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Gene expression in peripheral blood mononuclear cells in impulsive aggression: Intermittent explosive disorder compared with non-aggressive healthy and psychiatric controls

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ABSTRACT

Evidence of chronic, systemic, low levels of inflammation is present in several stress-related psychiatric conditions including schizophrenia, depression, and intermittent explosive disorder (IED). We analyzed leukocyte gene expression (mRNA) to quantify the activity of pro and anti-inflammatory signaling pathways. Work performed in non-aggressive populations has uncovered a Conserved Transcriptional Response to Adversity (CTRA) characterized by an upregulation of pro-inflammatory gene transcription in chronically stressed individuals. We used pathway-based bioinformatic analyses of genome-wide transcriptional profiles of peripheral blood leukocyte samples from IED study participants (N = 45) and controls [healthy (n = 45) and psychiatric (n = 34)], with analyses focusing on the pro-inflammatory transcription control pathway mediated by the NF-kB family of transcription factors (typically upregulated in CTRA) and the antiviral transcription control pathway mediated by anti-viral response (IRF) family transcription factors (typically downregulated in CTRA). Compared with both healthy and psychiatric controls, individuals with IED had upregulated transcriptional activity of the antiviral response (IRF), but no evidence of pro-inflammatory NF-kB activation. Analyses implicated CD4 + T cells, CD8 + T cells, and B lymphocytes in IED-related transcriptional alterations, but showed no significant indication of monocyte involvement. This suggests that the inflammatory profile of IED differs substantially from that observed previously in other stress-related disorders, and may involve a pathogen-driven adaptive immune etiology.

1. Introduction

Evidence of chronic, systemic, low levels of inflammation is present in a number of psychiatric conditions including schizophrenia and depression, among others (Eisenberger et al., 2017). While it is not known if the presence of inflammation is causal for these disorders, there is evidence that inflammation is associated with relevant effects on neuronal circuitry. For example, administration of endotoxin, which leads to a swift increase in systemic inflammation is associated with priming effects on the amygdala's response to social threat (Inagaki et al., 2012) and priming effects on orbitofrontal responses (Kullmann et al., 2013) to emotional stimuli.

In addition, evidence for a role of inflammation in impulsive aggressive behavior, both as a dimension and as a disorder codified in the DSM-5 as Intermittent Explosive Disorder (Coccaro, 2012) that affects at least 4% of adults and 8% of adolescents, lifetime (Coccaro and Lee, 2020). Dimensional measures of aggression correlate directly with circulating C-Reactive Protein (CRP; Marsland et al., 2008; Suarez, 2003) and the cytokine Interleukin-6 (IL-6; Marsland et al., 2008; Suarez, 2003) in healthy individuals while elevated levels of CRP (Coccaro, 2006; Coccaro et al., 2014), IL-6 (Coccaro et al., 2014), and soluble IL-1RII receptor protein (Coccaro et al., 2016a) have been observed in those with IED compared with both healthy and psychiatric controls. Evidence suggesting inflammatory mediators may be causal for aggression comes from animal studies reporting that direct application of pro-inflammatory proteins increases defensive rage in felines (Zalcman and Siegel, 2006). However, the etiology of IED-related elevations in blood inflammatory proteins remains unclear.

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To explore more deeply the underlying immunoregulatory origins of IED-related elevations in blood inflammatory markers, we compared genome-wide transcriptional profiles of circulating blood leukocytes (peripheral blood mononuclear cells; PBMC) from IED patients with those from healthy and psychiatric patient controls. Circulating leukocytes can contribute to the production of pro-inflammatory cytokines in circulating blood, and they can also serve as cellular reporters of physiological processes that modify cytokine production in other tissues such as the spleen, lymph nodes, and other lymphoid organs that serve as an important source of circulating cytokine proteins (e.g., effects of the nervous, endocrine, metabolic, or microbiotic systems). For example, work in other, non-aggressive, populations has led to evidence of a sympathetic nervous system-activated Conserved Transcriptional Response to Adversity (CTRA) manifest by a monocyte-mediated upregulation of pro-inflammatory gene transcription (and downregulation of Type I interferon gene transcription) in chronically stressed individuals with internalizing disorders (Cole, 2019). Aspects of CTRA are also observed in socially-defeated mice exposed to an aggressive mouse (McKim et al., 2018; Powell et al., 2013; Weber et al., 2017). While impulsive aggressive behaviors (and IED) are considered "externalizing" in nature, individuals with these behaviors also have significant histories of adversity in childhood and beyond (Fanning et al., 2014). Accordingly, it is an empirical question if gene expression studies of these individuals will yield results consistent with the CTRA profile, or whether a different immunoregulatory gene expression profile might drive the pro-inflammatory protein upregulation previously associated with IED. To clarify the biological pathways involved in IED-associated elevations in circulating inflammatory proteins, we used pathway-based bioinformatic analyses of genome-wide transcriptional profiles of peripheral blood leukocyte samples from IED patients and controls (both healthy and psychiatric), with analyses focusing on the pro-inflammatory transcription control pathway mediated by the NF-kB family of transcription factors (typically upregulated in CTRA) and the innate antiviral transcription control pathway mediated by IRF family transcription factors (typically downregulated in CTRA). Parallel transcript origin analyses examined whether IED-associated differences in gene expression derived predominately from monocytes and other myeloid lineage cells (typical of the CTRA) or from other cell subsets (e.g., CD4 + T cells, CD8 + T cells, or B cells of the adaptive immune system).

2. Methods

2.1. Study participants

One-hundred and twenty-four adult individuals participated in this study. All participants were physically healthy and systematically evaluated in regard to aggressive and other behaviors as part of a larger program designed to study correlates of impulsive aggressive, and other personality-related, behaviors in human participants. Participants were recruited through public service announcements, newspaper, and other media, advertisements seeking out individuals who: a) reported psychosocial difficulty related to anger or, b) had little evidence of psychopathology. All Participants gave informed consent and signed the informed consent document approved by our Institutional Review Board.

2.2. Diagnostic assessment

Psychiatric diagnoses were made according to DSM-5 criteria (American Psychiatric Association, 2013). Diagnoses were made using information from: (a) the Structured Clinical Interview for DSM Diagnoses (First et al., 1995) for syndromal (formally Axis I) disorders and the Structured Interview for the Diagnosis of Personality Disorder (Pfohl et al., 1997) for personality (formally Axis II) disorders; (b) clinical interview by a research psychiatrist; and, (c) review of all other available clinical data. Research diagnostic interviews were conducted by individuals with a masters, or doctorate, degree in Clinical Psychology. All diagnostic raters went through a rigorous training program that included lectures on DSM diagnoses and rating systems, videos of expert raters conducting SCID/SIDP interviews, and practice interviews and ratings until the rater was deemed reliable with the trainer. This process resulted in good to excellent inter-rater reliabilities (mean kappa of 0.84 ± 0.05; range: 0.79 to 0.93) across anxiety, mood, substance use, impulse control, and personality disorders. Final diagnoses were assigned by team best-estimate consensus procedures involving research psychiatrists and clinical psychologists (Coccaro et al., 2012). While information for assigning syndromal diagnoses were collected through the use of the SCID-1, more than sufficient information from was available to update syndromal diagnoses from DSM-IV to those of DSM-5; DSM-5 diagnoses for personality disorder, based on the SIDP, are the same for DSM-IV. Finally, participants with a current history of a substance use disorder or a life history of bipolar disorder, schizophrenia (or other psychotic disorder), or mental retardation were excluded from study, because, by definition, IED participants cannot have such comorbidity.

After diagnostic assignment, forty-five participants had no evidence of any psychiatric diagnosis (Healthy Controls: HC); thirty-four participants met criteria for a lifetime diagnosis of a syndromal psychiatric disorder or personality disorder other than IED (Psychiatric Controls: PC), and forty-five participants met criteria for a current DSM-5 diagnosis of intermittent explosive disorder. Of the seventy-nine PC/IED participants, about half (n = 42, 53.1%) reported either history of formal psychiatric treatment (37.9%) or history of behavioral disturbance during which the subject, or others, thought should have sought mental health services but did not (15.2%). Prevalence of syndromal and personality disorder diagnoses are listed in Table 1.

Table 1
Demographic and Psychometric Characteristics of Study Participants.

	HC (N = 45)	PC (N = 34)	IED (N = 45)	P*	Group Differences
Demographic Variables					
Age	32.1 + 9.0	29.1 + 8.1	37.2 + 11.9	= 0.002	HC = PC < IED
Sex (% Male)	36%	53%	53%	= 0.060	HC = PC = IED
Race (% White)	60%	65%	36%	= 0.016	HC = PC > IED
Education (% HS / Part College / Full College)	2%/ 42%/ 56%	0%/ 47%/ 53%	31%/ 45%/ 24%	< 0.001	HC = PC > IED
SES Score	41.3 + 12.3	37.1 + 14.1	34.2 + 14.0	= 0.045	HC > IED = PC
Other Covariates					
Body Mass Index	26.7 + 3.6	24.7 + 3.6	28.6 + 6.8	< 0.01	HC = IED > PC
Currently Drinking (%)	73%	77%	57%	= 0.135	HC = PC = IED
Drinks Per Week	3.2 + 3.3	7.2 + 6.6	5.0 + 5.7	= 0.02	HC = IED < PC
Current Smoking (%)	7%	18%	18%	= 0.227	HC = PC = IED
Packs Per Week	0.1 + 0.1	2.8 + 2.7	3.8 + 4.6	= 0.344	HC = PC = IED
Psychometric Variables					
Aggression: LHA	4.0 + 3.0	6.0 + 4.0	19.0 + 3.3	< 0.001	IED > PC > HC
Aggression BPA	25.8 + 8.4	29.0 + 7.5	46.5 + 12.3	< 0.001	IED > PC = HC
Impulsivity: LHIB	21.5 + 13.1	32.3 + 18.2	48.1 + 23.3	< 0.001	IED > PC > HC
Impulsivity: BIS-11	51.6 + 7.3	58.2 + 10.6	68.9 + 11.0	< 0.001	IED > PC > HC

2.3. Assessment of Aggression and Impulsivity

Aggression was assessed with the Aggression score from the Life History of Aggression assessment (LHA; Coccaro et al., 1997; α = 0.90) and the Aggression (Physical and Verbal) sub-score from the from the Buss-Perry Aggression questionnaire (BPA; Buss and Perry, 1992; α = 0.93). The LHA assesses history of actual aggressive behavior and BPA assesses aggressive tendencies as a personality trait. Impulsivity was assessed by the Life History of Impulsive Behavior (LHIB; Coccaro and Schimdt-Kaplan, 2012; α = 0.97) and Barratt Impulsivity Scale (BIS-11; Patton et al., 1995; α = 0.88). The LHIB assesses the number of times a person has engaged in impulsive behavior while the BIS-11 assesses the person's tendency to act impulsively as a personality trait. Each assessment has good to excellent psychometric properties.

2.4. Collection and processing of samples for gene expression

All study participants were medically healthy as assessed by medical history, physical examination, and laboratory testing. In addition, no study particpant were on any medication at time of sampling (only 10% of study participants reported having been on any psychotropics at any time; 12% for PC and 18% for IED). All study particpant were fasting on the morning of blood draw, negative on a breathalyzer test and urine drug screen, and had been instructed to abstain from exercise/drinking alcohol/smoking before the blood draw. Diet was not controlled for. Ten ml of whole blood was obtained on the study particpant by venipuncture of an antecubital vein. Samples were then transported to the Core Immunology Laboratory at the University of Chicago Medical Center for the processing of samples. Whole blood was placed in a 250 ml conical tube in which an equal volume of Dulbecco's Phosphate Buffered Saline (DPBS; Invitrogen) was added and mixed by inverting the tube ten times. In another 50 ml conical tube, 15 ml of Ficoll-Paque Plus (GE Healthcare, 1.077 g/ml) was added and the tube was centrifuged for 30 min at 800*g* without brake. The buffy coat was then pipetted from the interface into a new 50 ml conical tube and DPBS to a total volume of 40 ml was added. The tube was centrifuged for 10 min at 300*g*, the supernatant discarded and the cells washed again. The supernatant was discarded and cells were re-suspended in 10 ml of DPBS and counted. The cells were spun down again and re-suspended in a proper amount of freezing medium [DMSO (10%) + serum (90%)] so the concentration of cells reached 10×10^6 per ml (Invitrogen). Cells were aliquoted into pre-labeled cryovials and placed into Mr. Frosty (Fisher Scientific) which was immediately placed in a − 80°C freezer and then stored in a liquid nitrogen freezer until sent to the UCLA Social Genomics Core Laboratory for genome-wide transcriptional profiling.

2.5. Gene expression assay

Total RNA was extracted from cell samples (Qiagen RNeasy), checked for suitable mass (> 200 ng by NanoDrop spectrophotometry) and integrity (RNA integrity number > 3 by Agilent TapeStation capillary electrophoresis), and converted to complementary DNA libraries (Lexogen QuantSeq 3′ FWD) for multiplex DNA sequencing on an Illumina HiSeq 4000 instrument in the UCLA Neuroscience Genomics Core Laboratory (all following the manufacturers' standard protocols). Assays targeted > 10 million single-strand 65 nt sequence reads (achieved mean = 18.1 million), each of which was mapped to the GRCh38 human transcriptome sequence using the STAR aligner (Dobin et al., 2013; average mapping rate = 92.9%). Raw read counts were pre-normalized to gene transcript counts per million total transcripts (TPM) and subsequently normalized to standardize expression of 11 standard reference gene transcripts (Eisenberg and Levanon, 2013) as in previous research (Cole et al., 2020).

2.6. Statistical analysis

Normalized gene expression values were log2-transformed for analysis by standard linear statistical models relating transcript abundance to study group (IED vs. Controls) while controlling for age, sex, race, education, SES, body mass index (kg/m^2), alcohol use (drinks per week) smoking (packs per week), and excluding genes with minimal variation in expression (SD < 0.5 log2 expression units). These covariates were chosen because of evidence in the literature that these variables can affect inflammatory marker variables [age (Kim et al., 2012), sex (Klein and Flanagan, 2016), race (Haralambieva et al., 2013), SES and education (Schreier et al., 2014), BMI (Schmidt et al., 2015), current alcohol use (Neupane, 2016) and current smoking (Lee et al., 2012). Among the 60,679 gene transcripts assayed, 12,681 showed above-minimal variability and were used to quantify empirical differences in genome-wide transcriptional profiles. In each analysis, all genes showing a point estimate of > 1.5-fold difference in average transcript abundance in IED vs. Controls served as input into higher-order bioinformatics analyses using TELiS promoter sequence analysis (Cole et al., 2005) to test 2 a priori-specified hypotheses regarding activity of transcription control pathways relevant to CTRA biology: 1) the positive CTRA component of inflammation (NF-kB, as indicated by differential expression of genes bearing transcription factor-binding motifs matching the TRANSFAC position-specific weight matrices V$NFKAPPAB65_01 and/or V$NFKAPPAB50_01, as well as the AP-1 family of transcription factors indicated by V$AP1_C) and, 2) the inverse CTRA component of innate antiviral responses (interferon response factors, IRFs, as indicated by V$ISRE_01). TELiS analyses were conducted using 9 different parametric combinations of promoter DNA sequence length (−300, −600, and −1000 to +200 nucleotides surrounding the RefSeq-designated transcription start site) and transcription factor-binding motif (TFBM) detection stringency (TRANSFAC mat_sim values of 0.80, 0.90, and 0.95; Cole et al., 2005). Log2-transformed TFBM ratios (comparing prevalence in promoters of up- vs. down-regulated genes) were averaged across the 9 parametric combinations and tested for statistical significance using standard errors derived from bootstrap resampling of linear model residual vectors (controlling for potential correlation across genes). Parallel Transcript Origin Analyses (Cole et al., 2011) were applied to the same set of input genes to identify which of 6 major leukocyte subsets (CD4 + T cells, CD8 + T cells, B cells, NK cells, plasmacytoid dendritic cells, and monocytes) might represent a predominate source of IED-related differences in gene expression, using reference gene expression profiles from isolated leukocyte samples (Gene Expression Omnibus GSE1133) to compute a set of cell type diagnosticity scores for each gene, as previously described (Cole et al., 2011). Average cell type diagnosticity scores for genes up-regulated and down-regulated in association with IED were tested for statistical significance using standard errors derived from bootstrap resampling of linear model residual vectors as described above. Parallel Transcriptome Representation Analyses [refs] tested for differential expression of gene transcripts expressed selectively within each major leukocyte subset to determine whether differential cell type abundance might contribute to the observed differences in the overall PBMC transcriptome. Individual genes were not tested for statistically significant difference in expression because the goal of this study was solely to assess differences in average expression of sets of genes that reflect common transcription factor or cellular influences, and for this application point estimate-based screening has been shown to provide more reliable results than gene screening based on gene-specific p-values (Cole et al., 2005).

3. Results

3.1. Sample characteristics (Table 1)

The three groups differed modestly, but significantly, in age, sex, ethnicity, and in socioeconomic score (SES). Age was higher among IED

compared with PC but not compared with HC participants. Percent of males was lowest among HC, but similar among PC and IED participants. Percentage of African Americans was highest among IED participants and about the same among PC and HC participants. Level of education was lowest among the IED participants compared with the PC/HC participants who did not differ in this regard. SES was highest among HC compared with PC and IED participants who did not differ on this variable. Body mass index differed significantly between the groups but this was due to lower BMI among the PC participants. Percent of individuals who reported current drinking or smoking did not differ among the groups. Accordingly, subsequent analyses controlled for these differences since these variables could influence the results. As expected, IED participants had higher scores an aggression (LHA/BPA), impulsivity (LHIB/BIS), and state depression (BDI) measures with lower score for PC and even lower cores for HC participants. IED and PC groups did not differ in the frequency of current or lifetime Syndromal disorders but did differ in the frequency of Personality Disorders as expected. Details on the diagnostic characteristics of the sample are listed in Table 2.

3.2. Inflammatory gene expression

Genome-wide transcriptional profiling of peripheral blood leukocytes by RNA sequencing identified 3372 gene transcripts that differed in average expression level by 50% or more in the IED group relative to the pooled control group, after control for age, sex, race, education, SES, body mass index, current smoking, and previous heavy alcohol use (536 upregulated, 2836 downregulated). Promoter-based bioinformatics analyses of these genes (Fig. 1) indicated no significant difference in activity of the NF-kB p65 subunit (log2 ratio of transcription factor-binding motif prevalence in core promoter sequences of up- vs. down-regulated genes = −0.141 ± standard error 0.225, p = 0.5319), but significantly greater activity of the NF-kB p50 subunit in the IED group relative to controls (0.830 ± 0.312, p = 0.0084). Analyses indicated significant downregulation of AP-1 activity in IED vs. controls (−0.413 ± 0.165, p = 0.0130). Similar results emerged in follow-up analyses comparing IED to PC controls alone (although statistical significance decreased due to sample size; NF-κB p65: −0.804 ± 0.217, p = 0.0003; NF-κB p50: 0.449 ± 0.286, p = 0.1181; AP-1: −0.345 ± 0.144, p = 0.0174). Similar results also emerged in analyses of crude (unadjusted) group differences (NF-κB p65: 0.155 ± 0.206, p = 0.4538; NF-κB p50: 0.668 ± 0.265, p = 0.0124; AP-1: −0.257 ± 0.097, p = 0.0088).

Table 2
Syndromal and Personality Disorder Diagnoses Among Study Participants.

	PC (N = 34)	IED (N = 45)	P
Current Syndromal Disorders:			
Any Depressive Disorder	2 (5.9%)	9 (20.0%)	= 0.10
Any Anxiety Disorder	6 (17.6%)	7 (15.6%)	= 0.80
Stress and Trauma Disorders	5 (14.7%)	10 (22.2%)	= 0.40
Obsessive-Compulsive Disorders	0 (0.0%)	1 (2.2%)	= 0.38
Eating Disorders	0 (0.0%)	0 (5.1%)	= 0.99
Non-IED Impulse Control Disorders	0 (0.0%)	0 (0.0%)	= 0.99
Lifetime Syndromal Disorders:			
Any Depressive Disorder	18 (57.9%)	22 (48.9%)	= 0.07
Any Anxiety Disorder	11 (32.4%)	11 (24.4%)	= 0.44
Any Substance Use Disorder	14 (41.2%)	22 (48.9%)	= 0.50
Stress and Trauma Disorders	12 (35.3%)	14 (31.1%)	= 0.70
Obsessive-Compulsive Disorders	0 (0.0%)	1 (2.2%)	= 0.38
Eating Disorders	2 (5.9%)	1 (2.2%)	= 0.40
Non-IED Impulse Control Disorders	0 (0.0%)	1 (2.2%)	= 0.38
Personality Disorders:			
Any Personality Disorder	14 (41.2%)	41 (91.1%)	< 0.001*
Cluster A (Odd)	0 (0.0%)	6 (13.3%)	< 0.03
Cluster B (Dramatic)	2 (5.9%)	10 (22.2%)	= 0.03
Cluster C (Anxious)	4 (11.3%)	6 (13.3%)	= 0.84
PD-NOS	9 (26.5%)	24 (53.3%)	< 0.02

*p < 0.05 after correction for multiple comparisons (uncorrected p < 0.0025).

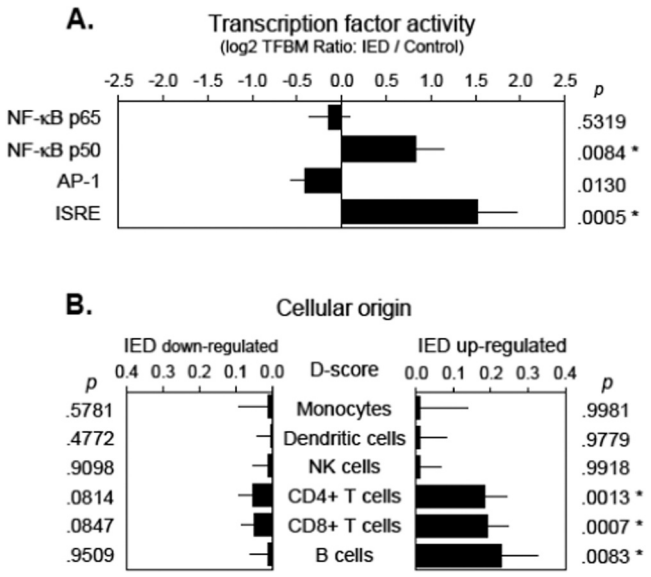

Fig. 1. (A) Promoter-based bioinformatics analyses indicating no significant difference in activity of the NF-κB p65 subunit (log2 ratio of transcription factor-binding motif prevalence in core promoter sequences of up- vs. down-regulated genes, but significantly greater activity of the NF-κB p50 subunit in the IED group relative to controls. Analyses indicated significant down-regulation of AP-1 activity in IED vs. controls. (B). Application of Transcript Origin Analysis to the same set of IED-related gene transcripts showed no indication of differential monocyte activity and, instead, identified CD4 + T cells, CD8 + T cells, and B cells and predominate sources of gene transcripts upregulated in association with IED. No specific leukocyte subtype was implicated as a predominate source of IED-downregulated genes.

3.3. Interferon gene expression

Parallel analyses of interferon signaling indicated significantly upregulated activity of the interferon-stimulated response element (ISRE) in IED vs. controls (1.521 ± 0.432, p = 0.0005). Similar results emerged in follow-up analyses comparing IED to PC controls alone (with statistical significance decreased due to diminished sample size: 0.634 ± 0.409, p = 0.1226) and unadjusted comparison of IED vs. controls (0.831 ± 0.489, p = 0.0907).

3.4. Cellular origins

Application of Transcript Origin Analysis to the same set of IED-related gene transcripts showed no indication of differential monocyte activity (Fig. 1b) and instead identified CD4 + T cells, CD8 + T cells, and B cells and predominate sources of gene transcripts upregulated in association with IED. No specific leukocyte subtype was implicated as a predominate source of IED-downregulated genes (Fig. 1b). In analyses testing for potential differences in leukocyte subset abundance within the PBMC transcriptome, Transcriptome Representation Analyses using major leukocyte subset-diagnostic gene sets showed no significant difference between IED and controls in the prevalence of CD4 + T, CD8 + T, B, NK cell, or monocyte-diagnostic gene transcripts (all p > 0.16).

4. Discussion

Results of these analyses identify significant differences in basal activity of key immunoregulatory transcription control pathways in circulating white blood cells from IED patients in comparison to controls either with or without a history of psychiatric diagnosis. These effects include increased activity of some members of the NF-kB transcription factor family (notably the p50 subunit), increased activity of interferon

signaling pathways, and decreased activity the AP-1 family of transcription factors. These alterations in immunoregulatory transcription factor activity clarify the molecular mechanisms involved in mediating previously observed elevations of circulating pro-inflammatory cytokine protein levels in IED patients relative to controls. The specific pattern of transcription factor activity observed here, however, is not consistent with the CTRA profile, which generally involves increased activity of pro-inflammatory signaling pathways (particularly NF-kB p65 and AP-1) accompanied by decreased activity of interferon signaling (IRF). The present analyses of leukocyte gene regulation in IED indicate either no significant alteration or downregulation of the CTRA-typical proinflammatory pathways (NF-kB p65, AP-1) accompanied by upregulated activity of interferon signaling and upregulated activity of the relatively anti-inflammatory p50 subunit of NF-kB.

Collectively, these results link IED to upregulated transcriptional activity of the adaptive immune system and innate antiviral/interferon response, and suggest that the biological correlates of this externalizing behavioral disorder differ substantially from the innate inflammatory upregulation observed previously in the context of internalizing disorders.

The biological basis for IED's distinctive immunoregulatory profile remain to be determined, but transcriptional activation of all three adaptive immune cell subsets (CD4 + T cells, CD8 + T cells, and B cells), in tandem with the interferon system, would be consistent with increased activity of intracellular pathogens (e.g., subclinical chronic viral or protozoan infection; Matta et al., 2019) or with some other form of antigen-driven immunologic activation (e.g., autoimmune processes). Such results would be consistent with previous observations linking IED to elevated rates of specific infectious agents such as Toxoplasma gondii (Coccaro et al., 2016b). The present analysis clearly indicates some form of immunologic activation in the context of IED (reflected in increased NF-kB p50 and interferon activity), but the cellular characteristics, molecular mechanisms, and biological origins of this pattern appear to differ from the classical monocyte-related CTRA profile previously observed in the context of threat responses and stress biology (Cole, 2019). These data are most consistent with the hypothesis that a chronic subclinical infection (e.g., with Toxoplasma gondii or other pathogens) may lead to chronic activation of Type I interferon activity and adaptive immune responses by T and B lymphocytes, with attending elevations in circulating cytokine levels that subsequently impact CNS function to increase negative affectivity, irritability, and other neurobiological processes driving IED manifestation. Alternatively, these immune-related neurobiological alterations may impair normal inhibitory control of aggressive impulses. Under either of these scenarios, intermittent explosive behaviors may arise from intermittent encounters with triggering environmental events amidst chronically elevated immune-mediated neurovulnerability, or may reflect intermittent peaks in immunological response and pathogen activity that trigger intermittent neurovulnerabilty.

The present analyses controlled for differences in age, sex, BMI, race, education, SES, heavy alcohol use, and smoking, so the differences observed here cannot be attributed to confounding by these factors. Transcriptome Representation Analyses also found no evidence that differences in leukocyte subset abundance (e.g., numbers of T cells, B cells, monocytes, etc.) differed in IED vs. controls and might thus contributed to the observed difference in overall PBMC transcriptomes. However, this is an observational study and it remains possible that other unmeasured factors may correlate with IED and thereby contribute to the differences observed here.

This study is subject to several important limitations. First, the present results come from a cross-sectional analysis in a single sample with less than 50 study participants per group, and it remains to be determined how general these effects are across other populations or whether they represent a causal relationship between IED and gene expression. It is plausible that IED-associated inflammatory biology might contribute to IED psychobiology (e.g., via effects of cytokine signaling to the brain or immune cell access to the CNS), but it is also possible the IED may cause changes in peripheral gene expression via autonomic or neuroendocrine alterations. Future research using interventions in randomized controlled experiments will be important to clarify the causal pathways underlying the immunoregulatory associations observed here. The present analysis of biological mechanisms is also limited by complex cellular composition of PBMC, and future research should directly assess leukocyte subset prevalence (e.g., by flow cytometry) and analyze gene expression in isolated leukocyte subsets (e.g., by immunomagnetic selection) to further clarify the cellular mechanisms involved. Finally, this study involved no direct assessment of health outcomes or longitudinal follow-up, so the health significance and relevance to pathophysiology of IED remain to be determined in future research.

5. Conclusion

The present analyses identify activation of multiple gene regulation pathways in leukocytes from patients with IED, including NF-kB p50 and interferon response systems. These effects are distinct from the classical monocyte-related CTRA profile previously observed in the context of internalizing disorders and suggest that externalizing disorders such as IED may involve a distinct profile of immunologic activation involving the adaptive immune system and innate antiviral response. These immune-regulatory alterations may contribute to previously observed increases in plasma inflammatory cytokine levels in IED, and future research will be required to define the basis for the gene regulatory alterations observed here.

Author contributions

Drs. Coccaro, Irwin and Cole designed the study, Ms. Arevalo and Mr. Dizon conducted the assays and initial data analyses, and Dr. Cole did the final data analysis, and Drs. Coccaro, Irwin and Cole wrote the manuscript.

Conflicts of interest

Dr. Coccaro reports being a consultant to and being on the Scientific Advisory Boards of Azevan Pharmaceuticals, Inc. and of Avanir Pharmaceuticals, Inc., and being a current recipient of Grant awards from the NIMH and NIAAA. The remaining authors have nothing to disclose.

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References

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders (5th ed.). Washington, DC.

Buss, A.H., Perry, M., 1992. The aggression questionnaire. J. Pers. Soc. Psychol. 63 (3), 452–459.

Coccaro, E.F., 2006. Association of C-reactive protein elevation with trait aggression and hostility in personality disordered study particpant: a pilot study. J. Psychiatr. Res. 40 (5), 460–465.

Coccaro, E.F., 2012. Intermittent explosive disorder as a disorder of impulsive aggression for DSM-5. Am. J. Psychiatry 169 (6), 577–588.

Coccaro, E.F., Lee, R.J., 2020. Disordered aggression and violence in the United States. J. Clin. Psychiatry 81 (2), 19m12937.

Coccaro, E.F., Schmidt-Kaplan, C.A., 2012. Life history of impulsive behavior: development and validation of a new questionnaire. J. Psychiatr. Res. 46, 346–352.

Coccaro, E.F., Berman, M.E., Kavoussi, R.J., 1997. Assessment of life history of aggression: development and psychometric characteristics. Psychiatry Res. 73 (3), 147–157.

Coccaro, E.F., Nayyer, H., McCloskey, M.S., 2012. Personality disorder-not otherwise specified evidence of validity and consideration for DSM-5. Compr. Psychiatry 53, 907–914.

Coccaro, E.F., Lee, R., Coussons-Read, M., 2014. Elevated plasma inflammatory markers in individuals with intermittent explosive disorder and correlation with aggression in humans. JAMA Psychiatry 71 (2), 158–165.

Coccaro, E.F., Lee, R., Fanning, J.R., Fuchs, D., Goiny, M., Erhardt, S., Christensen, K., Brundin, L., Coussons-Read, M., 2016a. Tryptophan, kynurenine, and kynurenine metabolites: relationship to lifetime aggression and inflammatory markers in human study particpant. Psychoneuroendocrinology 71, 189–196.

Coccaro, E.F., Lee, R., Groer, M.W., Can, A., Coussons-Read, M., Postolasche, T.T., 2016b. *Toxoplasma gondii* infection: relationship with other and self-directed aggression in human study particpant. J. Clin. Psychiatry 77 (3), 334–341.

Cole, S.W., 2019. The conserved transcriptional response to adversity. Curr. Opin. Behav. Sci. 28, 31–37.

Cole, S.W., Yan, W., Galic, Z., Arevalo, J., Zack, J.A., 2005. Expression-based monitoring of transcription factor activity: the TELiS database. Bioinformatics 21 (6), 803–810.

Cole, S.W., Hawkley, L.C., Arevalo, J.M., Cacioppo, J.T., 2011. Transcript origin analysis identifies antigen-presenting cells as primary targets of socially regulated gene expression in leukocytes. Proc. Natl. Acad. Sci. USA 08 (7), 3080–3085.

Cole, S.W., Shanahan, M.J., Gaydosh, L., Harris, K.M., 2020. Population-based RNA profiling in Add Health finds social disparities in inflammatory and antiviral gene regulation to emerge by young adulthood. Proc. Natl. Acad. Sci. USA 117 (9), 4601–4608.

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T.R., 2013. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29 (1), 15–21.

Eisenberg, E., Levanon, E.Y., 2013. Human housekeeping genes, revisited. Trends Genet. 29 (10), 569–574.

Eisenberger, N.I., Moieni, M., Inagaki, T.K., Muscatell, K.A., Irwin, M.R., 2017. In sickness and in health: the co-regulation of inflammation and social behavior. Neuropsychopharmacology 42 (1), 242–253.

Fanning, J., Meyerhoff, J., Lee, R., Coccaro, E.F., 2014. Intermittent explosive disorder and suicidal behavior as a function of trauma experienced in childhood. J. Psychiatr. Res. 56, 10–17.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B., 1995. Structured Clinical Interview for DSM-IV Axis I Disorders. New York State Psychiatric Institute, New York.

Haralambieva, I.H., Ovsyannikova, I.G., Kennedy, R.B., Larrabee, B.R., Shane Pankratz, V., Poland, G.A., 2013. Race and sex-based differences in cytokine immune responses to smallpox vaccine in healthy individuals. Hum. Immunol. 74 (10), 1263–1266.

Inagaki, T.K., Muscatell, K.A., Irwin, M.R., Cole, S.W., Eisenberger, N.I., 2012. Inflammation selectively enhances amygdala activity to socially threatening images. Neuroimage 59 (4), 3222–3226.

Kim, O.Y., Chae, J.S., Paik, J.K., Seo, H.S., Jang, Y., Cavaillon, M.C., Lee, J.H., 2012. Effects of aging and menopause on serum interleukin-6 levels and peripheral blood mononuclear cell cytokine production in healthy nonobese women. Age 34 (2), 415–425.

Klein, S., Flanagan, K., 2016. Sex differences in immune responses. Nat. Rev. Immunol. 16, 626–638.

Kullmann, J.S., Grigoleit, J.-S., Lichte, P., Kobbe, P., Rosenberger, C., Banner, C., Wolf, O. T., Engler, H., Oberbeck, R., Elsenbruch, S., Bingel, U., Forsting, M., Gizewski, E.R., Schedlowski, M., 2013. Neural response to emotional stimuli during experimental human endotoxemia. Hum. Brain Mapp. 34, 2217–2227.

Lee, J., Taneja, V., Vassallo, R., 2012. Cigarette smoking and inflammation: cellular and molecular mechanisms. J. Dent. Res 91 (2), 142–149.

Marsland, A.L., Prather, A.A., Petersen, K.L., Cohen, S., Manuck, S.B., 2008. Antagonistic characteristics are positively associated with inflammatory markers independently of trait negative emotionality. Brain Behav. Immun. 22 (5), 753–761.

Matta, S.K., Olias, P., Huang, Z., Wang, Q., Park, E., Yokoyama, W.M., Sibley, L.D., 2019. Toxoplasma gondii effector TgIST blocks type I interferon signaling to promote infection. Proc. Natl. Acad. Sci. 116 (35), 17480–17491.

McKim, D.B., Yin, W., Wang, Y., Cole, S.W., Godbout, J.P., Sheridan, J.F., 2018. Social stress mobilizes hematopoietic stem cells to establish persistent splenic myelopoiesis. Cell Rep. 25 (9), 2552–2562 e3.

Neupane, S.P., 2016. Neuroimmune interface in the comorbidity between alcohol use disorder and major depression. Front. Immunol. 7, 655.

Patton, J., Stanford, M., Barratt, E., 1995. Factor structure of the Barratt Impulsiveness Scale. J. Clin. Psychol. 51, 768–774.

Pfohl, B., Blum, N., Zimmerman, M., 1997. Structured Interview for DSM-IV Personality Disorder: SIDP-IV. American Psychiatric Press, Washington, D.C.

Powell, N.D., Sloan, E.K., Bailey, M.T., Arevalo, J.M., Miller, G.E., Chen, E., Kobor, M.S., Reader, B.F., Sheridan, J.F., Cole, S.W., 2013. Social stress up-regulates inflammatory gene expression in the leukocyte transcriptome via β-adrenergic induction of myelopoiesis. Proc. Natl. Acad. Sci. USA 110 (41), 16574–16579.

Schmidt, F.M., Weschenfelder, J., Sander, C., Minkwitz, J., Thormann, J., Chittka, T., Mergl, R., Kirkby, K.C., Faßhauer, M., Stumvoll, M., Holdt, L.M., Teupser, D., Hegerl, U., Himmerich, H., 2015. Inflammatory cytokines in general and central obesity and modulating effects of physical activity. PLoS One 10 (3), e0121971.

Schreier, H.M., Roy, L.B., Frimer, L.T., Chen, E., 2014. Family chaos and adolescent inflammatory profiles: the moderating role of socioeconomic status. Psychosom. Med. 76 (6), 460–467.

Suarez, E.C., 2003. Joint effect of hostility and severity of depressive symptoms on plasma interleukin-6 concentration. Psychosom. Med. 65 (4), 523–527.

Weber, M.D., Godbout, J.P., Sheridan, J.F., 2017. Repeated social defeat, neuroinflammation, and behavior: monocytes carry the signal. Neuropsychopharmacology 42 (1), 46–61.

Zalcman, S.S., Siegel, A., 2006. The neurobiology of aggression and rage: role of cytokines. Brain Behav. Immun. 20 (6), 507–514.