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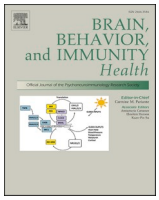

Immunological correlates of suicidality among adolescents with internalizing symptoms

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ABSTRACT

Background: Suicide is a leading cause of death in adolescents and young adults globally. Well-established risk factors for suicide are depression and past suicide attempts. People experiencing suicidality may represent a distinct neurobiological group of people with depression. Because converging evidence has implicated inflammation in depression, we sought to investigate relationships between suicidality and immune markers in youth experiencing diverse mood and anxiety symptoms. We hypothesized that adolescents with suicidality would exhibit a unique immune signature.

Methods: Adolescents underwent semi-structured interviews and completed self-reported measures to assess psychopathology, including suicidality (suicidal ideation, plans, or attempts). Fasting blood samples were collected, cultured with and without lipopolysaccharide (LPS) to stimulate an inflammatory response, and analyzed for 41 immune analytes. To assess how immune function related to suicidality categorically and dimensionally, we conducted group comparisons and correlations while controlling for multiple comparisons using false discovery rate (FDR). To further uncover subtle immune-suicidality relationships, we employed a data-driven approach using factor analysis to extract major immune factors, each of which was subsequently correlated with suicidality measures.

Results: Among 126 participants, 29 were healthy controls and 97 participants had internalizing symptoms; within the clinical group, 57 experienced suicidality. Three immune analytes differed between healthy controls, suicidal, and non-suicidal adolescents with internalizing symptoms in the LPS condition: Flt-3L ($p_{FDR} = 0.0246$), GM-CSF ($p_{FDR} = 0.0246$), and IFN-γ ($p_{FDR} = 0.0246$). These analytes were negatively correlated with the Beck Scale for Suicide Ideation (BSSI): Flt-3L ($\rho = -0.19$, $p = 0.04$); GM-CSF ($\rho = -0.26$, $p = 0.004$); IFN-γ ($\rho = -0.33$, $p = 0.0003$). GM-CSF also negatively correlated with number of suicide attempts ($\rho = -0.39$, $p = 0.003$). Factor analysis reduced 41 analytes to several common immune factors across experimental conditions, with Flt-3L, GM-CSF, and IFN-γ all loading heavily onto immune factors that were hypoactive in suicidality. Through this data-driven approach, we detected further associations between suicidality and immune factors across all conditions.

Conclusions: Peripheral immune function may be distinctly altered in adolescent suicidality. Future work should examine immune-suicidality relationships longitudinally.

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1. Introduction

For adolescents, suicide is among the most serious health problems (Curtin and Garnett, 2023). Based on the World Health Organization's reporting, suicide is a leading cause of death in adolescents and young adults ages 10-19 (World Health Organization, 2023). In the United States, where our study took place, suicide was the second leading cause of death among adolescents and young adults ages 10–24. (Center for Disease Control, 2023). Despite suicide's massive disease burden, its etiology remains elusive. This lack of predictability presents a major barrier for identification and prevention of suicidal behavior. Importantly, over 80% of suicidal youth present with depressive symptoms at the time of the attempt. However, only 30% of depressed youth will ever attempt suicide (Cash and Bridge, 2009). Some of the most reliable predictors of a future suicide attempt among depressed individuals are a past attempt, suicidal ideation, and non-suicidal self-injury (Lewinsohn et al., 1994; Mars et al., 2019; May and Klonsky, 2016; Ribeiro et al., 2016; Shaffer et al., 1996; Wong et al., 2008), suggesting that those at risk for suicide may represent a distinct neurobiological group.

One possible pathway contributing to suicidality may be immune system activation. Mounting evidence suggests that the immune system may be implicated in psychiatric conditions like depression (Shaffer et al., 1996; Bradley et al., 2015, 2019; Dantzer et al., 2008; Ely et al., 2021; Gabbay et al., 2010, 2012; Miller and Raison, 2016; Réus et al., 2015; Zunszain et al., 2013). Previous work has also endorsed the possibility of a unique relationship between suicidality and the immune system. For example, acute suicidality, defined as suicidal ideation or behavior, has been reported in non-psychiatric patients treated with immunological mediators (e.g., interferons) for melanoma and Hepatitis C (Pandey, 2015). Supporting this observation, a meta-analysis reported higher levels of pro-inflammatory cytokines in the cerebrospinal fluid and plasma of people with suicidality (Ducasse et al., 2015). Further, several studies have also found that certain cytokines, such as IL-2, TNF-α, and IL-6 differ between suicidal patients, non-suicidal patients, and healthy controls (Ducasse et al., 2015; Janelidze et al., 2011; Serafini et al., 2020). However, it is inconsistent whether cytokine levels are elevated or deficient among people with more severe suicidality.

Work on adolescents specifically is sparse but critical because adolescence is a vulnerable period with high suicide risk (Shaffer et al., 1996). Adolescents experience heightened stress (e.g., changing biology and social networks) that may induce inflammation (Clayton et al., 2023; Miller and Prinstein, 2019). As such, findings in adults may not translate to youth. Our group previously reported that suicidal adolescents with major depressive disorder (MDD) had lower levels of plasma TNF-α compared to non-suicidal adolescents with MDD. Conversely, elevated IFN-γ was found to similar degrees in both suicidal and non-suicidal adolescents with MDD compared to controls (Gabbay et al., 2009a). Additionally, we found that levels of metabolites from the neuroimmunological kynurenine pathway differed between depressed suicidal adolescents, depressed non-suicidal adolescents, and healthy controls (Bradley et al., 2015), indicating unique immune profiles. Our finding of decreased peripheral TNF-α in suicidal adolescents conflicts with much of the literature in adult cohorts, supporting a potential difference in the cytokine profile of suicidal teenagers that is critical to examine. One study has also reported elevated TNF-α, IL-1β, and IL-6 in the brains of teenagers who died by suicide vs. other causes of death (Pandey et al., 2012), highlighting an ongoing need for larger replication samples.

Here, we sought to expand upon others and our previous research and examine immune profiles in a cohort of adolescents focusing on 41 immune mediators and using data-driven analytical approaches. Unlike other studies to date, our immune approach examined immune responses to toll-like receptor 4 (TLR4) agonist lipopolysaccharide (LPS), a well-established and characterized immune trigger, using an *in vitro* model with whole blood from participants cultured with and without LPS. The immunological challenge with LPS is an established method and has been utilized in investigations of psychiatric populations (Lacosta et al., 1999; Linthorst and Reul, 1998; Lu et al., 2008). This method complements measuring analytes in a classic approach as it assesses immune function in response to stress in addition to capturing baseline immune profiles (Freed et al., 2019).

As done in our previous studies, we adopted an NIH Research Domain Criteria (RDoC) framework including adolescents with a range of internalizing (mood and anxiety) symptoms rather than limiting our enrollment to those who met categorical diagnostic criteria for MDD (Gabbay et al., 2015; Henderson et al., 2013) as well as healthy controls. We hypothesized that: a) specific immune profiles would be associated with suicidality, assessed both categorically and dimensionally, across three groups (clinical with suicidality, clinical without suicidality, and healthy controls) and b) suicidality would be associated with dysregulated immune function.

2. Materials and methods

2.1. Participants

We recruited adolescents, ages 12–20, in the New York City area from affiliated hospitals and social media advertisements. Participants were divided into three analysis groups: (1) a clinical group (i.e., had a current psychiatric diagnosis or endorsement of mood and anxiety symptoms on assessments) with history of suicidality (suicidal ideation, plans, or attempts), (2) a clinical group with no history of suicidality, and (3) a healthy control (HC) group with no psychiatric history. To ensure participants were medically healthy, medical assessments and laboratory tests (complete blood count, metabolic panel, liver function, thyroid function) were conducted. Participants also had a urine toxicology test and, in biological females, a pregnancy test.

Exclusion criteria for all participants were a) immune-affecting medications taken in the past six months, b) any immunological or hematological disorder, c) chronic fatigue syndrome, d) any infectious disease in the month prior, e) significant medical or neurological disorders, f) developmental, bipolar spectrum, or psychotic disorders, g) substance use disorder or a positive urine toxicology test, h) a positive urine pregnancy test, and i) use of psychotropic medications at least one month prior to blood collection (or 3 months for drugs with a longer half-life such as fluoxetine). For participants over 18, informed consent was obtained. Those under 18 provided assent, and a parent or guardian provided consent. All procedures were approved by the Institutional Review Boards of the affiliated institutions. Some data for the study were previously reported in prior work (Freed et al., 2019; Nguyen et al., 2022) but are used in the context of new analyses in the current report.

2.2. Clinical evaluations and assessments

Clinician-administered procedures: A licensed clinician administered the Kiddie Schedule for Affective Disorders and Schizophrenia—Present and Lifetime Version for Children (K-SADS (Kaufman et al., 1997)), a semi-structured psychiatric diagnostic interview, as well as the Children's Depression Rating Scale-Revised (CDRS-R; ranges from 17 to 113 with higher scores indicating greater depressive symptoms (Poznanski et al., 1984)) to the participant and a parent. Both interviews were used to generate a comprehensive report.

Self-report measures: *Suicidality*: The Beck Scale for Suicide Ideation (BSSI; ranges from 0 to 38 (Beck and Steer, 1991)) is a self-report measure consisting of 19 items (each scored 0, 1, or 2 with higher values indicating greater degrees of suicidality) that was used to characterize suicidal thoughts and behaviors over the past week. All individuals completed the first five screening items, with those scoring above a 0 on any screening item directed to complete the additional 14 follow-up items.

Mood and anxiety symptoms: Depression severity was assessed with the self-rated Beck Depression Inventory, 2nd edition (BDI; scores range

from 0 to 63; (Beck et al., 1987)). Anxiety was assessed by the self-rated Multidimensional Anxiety Scale (MASC; ranges from 0 to 117; (March et al., 1999)). Additionally, anhedonia severity was assessed using the Snaith-Hamilton Pleasure Scale (SHAPS; ranges from 14 to 56; (Snaith et al., 1995)) as our previous findings linked suicidality to anhedonia severity (Ely et al., 2021; Gabbay et al., 2015).

2.2.1. Immunological procedures and measurements

Participants underwent a fasting blood draw in the morning to address the diurnal variability of cytokine production (Galbo and Kall, 2016; Nilsonne et al., 2016). Laboratory staff were blinded to participants' clinical status. As we sought to assess participants' functional immune responses, we modeled each participant's immune profiles under two conditions using *in vitro* experiments. Whole blood samples were cultured on RPMI Medium 1640 (1x) with L-Glutamine, Cat# 11, 875–093 (Invitrogen, Waltham, MA) both with and without LPS at the final concentration 0.1 μg/ml at 37 °C in a 5% CO_2 tissue culture incubator. We utilized LPS since it is a well-established and well-characterized immune trigger (van Eeden et al., 2020). Samples cultured on standard growth medium alone served as our baseline ("Medium") condition, while samples cultured on standard growth medium plus LPS served as our immune-activation ("LPS") condition. The cultured whole blood samples were centrifuged after 6 h of incubation. Supernatant was collected and stored at −20 °C. Using a bead-based Luminex-200 system and the XMap Platform (Luminex Corporation, Austin, TX), levels of 41 immune analytes were measured. Selection of these 41 analytes was based on available panels at the Human Immune Monitoring Center at Mount Sinai, which were determined by their team of immunologists. The panel used was the multiplex panel (MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel - Premixed 41 Plex, Millipore Corp., Burlington, MA). See Supplementary Table 1 for additional information on immune analytes.

Median fluorescence intensity (MFI) values were measured in duplicate wells for each of the two peripheral whole blood culture growth conditions (i.e., 2 × Medium and 2 × LPS samples per subject). Duplicate MFI values for each participant/condition were then averaged for use in analyses, as this approach optimizes statistical power (Nguyen et al., 2022; Breen et al., 2015, 2016). For detailed immunological procedures, see Nguyen et al., 2022.

2.2.2. Statistical analyses

Participant characteristics including demographics, assessments, and diagnoses were summarized for the analysis groups. Continuous variables were compared between groups using a Kruskal-Wallis test (Wilcoxon rank sum tests for pairwise comparison) while categorical variables were compared using Pearson's chi-squared test or Fisher's exact test when the frequency count in cells were ≤5.

We examined immune analytes under the two experimental conditions, Medium and LPS. We also examined the ratio of LPS/Medium to reflect relative changes induced by the inflammatory condition. Immune analytes were summarized and compared among the three participant groups (clinical with suicidality, clinical without suicidality, and HC) using Kruskal-Wallis tests. Multiple comparisons were corrected for using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995). Partial Spearman correlations adjusting for age, sex, and body mass index (BMI) were used to assess associations of each immune analyte with BSSI and number of suicide attempts. Analyses were done separately for each immune condition.

As there is growing evidence that combinations of immune markers, rather than individual analytes, act simultaneously in psychopathology contexts (Audet and Anisman, 2013; Himmerich et al., 2019), we also used factor analysis to further explore the relationship between suicidality and immune interactions. Factor analysis is a data-driven technique, allowing us to identify groups of immune proteins that vary together. Additionally, factor analysis reduces the dimensionality of complex, multidimensional data, like our panel of 41 immune analytes, improving sensitivity following multiple comparison correction. Using factor analysis with "varimax" rotation, we extracted the factors explaining most of the variance across the whole sample for each of the three immune conditions. Factors explaining greater than 5% of variance were used for subsequent analyses. Immune factors were compared between analysis groups in each immune condition adjusting for age, sex, and BMI. To further examine associations between immune function and suicidality severity across a continuum, immune factors were correlated with BSSI and number of suicide attempts using Spearman partial correlations to control for age, sex, and BMI. For the 41 immune analytes of 126 individuals from both the Medium and LPS conditions (10,332 data points), we only had 19 missing data points, accounting for <0.2% of all the data.

All analyses were conducted in the statistical software SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Significance was set at FDR-corrected, two-tailed $p < 0.05$.

3. Results

3.1. Participant characteristics

The analysis sample included 126 participants: 57 in the clinical group with suicidality, 40 in the clinical group without suicidality, and 29 HC. Within the clinical group with suicidality, 25 participants had attempted suicide at least once (see distribution in Table 1).

Demographic characteristics between participant groups were similar. As expected, HCs significantly differed from the clinical group in their self-reported symptoms. Groupwise comparisons showed that the three groups significantly differ in levels of suicidality, depression, anxiety, and anhedonia. Participants in the clinical group with suicidality had depressive disorder diagnoses more often than those in the clinical group without suicidality ($p = 0.0006$; Table 1), while the other psychiatric diagnoses were not statistically different between the two groups.

3.2. Statistical analyses of individual immune analytes

Group Comparisons: As shown in Supplementary Table 2 and in the LPS (immune triggered) condition, FDR-corrected Kruskal-Wallis tests indicated that levels of three immune analytes differed significantly across the three groups: Flt-3L ($p_{FDR} = 0.0246$), GM-CSF ($p_{FDR} = 0.0246$), and IFN-γ ($p_{FDR} = 0.0246$). No immune analytes significantly differed by group in the Medium or LPS/Medium conditions.

Post Hoc Pairwise Comparisons (LPS condition only): Also shown in Supplementary Table 2, the clinical group with suicidality expressed significantly lower levels of Flt-3L, GM-CSF, and IFN-γ than the clinical group without suicidality as well as significantly lower levels of IFN-γ than the HC group. Additionally, the clinical group without suicidality had elevated GM-CSF compared to HC. All other pairwise comparisons were non-significant.

Correlations: Associations between LPS-stimulated Flt-3L, GM-CSF, and IFN-γ levels and suicidality measures were examined using partial Spearman correlations controlled for age, sex, and BMI. In the whole sample ($N = 126$), suicidality severity (BSSI) negatively correlated with Flt-3L ($\rho = -0.19$, $p_{FDR} = 0.04$), GM-CSF ($\rho = -0.26$, $p_{FDR} = 0.004$), and IFN-γ ($\rho = -0.33$, $p_{FDR} = 0.0003$). Similarly, in the combined clinical sample with and without suicidality ($n = 97$), BSSI negatively correlated with Flt-3L ($\rho = -0.30$, $p_{FDR} = 0.006$), GM-CSF ($\rho = -0.40$, $p_{FDR} < 0.0001$), and IFN-γ ($\rho = -0.38$, $p_{FDR} = 0.0002$). Within the clinical group with suicidality, GM-CSF negatively correlated with number of suicide attempts ($\rho = -0.39$, $p_{FDR} = 0.003$; Fig. 1). No significant correlations were observed between suicidality measures and immune analytes in the Medium or LPS/Medium conditions.

Table 1
Participant characteristics for three participant groups.

Measure	Clinical with Suicidality (n = 57)	Clinical without Suicidality (n = 40)	Healthy Control (n = 29)	p[a]
Demographic Characteristics				
Age, years, mean (SD), [range]	15.0 (2.1) [12, 20]	15.1 (2.0) [12, 19]	14.8 (2.3) [12, 20]	0.91
Female, n (%)	40 (70.2)	24 (60.0)	15 (51.7)	0.23
Race/ethnicity, n (%)				0.12
Hispanic	26 (45.6)	23 (57.5)	7 (24.1)	
Non-Hispanic Black	16 (28.1)	8 (20.0)	9 (31.0)	
Non-Hispanic White	10 (17.5)	6 (15.0)	11 (37.9)	
Non-Hispanic Other	5 (8.8)	3 (7.5)	2 (6.9)	
BMI, kg/m^2, mean (SD), [range]	25.1 (6.4) [16.7, 43.6]	25.1 (6.7) [15.9, 44.8]	23.1 (5.5) [15.3, 34]	0.41
Clinical Characteristics				
Symptom scales, mean (SD), [range]				
BSSI	6.8 (9.3) [0, 35]	0.7 (2.2) [0, 10]	0	<0.0001
BDI	23.7 (14.5) [0, 50]	10.7 (7.7) [0, 29]	2.1 (2.7) [0, 10]	<0.0001
CDRS-R	44.1 (15.1) [18, 85]	33.9 (11.6) [17, 53]	18.3 (1.5) [17, 22]	<0.0001
MASC	51.1 (20.3) [13, 107]	45.6 (18.3) [11, 99]	28.2 (13.0) [2, 57]	<0.0001
SHAPS	26.7 (6.6) [14, 43]	22.2 (5.3) [14, 36]	19.6 (5.4) [14, 34]	<0.0001
Number of suicide attempts, n (%)				
0	32 (56.14)	–	–	
1	13 (22.81)	–	–	
2	6 (10.53)	–	–	
3	2 (3.51)	–	–	
4	2 (3.51)	–	–	
5	2 (3.51)	–	–	
Psychiatric conditions[b], n (%)				
Depressive Disorders	55 (96.5)	28 (70.0)	–	0.0006
Anxiety Disorders	41 (71.9)	26 (65.0)	–	0.47
PTSD	12 (21.1)	3 (7.5)	–	0.09
ADHD	19 (33.3)	11 (27.5)	–	0.54
ODD	5 (8.8)	9 (22.5)	–	0.08
OCD	5 (8.8)	4 (10.0)	–	>0.99

[a] Continuous variables were compared between groups using a Kruskal-Wallis test (Wilcoxon rank sum tests for pairwise comparison) while categorical variables were compared using Pearson's chi-squared test or Fisher's exact test when the frequency count in any cell was ≤5.

[b] Depressive Disorders = DSM diagnoses of Disruptive Mood Dysregulation Disorder, Major Depressive Disorder, Persistent Depressive Disorder, and Other Specified or Unspecified Depressive Disorder. Anxiety Disorders = DSM diagnoses of Generalized Anxiety Disorder, Social Phobia, Specific Phobia, and Anxiety Disorder Not Otherwise Specified. ADHD = Attention-Deficit/Hyperactivity Disorder. ODD= Oppositional Defiant Disorder. OCD = Obsessive-Compulsive Disorder.

3.3. Suicidality trait vs. state

To examine whether a potential relationship between suicide and cytokines would be trait-based or state-based, we compared immune analyte concentrations of participants with current versus past suicidality, elicited from the BSSI and clinician interviews, across all immune conditions. We found no difference in these two subgroups.

3.4. Factor analysis

As shown in Fig. 2, in the Medium condition, factor analysis yielded 6 factors explaining 83% of the variance. In the LPS condition, 5 factors were obtained and explained 72% of total variance. In the LPS/Medium condition, 5 factors were extracted, explaining 63% of total variance. For numeric values, see Supplementary Table 3.

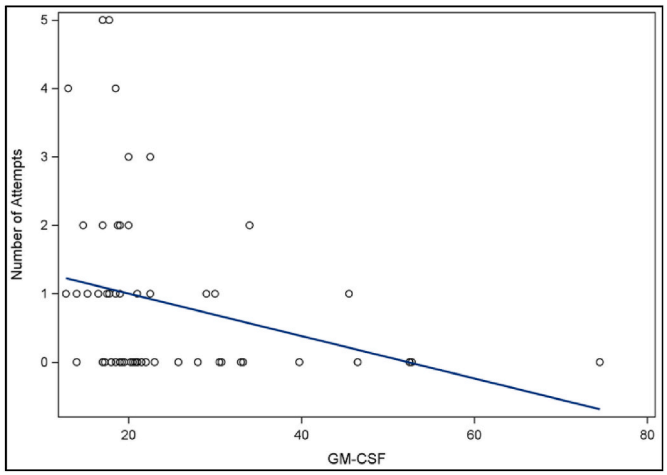

Fig. 1. Correlation between number of suicide attempts and LPS-stimulated GM-CSF level in the clinical group with suicidality.

Immune Factor Group Comparisons: In the Medium condition, group status was not associated with any factors. In the LPS condition, clinical participants with suicidality expressed lower Factor 2 scores relative to those without suicidality ($p = 0.002$). In the LPS/Medium condition, clinical participants with suicidality differed from clinical participants without suicidality by lower Factor 1 scores ($p = 0.0043$) and Factor 4 scores ($p = 0.0091$). Clinical participants with suicidality differed from HCs by decreased Factor 1 scores ($p = 0.0346$) and increased Factor 5 scores ($p = 0.0044$).

Immune Factors Correlations: In the Medium condition, Factor 1 was negatively associated with number of suicide attempts in the clinical group with suicidality ($\rho = -0.295$, $p = 0.0276$). In the LPS condition, number of suicide attempts in the clinical group with suicidality was negatively associated with Factor 2 ($\rho = -0.300$, $p = 0.0245$) and Factor 5 ($\rho = -0.266$, $p = 0.048$). In the LPS/Medium condition, suicidality severity (BSSI) across the whole sample was negatively associated with Factor 1 ($\rho = -0.221$, $p = 0.015$) but positively associated with Factor 5 ($\rho = 0.186$, $p = 0.04$). For the combined clinical group in the LPS/Medium condition, Factor 1 was negatively associated with BSSI ($\rho = -0.219$, $p = 0.036$). Finally, for the clinical group with suicidality in the LPS/Medium condition, Factor 3 was positively associated with number of suicide attempts ($\rho = 0.271$, $p = 0.047$). See Supplementary Table 4.

4. Discussion

4.1. Adolescents with suicidality showed differential immunological profiles

The present study investigated the relationships between suicidality and 41 immune analytes in 126 adolescents with mood and anxiety conditions. Supporting our hypothesis, we detected lower levels of LPS-stimulated Flt-3L, GM-CSF, and IFN-γ in clinical participants with suicidality relative to those without. These analytes were inversely correlated with self-reported suicidality severity and number of suicide attempts.

In addition, factor analysis revealed stable groupings of analytes that function together. Interestingly, all but two factors that had significant associations with suicidality had strong loadings of the three analytes we uncovered with traditional statistical tests. The presence of these analytes within such factors emphasizes their consistent contribution to suicidality. These findings also highlight the complexity of immunological mechanisms underlying suicidality. Further, while traditional statistical methods showed differences in only the LPS condition, factor

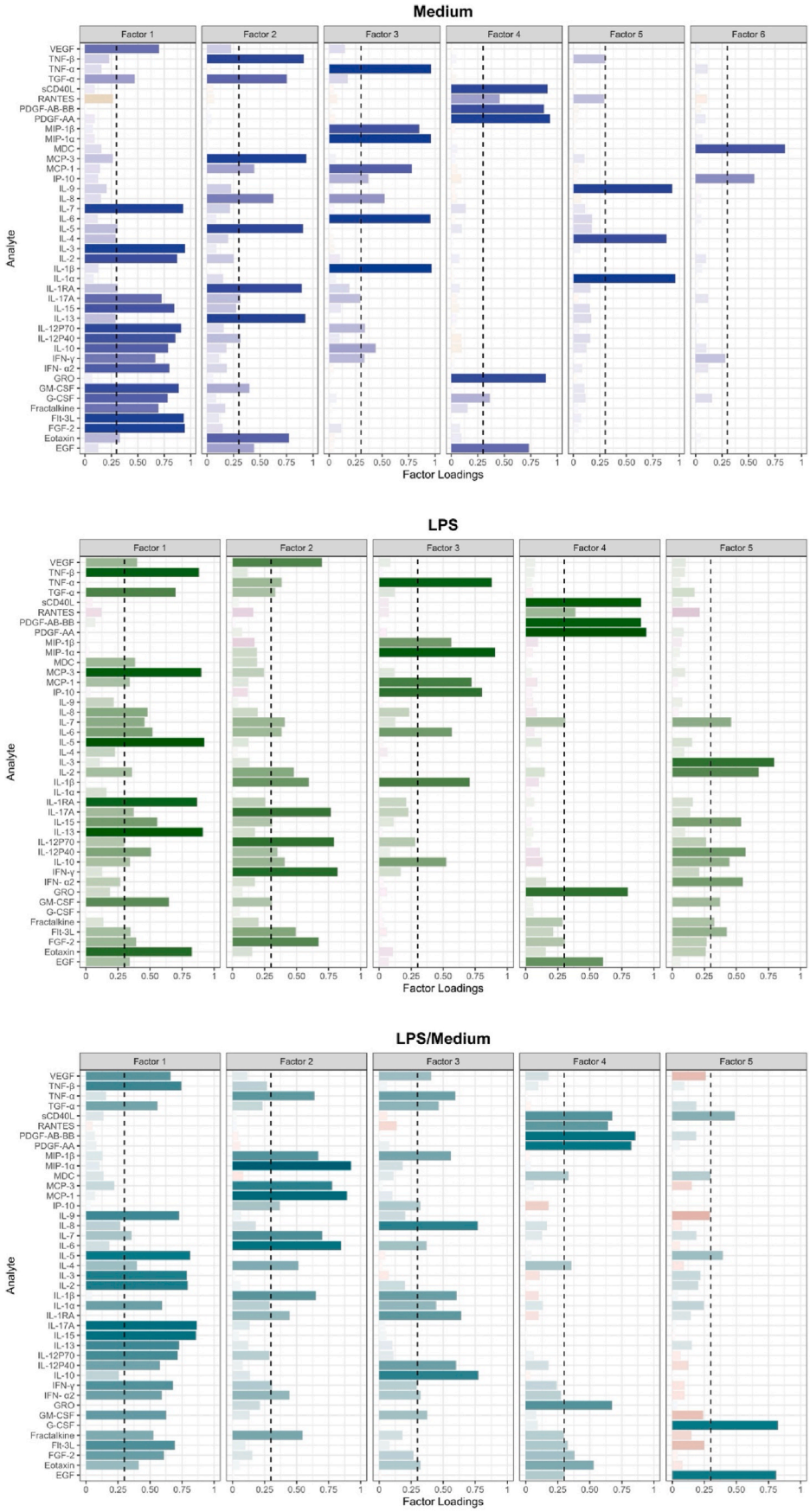

Fig. 2. Loadings for each factor across all immune conditions.

analyses detected associations with suicidality across all immune conditions, underscoring nuances of immune interactions and the enhanced sensitivity of this data-driven approach.

4.2. Inflammatory dysfunction in suicidality

After correcting for multiple comparisons, group differences in levels of individual immune analytes were only significant in the LPS condition. This finding supports previous work suggesting that acute stress, particularly interpersonal stress, may alter immune responses, thus precipitating depression and suicidality (Dantzer et al., 2008; Miller and Raison, 2016; Zunszain et al., 2013; Pandey et al., 2012; Slavich et al., 2010). Additionally, within the clinical group with suicidality, we did not find a difference in the immune profiles of participants with current vs. past suicidality. The homogeneity within the clinical group with suicidality, regardless of current suicidality status, supports work suggesting that suicidality is not state-based (Janelidze et al., 2011; Serafini et al., 2020), potentially highlighting stable underlying neurobiological risks.

Prior reports indicate that production of immune proteins can be either elevated or suppressed when people with internalizing symptoms are stressed. In our study, Flt-3L, GM-CSF, and IFN-γ were negatively correlated with suicidality severity and number of suicide attempts, indicating immune suppression in suicidality. Deficits in LPS-stimulated secretion of these analytes are in line with our previous findings showing decreased TNF-α in depressed suicidal vs. non-suicidal participants (Gabbay et al., 2009a). To our knowledge, we are the first to report links between suicidality and Flt-3L and GM-CSF. A brief overview of the known functions of Flt-3L, GM-CSF, and IFN-γ is provided below.

Flt-3L is a pro-inflammatory cytokine involved in the production of several immune molecules including monocytes (macrophages and dendritic cells), natural killer cells, and B cell maturation. Flt-3L is strongly expressed in both the central and peripheral nervous systems (O'Shea et al., 2013). A study found that Flt-3L and nerve growth factor promoted the survival of cultured dorsal root ganglion neurons. However, Flt-3L could not produce this effect alone (Brazel et al., 2001). On the other hand, GM-CSF is a growth factor that induces granulocyte and macrophage proliferation. It has been shown to assist in monocyte migration across the endothelial layer of the blood brain barrier, leading to neuroinflammation (Vogel et al., 2015). Our study also supports the previously reported role of IFN-γ in adolescent suicidality. IFN-γ is another cytokine that acts in concert with other inflammatory molecules and pathways to heighten the overall immune response. In the central nervous system, IFN-γ may upregulate microglial activity, which may result in dopaminergic neuron loss (Kak et al., 2018; Mount et al., 2007; Ottum et al., 2015).

Studies have examined associations between GM-CSF and depression, but this work has been limited and inconsistent. Two studies found no relationship between GM-CSF and depression (Mindt et al., 2020; Naudé et al., 2022). One study found that treatment with duloxetine, a serotonin-norepinephrine reuptake inhibitor (SNRI) antidepressant, decreased plasma levels of GM-CSF (Miyauchi et al., 2019). Interestingly, another study found that among people taking selective serotonin reuptake inhibitor (SSRI) antidepressants, baseline GM-CSF level was higher in people in depression remission than those currently depressed, suggesting that low GM-CSF may be related to depression chronicity (Atake et al., 2022). Similarly, lower levels of GM-CSF were associated with depression in breast cancer patients (Kim et al., 2012). It is possible that the varied relationships between GM-CSF and more severe depression in these studies partially reflects this analyte's contribution to suicidality, a prominent symptom of depression. Importantly, all GM-CSF findings in depression to date were based on studies of adults. Our findings extend the literature by highlighting the presence of immune dysregulation underlying suicidality even in adolescence, emphasizing the need to further investigate interactions between the immune system and brain across the lifespan.

Although IFN-γ has been implicated in suicidality, the literature lacks consensus about whether higher or lower levels of IFN-γ drive this association (Serafini et al., 2020; Ganança et al., 2016). Some studies have shown that higher levels of IFN-γ are associated with major depression, seasonal affective disorder, and suicidal behavior, including work from our laboratory in youth (Gabbay et al., 2009a, 2009b; Omrani et al., 2009; Wang et al., 2020). However, other studies demonstrated that lower levels of IFN-γ are associated with major depression and suicidality in adults (Kim et al., 2008; Pavón et al., 2006). Notably, these studies examined levels of IFN-γ in circulation, whereas our current study assessed production of IFN-γ and other analytes using an *in vitro* culture model under baseline and immune-stressor conditions. This approach provided a more comprehensive way to probe the relationship between immune activity and suicidality, with our findings underscoring the importance of studying dynamic immune function beyond static profiles.

Based on current understanding, Flt-3L, GM-CSF, and IFN-γ all provide important support to various immune domains and foster communication between peripheral and central immunity. Reports detailing their diverse participation in neuro-immune pathways underscore the complexity of these systems, where groups of specific immune markers interact in an interdependent fashion to maintain homeostasis in health and respond to disease. The current findings suggest that altered Flt-3L, GM-CSF, and IFN-γ expression following an acute immune challenge may provide a unique signature for a dysregulated immune response associated with suicidality in youth. Additionally, LPS stimulates dendritic cells (DCs; a type of antigen-presenting cell), which immune analytes like Flt-3L, GM-CSF, and IFN-γ act jointly upon. GM-CSF and Flt-3L have been documented to enhance the antigen uptake capabilities of DCs. Specifically, GM-CSF acting in conjunction with Flt-3L significantly improves the functional performance of DCs, such as their ability to take up dextran, which is vital for antigen presentation and subsequent T-cell activation (Liu et al., 2022; Lellahi et al., 2023). When activated by immune markers such as GM-CSF and Flt-3L, DCs can influence the immune environment in the central nervous system. This includes the modulation of T cell responses and the maintenance of immune tolerance, which are crucial in preventing excessive neuroinflammation and autoimmunity (Liu et al., 2022). Taken together, our results may suggest a defect in antigen-presenting cell activation in the context of suicidality. We speculate that the observed defects may be due to heightened stress related to suicidality altering dendritic cell functions.

Another possible biological driver of suicidality-related immune dysfunction is cortisol response. Many studies have found higher levels of cortisol, a hormone related to stress, in adolescents with depression (Stetler and Miller, 2011; Joseph and Golden, 2017). Chronic stress can have harmful effects (McEwen, 1998), one of which being the suppression or reduction of immune responses (Irwin et al., 1990; Dhabhar, 2014). Because cytokines influence the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the release of cortisol (Makhija and Karunakaran, 2013), there may be a bi-directional relationship between cytokines and cortisol that explains the immune dysregulation that we see.

4.3. Nuances in immune profiles revealed by factor analysis

When we used factor analysis to group closely related analytes in the Medium, LPS, and LPS/Medium conditions, Flt-3L, GM-CSF, and IFN-γ all loaded heavily onto immune factors that were hypoactive in suicidality. These data-driven results further confirm our earlier findings of suppression of these analytes in relation to suicidality and revealed subtle differences in the immune profiles of adolescents with suicidality even in the absence of biological stressors. Interestingly, we also found that Factor 3 (comprising a mix of anti-inflammatory and pro-inflammatory immune proteins) and Factor 5 (comprising predominantly growth factors) in the LPS/Medium condition were hyperactive

in suicidality. These positive associations only emerged with a data-driven approach assessing relative immune response, suggesting subtle compensatory mechanisms in response to immune dysregulation in youth.

Our results are in line with reports from Clayton et al. (2023) showing that associations between interpersonal stress and heightened risk for suicidal behavior only occurred in conditions of blunted pro-inflammatory reactivity (Clayton et al., 2023). Recent work also found immune dysregulation in young people with suicidal thoughts and behaviors (Bellato et al., 2023). Our new findings, together with our prior reports, support the idea that immune activity is overall suppressed in suicidal adolescents (Gabbay et al., 2009a); this effect might be moderated by chronic stress (Leonard, 2000). In addition, previous literature has described relationships between suicidality and an array of cytokines, including IL-2, IL-6, IL-1β, and TNF-α. While we found no difference in IL-2 levels when compared across groups, we observed that IL-2 loaded heavily onto factors in all three conditions that were negatively correlated with suicidality measures, supporting previous work that found lower IL-2 in suicidal participants (Serafini et al., 2020; Ganança et al., 2016). Similarly, although levels of IL-6, IL-1β, and TNF-α did not differ across groups, factor analysis yielded evidence for their complex roles in suicidality.

In the LPS condition, these three immune analytes loaded heavily onto factors that were negatively correlated with suicidality measures. This supports our previous finding on low TNF-α in suicidal adolescents (Gabbay et al., 2009a). However, in the LPS/Medium condition, these cytokines, along with GM-CSF, loaded heavily onto Factor 3, which was positively correlated with number of suicide attempts. While there is a lack of investigation of immune function in adolescent suicidality, a prior study using RNA analysis reported elevated IL-6, IL-1β, and TNF-α in postmortem brain samples of adolescents who died by suicide (Pandey et al., 2012), aligning with our positive finding.

Altogether, our factor analysis results emphasize the advantage of employing data-driven approaches in addition to hypothesis testing in adolescent studies to capture the complexity of the immune system in potentially modulating suicidality.

4.4. Limitations and future directions

There are several limitations in our study. First, although we controlled for variances in age, sex, and BMI in our analyses, we were unable to account for other behavioral factors that might affect the immune system, such as exercise, diet, stress, and sleep. Future studies should collect these data to better control for confounds. Additionally, because we assessed peripheral immune analytes, we did not have direct information on how levels of immune analytes impact the brain. Future studies can correct for this by using other methodology, such as laboratory models of the blood brain barrier (Eugenin and Berman, 2003). Additionally, as those with suicidality were more likely to meet DSM criteria for a depressive disorder and experienced more severe depression symptoms compared those without, future work should examine the relationship between immune analytes and depression. Furthermore, because our study design is cross-sectional, we are limited in our ability to infer whether these associations are stable over time. Replication of these findings and extension to longitudinal cohorts is indicated. Longitudinal data will allow us to predict future clinical suicidality based on earlier immune profiles, providing potential clinical utility for identifying teens with elevated suicide risks.

4.5. Conclusions

Our results provide support for the idea that adolescents with suicidality have an immune profile that differs from those without suicidality. Suppressed levels of individual cytokines, including Flt-3L, GM-CSF, and IFN-γ, in response to an inflammatory stressor appear to reflect a pro-suicidal state of immune dysregulation. Combining data from all available immune analytes via exploratory factor analysis further revealed subtle associations between suicidality and immune function in the absence of an immune challenge as well as in the relative response to such a stressor. Our findings warrant further replication studies on adolescent suicidality, ideally integrating our comprehensive immune and clinical profiling approach with a longitudinal study design. We believe a refined understanding of these neuroinflammatory processes will help detect, treat, and ultimately prevent suicide in youth.

CRediT authorship contribution statement

Chloe Roske: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Tram N.B. Nguyen:** Writing – review & editing, Validation, Investigation, Funding acquisition, Methodology, Software, Visualization. **Joshua J. Schwartz:** Writing – review & editing, Project administration, Methodology, Investigation, Data curation. **Ava Erulker:** Writing – review & editing. **Kai Nie:** Methodology, Investigation. **Hui Xie:** Methodology, Investigation. **Seunghee Kim-Schulze:** Writing – review & editing, Validation, Supervision, Methodology, Investigation. **Benjamin A. Ely:** Writing – review & editing, Validation, Funding acquisition. **Russell H. Tobe:** Writing – review & editing, Validation, Data curation. **Wenzhu Mowrey:** Writing – review & editing, Visualization, Supervision, Software, Project administration, Methodology, Formal analysis. **Vilma Gabbay:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

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Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbih.2024.100866.

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