%) (see Fig. 3). Inclusion of covariates did not change the overall pattern of results.

3.4. Exploratory follow-up and mechanistic analyses

Fig. 5 depicts a protein–protein interaction network of the M1/M2 genes that encode proteins (Cytoscape & STRING Application Program
Table 3
Pre-Treatment Biomarkers Predicting Maternal Non-Response.

| Pre-Treatment Biomarker | Clinical Non-Response, Maternal PTSD |
|------------------------|-------------------------------------|
|                        | B (SE) | Wald's χ² | p-value | OR | n (% Non-Response) |
| M1/M2-like Phenotype   | 1.213  | 5.927  | 0.015* | 3.364 | 12 of 34 (35%) |
| Serum                  | 0.254  | 0.631  | 0.427  | 1.289 | 17 of 43 (40%) |
| Interleukin-1β Reactive| −0.345 | 1.179  | 0.277  | 0.708 | 17 of 43 (40%) |
| Protein                | 0.318  | 1.179  | 0.277  | 0.708 | 17 of 43 (40%) |

M1/M2-like Phenotype: Clinical non-response (n = 22 (65%) vs. responders n = 20 (40%); χ² = 8.735, p = .006*).

Note: *p < 0.05, †p < 0.10. n (%) refers to the number of mothers deemed PTSD non-responders, per published validated cut-offs (see methods), using the PTSD Symptom Scale Interview (PSSI). Clinical depression non-response was not assessed due to insufficient variance (see results). Results were computed by separate logistic regression models, each specifying a biomarker as the independent variable and the dichotomous PTSD response/non-response variable as the outcome. All biomarkers were normalized prior to entry to facilitate statistical interpretability.

Pre-Treatment Biomarker | Clinical Non-Response, Maternal Depression |
|------------------------|------------------------------------------|
|                        | B (SE) | Wald's χ² | p-value | OR | n (% Non-Response) |
| M1/M2-like Phenotype   | 0.705  | 2.835  | 0.092  | 2.024 | 11 of 34 (32%) |
| Serum                  | 0.320  | 0.979  | 0.322  | 1.377 | 17 of 43 (40%) |
| Interleukin-1β Reactive| −0.300 | 0.903  | 0.342  | 0.741 | 17 of 43 (40%) |
| Protein                | 0.316  | 0.903  | 0.342  | 0.741 | 17 of 43 (40%) |

Note: *p < 0.05. Means and their standard errors are given (M ± SEM), with p-values derived from logistic regression analyses predicting clinical treatment response, using a critical alpha of 0.05. Maternal M1/M2 phenotypes were significantly higher among women who were classified as clinical non-responders per their PTSD diagnosis (0.351 ± 0.003; n = 22 (65%) compared to responders (0.341 ± 0.002; n = 12 (35%) (F(1, 32) = 5.927, p = .006*) in unadjusted analyses, and this difference remained significant when adjusting for all demographic, socioeconomic, and health covariates in sets 1 and 2 (see methods; Table 3).

HLA-DRB9. While acknowledging that these exploratory AUC values are overfitted, nonetheless, it is noteworthy that the 9-gene subset version of M1/M2 (compared to the full 23-gene set) produced a maternal non-response AUC of 83% (compared to 78%) and a child non-response AUC of 71% (compared to 74%), thereby assisting future investigations to optimize M1/M2 as a biomarker of treatment response.

Fig. 2. Pre-Treatment Immune Biomarkers as Predictors of Clinical Non-Response in Mothers. Note: *p < .05. Means and their standard errors are given (M ± SEM), with p-values derived from logistic regression analyses predicting clinical treatment response, using a critical alpha of 0.05. Maternal M1/M2 phenotypes were significantly higher among women who were classified as clinical non-responders per their PTSD diagnosis (0.351 ± 0.003; n = 22 (65%) compared to responders (0.341 ± 0.002; n = 12 (35%) (F(1, 32) = 5.927, p = .006*) in unadjusted analyses, and this difference remained significant when adjusting for all demographic, socioeconomic, and health covariates in sets 1 and 2 (see methods; Table 3).

HLA-DRB9. While acknowledging that these exploratory AUC values are overfitted, nonetheless, it is noteworthy that the 9-gene subset version of M1/M2 (compared to the full 23-gene set) produced a maternal non-response AUC of 83% (compared to 78%) and a child non-response AUC of 71% (compared to 74%), thereby assisting future investigations to optimize M1/M2 as a biomarker of treatment response.

4. Discussion

It is now recognized that the peripheral immune system can regulate brain, behavior, and mental health (Bechter et al., 2019; Miller and Raison, 2016; Wohleb, 2016). These transformative discoveries have opened the door to a new wave of Precision Psychiatry treatments, which either directly target the immune system or employ basal immune biomarkers to provide tailored pharmacotherapy (Kappelmann et al., 2016; Raison et al., 2013). Given that non-response of PTSD symptoms can be as high as 50% for leading behavioral and pharmaceutical treatments (Brady et al., 2000; Davidson et al., 2001; Kar, 2011; Steenkamp et al., 2015), advancements are needed. The current study identifies an immunogenomic signature, reflecting a relative imbalance of pro-inflammatory versus pro-resolving macrophage-associated gene expression, which predicts poorer recovery from symptoms of PTSD and depression, following engagement in an empirically-supported behavioral trauma treatment. This study also identified a similar pattern of findings with pre-treatment levels of the pro-inflammatory cytokine IL-1β, further reinforcing the role of systemic inflammation. Notably, the overarching pattern of these predictive biomarkers held after adjusting for demographic, health, and socioeconomic factors. As we have
therefore not driving these effects. Hence, this study symptoms in mothers or their children here (data not shown), and were outcome. The positive standardized regression coefficients shown indicate that maternal phenotypes with relatively greater M1-like polarization were prospectively.

Note: **

Pre-Treatment Maternal Immune Markers Predicting Child Symptom Improvement During Child-Parent Psychotherapy.

Table 4

| Treatment-Related Change (Δ) | Pre-Treatment Maternal M1/M2-like Phenotype | Pre-Treatment Maternal Interleukin-1β (IL-1β) | Pre-Treatment Maternal C-Reactive Protein (CRP) |
|-----------------------------|---------------------------------------------|---------------------------------------------|---------------------------------------------|
|                            | β   | t-test     | p-value | β   | t-test     | p-value | β   | t-test     | p-value |
| Δ Child PTSD (TSC)          |     |            |         |     |            |         |     |            |         |
| Unadjusted (df = 32, 41)    | 0.333 | 1.999     | 0.054   | 0.429 | 3.037     | 0.004** | 0.295 | 1.976     | 0.055   |
| Adjusted Model 1 (df = 28, 37) | 0.418 | 2.171     | 0.039*  | 0.418 | 2.959     | 0.005** | 0.339 | 1.515     | 0.138   |
| Adjusted Model 2 (df = 29, 38) | 0.330 | 1.799     | 0.082   | 0.397 | 2.800     | 0.008** | 0.348 | 1.969     | 0.056   |
| Δ Child Depression (TSC)    |     |            |         |     |            |         |     |            |         |
| Unadjusted (df = 32, 41)    | 0.189 | 1.073     | 0.291   | 0.318 | 2.125     | 0.040*  | 0.145 | 0.924     | 0.361   |
| Adjusted Model 1 (df = 28, 37) | 0.282 | 1.354     | 0.187   | 0.313 | 2.021     | 0.051†  | 0.406 | 1.762     | 0.087†  |
| Adjusted Model 2 (df = 29, 38) | 0.101 | 0.550     | 0.587   | 0.321 | 2.158     | 0.037*  | 0.322 | 1.784     | 0.083*  |

Note: **p < 0.01, *p < 0.05, |p < 0.10 TSC = Trauma Symptom Checklist for Young Children, PTSD and depression symptom subscales. Separate linear regression models were fitted, each specifying the given biomarker as the independent variable and a residualized mental health symptom change score (pre-post CPP) as the outcome. The positive standardized regression coefficients shown indicate that maternal phenotypes with relatively greater M1-like polarization were prospectively associated with lesser reductions in children’s symptoms of PTSD and depression over the subsequent year of treatment with CPP. Adjusted model 1 includes the covariates: maternal age, BMI, and non-white Hispanic race/ethnicity and current antidepressant use (n = 6). Adjusted model 2 includes socioeconomic status factors including: high school education, US born, and meeting the census criteria for family poverty. Degrees of freedom (df) are shown first for the M1/M2 phenotype, then for serum protein markers (IL-1β and CRP).
concomitant change in maternal symptoms over the same time period, such that the greater prevalence of red/orange colors toward the upper right corner reflects how maternal and child responses to treatment tracked together. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Importantly, these clinical results represent a translation of numerous prior animal studies of repeated social defeat, which have already highlighted a key role for social stress-induced expression of monocyte/macrophage and brain IL-18 expression, together with other IL-1 family receptors and antagonists, in prolonging fear expression and impairing fear extinction (Lisboa et al., 2018). We tested IL-1β in conjunction with the M1/M2 phenotype, because IL-1β is primarily secreted by monocyte/macrophages (Dinarello, 2018), and it amplifies the permeability of the blood brain barrier to permit monocyte infiltration into the central nervous system (Weber et al., 2017). Indeed, the protein–protein network analysis of the M1/M2 gene set with co-expression data from this study overlaid (Fig. 5) identifies the IL-1 receptor antagonist (IL-1RA, or IL1RN as the expressed gene), which blocks IL-1β signaling, as a central hub in the network. In sum, these findings point to a trauma-related inflammatory phenotype that appears to be more transdiagnostic and transtherapeutic than previously recognized.

Many women in this sample (53%) had elevated levels of CRP, a clinically meaningful pro-inflammatory biomarker. Specifically, 23% of women had basal levels of CRP at or above cut-offs associated with moderate cardiovascular risk (1–3 mg/L), and 30% had CRP levels consistent with high risk (>3 mg/L) (Myers et al., 2004). This observation was noteworthy, given the women’s relatively young age (M = 32 years) and the absence of major chronic disease or autoimmune diagnoses. CRP is strongly associated with BMI (Mora et al., 2006), and the average BMI of women in this study was overweight (ranging from normal to obese); nonetheless, predictive biomarker relationships remained significant when adjusting for BMI and other relevant covariates. Similarly, the sample was low-income and racially/ethnically diverse; yet, the biomarker predictions also held when controlling for socioeconomic factors. In contrast, the failure of CRP to significantly predict treatment response in this sample is clinically relevant, because previous studies of major depression have used high CRP levels as the primary biomarker to guide precision medicine treatment with TNF-α antagonists (Miller and Raison, 2016). Thus, this study’s findings suggest that CRP may not be a sufficiently sensitive biomarker to predict behavioral treatment response for this trauma-related phenotype.

Response rates to the leading empirically supported treatments, both pharmacological and behavioral, underscore the need for a Precision Medicine approach to augment efficacy. Prior RCTs of SSRIs, a first-line pharmacotherapy for PTSD, report non-response rates of 40–47% for sertraline versus 62–68% for placebo (Brady et al., 2000; Davidson et al.,

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### Table 5

| Pre-Treatment Biomarker | Clinical Non-Response, Child PTSD | B (SE) | Wald's χ² | p-value | OR (95% CI) | n (%) Non-Response |
|-------------------------|----------------------------------|--------|-----------|---------|------------|-------------------|
| M1/M2-like Phenotype    |                                  | 0.577  | 1.595     | 0.207   | 1.782      | 7 of 34 (21%)     |
| Serum                   |                                  | 0.638  | 2.861     | 0.091†  | 1.893      | 12 of 43 (28%)   |
| Interleukin-1β          |                                  | 0.357  | 1.043     | 0.307   | 1.429      | 12 of 43 (28%)   |

Note: *p ≤ 0.05, †p ≤ 0.10. n (%) refers to the number of children deemed PTSD non-responders, per published validated cut-offs (see methods), using the Trauma Symptom Checklist for Young Children (TSC). Clinical depression non-responders, per published validated cut-offs (see methods), using the Protein

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**Fig. 4.** Pre-Treatment Maternal Immune Biomarkers as Predictors of Child PTSD Symptom Improvement Across Treatment. Note: **p < .01, †p < .10. The scatterplots above depict the relationships between pre-treatment, maternal inflammatory biomarkers (IL-1β left and M1/M2 right) as predictors of changes (Δ) in child symptoms of PTSD, over roughly 9 months of behavioral treatment for trauma (details in Table 4). Higher scores on the y-axis indicate poorer treatment response, as indexed per lesser decreases in symptoms over time. Unadjusted regression coefficients and p-values are given at the top; Symptom change outcomes are standardized residualized change scores that adjust for pre-treatment symptom levels. Serum IL-1β levels are Blom-transformed to improve normality of the distribution. Colors display the network derived from Pearson correlations of the current study's data. Red paths indicate stronger positive correlations and blue paths indicate stronger negative correlations, while grey paths indicate the STRING-derived interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

**Fig. 5.** Protein-Protein Interaction Network of M1/M2 Genes with Sample Co-Expression Overlaid. Note: Protein-protein interactions amongst proteins encoded for by genes included in the M1/M2 signature, as modeled by CytoScape v.3.7.2 and the STRING database, with overlaid gene co-expression network derived from Pearson correlations of the current study’s data. Red paths indicate stronger positive correlations and blue paths indicate stronger negative correlations, while grey paths indicate the STRING-derived interactions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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**Table 5**

Pre-Treatment Biomarkers Predicting Child’s PTSD Non-Response.

| Pre-Treatment Biomarker | Clinical Non-Response, Child PTSD | B (SE) | Wald's χ² | p-value | OR (95% CI) | n (%) Non-Response |
|-------------------------|----------------------------------|--------|-----------|---------|------------|-------------------|
| M1/M2-like Phenotype    |                                  | 0.577  | 1.595     | 0.207   | 1.782      | 7 of 34 (21%)     |
| Serum                   |                                  | 0.638  | 2.861     | 0.091†  | 1.893      | 12 of 43 (28%)   |
| Interleukin-1β          |                                  | 0.357  | 1.043     | 0.307   | 1.429      | 12 of 43 (28%)   |
In contrast, in the current study, women participating in CPP exhibited a 36% non-response rate for PTSD symptom reduction, when using comparable criteria for calculation of non-response as in the SSRI studies. Hence, pre-treatment biomarkers of non-response are needed, in order to target these individuals for adjunctive interventions, whether pharmacologic, behavioral, or both. As these data demonstrate, immunophenotyping may have promise for guiding Precision Psychiatry in PTSD and trauma, but we have to “look beyond the streetlight” of the few popular pro-inflammatory cytokines (Bush and Aschbacher, 2020).

Building on evidence from animal models, these findings advance translational medicine in the neuroimmunology of traumatic stress in humans and its treatment. Animal models of repeated social defeat have highlighted the central role of monocytes, whereby stress exposure causes monocytes to traffic to the brain, elicit neuroinflammation, and amplify PTSD-like behaviors (Weber et al., 2017). In socially agressed mice, monocytes upregulate expression of M1-associated genes (IL-1β, TNF-α, and TLRs), which render monocytes “primed,” or more reactive to secrete high levels of inflammatory cytokines upon exposure to future stressors (Weber et al., 2017), both psychological and immunological (Fleshner et al., 2017). In animal models, an M1-like polarization imbalance in meningeal macrophages is associated with deficits in learning and memory (Derecki et al., 2011). Moreover, these deficits were successfully reversed by treatment with monocytes treated ex-vivo to acquire an M2-like phenotype and intravenously re-injected (Derecki et al., 2011). Such evidence suggests the possibility that monocyte phenotypes may have a causal role, which could be therapeutically exploited.

Three prior studies of Cognitive Behavioral Therapy for major depression (Lopresti, 2017) and one exercise intervention for the treatment major depression (Rethorst et al., 2013) have reported that basal inflammatory markers predicted poorer treatment response. However, this is the first study to our knowledge to demonstrate such findings in relation to decreases in symptoms of PTSD in response to behavioral therapy. Collectively, the results of these studies invite the question of why inflammation is associated with poorer behavioral treatment outcomes. While future studies are needed to fully address these questions, it is noteworthy that M1-associated inflammation, and the relative deficit of M2-associated anti-inflammatory and pro-resolving functions (anti-inflammatory cytokines, T-cell interactions, autophagy/mitophagy, scavenging) have adverse effects on neuroplasticity, neurorepair and learning (Diniz et al., 2019; Hu et al., 2015). It is conceivable that either the presence of inflammation or the lack of M2-associated pro-resolving functions could aggravate cognitive processes that underlie PTSD symptoms (e.g., overgeneralization, attentional threat biases) or could interfere with acquisition of new skills and learning processes (e.g., fear extinction). In sum, future research should examine the links between inflammation and neuroplasticity with regards to the core behavioral skills that mediate behavior change efficacy.

If replicated, the present findings may contribute to a paradigm shift in the understanding and treatment of the inflammatory phenotype of PTSD and depressive symptoms. Specifically, a recent review of treatments for the inflammatory phenotype of depression reported significant symptom reductions with TNF-α antagonists (Raison et al., 2013). However, we caution that in this current study, the findings are driven by the concomitant expression of genomic markers that reflect both inflammation and immunosuppression. In other words, the signature was not “purely” pro-inflammatory. For example, we found that relatively lower CD14+ monocytic expression of HLA-DR genes were key drivers in the prediction of maternal PTSD treatment non-response (of note, HLA-DR mRNA is associated with protein expression (Pachot et al., 2005)). CD14+ HLA-DR<sup>BW</sup> cells have been described as immunosuppressive monocytes, in part, due to their capacity to diminish adaptive immunity and suppress T cells (Mengos et al., 2019). Higher CD14+ HLA-DR<sup>BW</sup> counts predict poor outcomes in sepsis, and are associated with poorer response to various therapeutics, including cancer vaccines, hematopoietic stem cell transplantation, surgical recovery and COVID-19 outcomes (Mengos et al., 2019; Benlyamani et al., 2020). This potential for immunosuppression may be a crucial observation because it is not known whether prescribing TNF-α antagonists (Raison et al., 2013), which would further suppress the immune system, could be harmful to individuals exhibiting immunosuppressive biomarkers (e.g., highly traumatized women or those with early life adversity (Aschbacher et al., 2021)). In sum, precision medicine trials of immunomodulatory drugs for mental health indications should assess exposure to interpersonal violence and employ deeper immunophenotyping to quantify immunosuppressive markers.

This study has several limitations. First, the lack of a control group limits certainty about whether some symptom changes may have occurred without treatment. Nonetheless, as multiple RCTs have validated the efficacy of CPP relative to rigorous control or comparison groups (Cicchetti et al., 2006; Cicchetti et al., 2000; Lieberman et al., 2005; Lieberman et al., 1991; Toth et al., 2006; Toth et al., 2002), it was unethical to randomize a vulnerable patient group to a no-treatment condition or a treatment known to be less effective than CPP. Thankfully, evidence suggests that these results are not explained by naturalistic change. The effect size of the change over time during the course of treatment was large, exceeding r = 0.80. In contrast, a prior RCT that computed the effect size relative to a control group, but also utilized a less powerful research design in some respects (i.e., less experienced clinicians and a non-specific measure of maternal distress), found the relative effect size of CPP was d = 0.38 (Lieberman et al., 2006). Hence, it has already been established that CPP results in significant effects relative to a control condition, and the strong effect sizes in this sample are therefore highly unlikely to be attributable solely to naturalistic change, placebo, or non-specific effects.

Although a consideration herein and for future inquiries, the fact that associations between basal immune markers and symptom trajectories remained significant when adjusting for antidepressant use by a handful of women (n = 6) shows that antidepressant use does not explain these results. In terms of generalizability, this study cannot establish whether or not these results would apply to other, non-IPV forms of trauma, or to...
men. The fact that the sample was highly traumatized, low-income, and diverse could conceivably have made these effects easier to detect in this sample. Nonetheless, with IPV affecting an estimated 1 in every 3 women in the world (World Health Organization, 2013), and approximately 70.8 million displaced individuals fleeing trauma and violence (The UN Refugee Agency, n.d.), these results arguably apply to a substantial portion of the adult population and to their children. That being said, these results may not apply to other types of PTSD, such as that resulting from combat-exposure. In addition, we were not able to ascertain the potential role of hormonal contraception use within this sample, which may be a factor for future consideration. The present RNA analyses focused on specific biological hypotheses regarding M1/M2 biology and were not powered for hypothesis-free exploratory/discovery analyses. As such, other biological pathways may well also associate with treatment responses, and future research in larger samples will be required to identify such results. Finally, we acknowledge that the M1 and M2 phenotypes represent more of a spectrum than discrete and defined categories (Murray et al., 2014), it is unclear whether these results reflect co-expression of M1 and M2-like markers within single cells versus alterations of subpopulations (Kim et al., 2016), and that the in vivo central nervous system expression and functional relevance of M1 and M2-like phenotypes remains an active area of scientific discovery. Given this study’s assessment of gene expression by RNA-seq, confirmation by other RNA assay methods would be ideal and future studies could potentially extend these findings using focused RT-PCR to assess M1 and M2 indicator genes (although such analyses would be more expensive than RNA seq given the total number of indicator genes involved and complicated by the normalization requirements of RT-PCR). Future studies should assess LPS-stimulated rather than basal IL-16 values to obtain more reliable measurements; nonetheless, this study’s results reinforce the importance of the IL-1 family pathway, particularly in context with the M1/M2 network results in Fig. 5.

When the body is sick or inflamed, the brain knows. Subsequent to sensing peripheral inflammatory inputs, the central nervous system acts to influence behaviors relevant to safety, social bonding, and survival. Over the past decade, our awakening recognition of these intertwined connections among the immune system, brain function, and mental health has fueled a transformation in Psychiatry. The current study’s findings show that inflammatory biomarkers have broader relevance as predictors of treatment response than previously recognized, not only in depression but in PTSD and trauma-exposed individuals, and not only in response to antidepressants, but in response to behavioral treatments. These genomic signatures implicate an imbalance in the homeostatic regulation of inflammation, the prognostic value of which cannot be captured solely through current clinical biomarkers such as CRP. Deeper phenotyping of both the immune system and trauma exposures may hold the key for Precision Psychiatry treatments, which redefine trauma-related symptoms of depression and PTSD as a transdiagnostic neuro-inflammatory phenotype, and call for treating it as such.

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Author Contributions

K.A. conceived of the presented idea, took the lead in writing and revising the manuscript, conducted the statistical analyses, and programmed visualizations and data science components. N.B. initiated, carried out, and supervised the intervention biomarker study, and contributed to the integrative theory, writing, and editing of the manuscript. S.C. supervised the RNA-seq assays and provided intellectual guidance in analyses. A.B. prepared the RNA-seq transcriptomic data for analyses and wrote the related methods. A.L. developed and led the clinical research intervention and supervised the clinicians. M.H. and L.R. assisted with the clinical research program, clinical data management, and helped write the associated methods. S.C., O.W., A.L., M.H., and L.R. provided critical feedback on the manuscript.

Data and Materials Availability

FASTQ files are available from the NCBI Sequence Read Archive (SRA) under accession number BioProject PRJNA626346.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.07.012.

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