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The expression of cytokines and chemokines in the blood of patients with severe weight loss from anorexia nervosa: An exploratory study

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ABSTRACT

Anorexia nervosa (AN) is a serious, potentially life-threatening disorder characterized by severe weight loss, dysregulated eating, and often excessive exercise. While psychiatric illnesses such as depression are associated with increased levels of pro-inflammatory mediators, evidence for such disturbances in patients with AN has been less clear. In an exploratory study of possible disturbances in immune responses in AN, we assayed a panel of cytokines and chemokines in the blood of patients undergoing inpatient treatment, testing the hypothesis that metabolic disturbances in this disease would lead to a pattern of immune disturbances distinct from that of other psychiatric diseases. For this purpose, we evaluated patients by the Beck Depression Inventory-II (BDI-II) and the Eating Disorders Examination-Questionnaire and assessed cytokines and chemokines by enzyme-linked immunosorbent assays. Patients reported a moderate level of depression (mean BDI-II = 22.6) but exhibited few immunologic abnormalities of the kind associated with major depressive disorder [e.g., increased interleukin (IL)-6]; RANTES showed the most frequent elevations and was increased in 4 of the patients studied. Together, these findings suggest that features of AN such as loss of adipose tissue and excessive exercise may attenuate cytokine production and thus modulate the experience of illness that impacts on core features of disease.

1. Introduction

AN is a serious, potentially life-threatening psychiatric illness that affects ~1% of the population. AN disproportionately afflicts females, and is associated with severe weight loss, dysregulated eating, distorted body image, and, often, excessive exercise [1–3]. AN carries substantial morbidity and mortality as well as personal, familial, and societal costs. Nevertheless, patients affected appear to value the ill state and can make extensive efforts to achieve and maintain the starvation that characterizes the illness. Given the clinical features of AN, treatment is uncertain and current approaches to therapy carry significant costs despite limited efficacy [4–6]. Medications targeting the core symptoms of the disorder currently are not available [7–9]. Elucidating the pathogenesis of AN and identifying biomarkers are therefore essential for developing more effective interventions.

AN presents a very complex biological setting that encompasses biochemical, metabolic, and sensory abnormalities [10–17]. Importantly, changes in visceral experience occur prominently in the ill state (e.g., reduced pain sensitivity and reduced detection of visceral changes such as heart beat), with findings that differ from alterations in experience accompanying food restriction that occurs independently of AN [18,19]. Biological alterations that regularly occur during starvation, combined with unique features of the ill state of AN, may help to explain why the ill state appears to be so reinforcing for patients.

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A potentially important but understudied aspect of AN is the effect of this disorder on the immune system. Data from existing studies are conflicting and, while some studies suggest a pro-inflammatory state, others indicate few abnormalities [20–30]. In contrast, depression and schizophrenia, among other psychiatric diseases, show abundant cytokine disturbances and define pro-inflammatory states that co-occur with the experience of mental illness [31–35]. The lack of consistent evidence of such a relationship in AN suggests that AN may involve a unique interplay between nervous and immune systems and dissociation of expected cytokine abnormalities from mood and affect.

The lack of more decisive evidence for cytokine disturbances in AN is notable, particularly given that the severe loss of fat in AN would be expected to have important effects on immunity. As now recognized, adipose tissue produces many bioactive substances, including pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 [36–39]. In addition to their immunological and metabolic effects, these cytokines could play a role in the initiation and maintenance of psychiatric manifestations such as disturbances in mood and affect. Notably, in addition to extreme dietary restriction with subsequent loss of fat mass, AN can be characterized by engagement in a determined excessive exercise routine. Extensive exercise may be relevant to the overall state of the immune system in AN since muscle cells, when undergoing contraction, can also produce cytokines, known as myokines [40,41]. Of the myokines, IL-6 may have anti-inflammatory action at least locally although systemically it can lead to immune activation. Because of extensive exercise, the production of myokines could increase in patients and, depending on the array of these mediators produced, attenuate inflammation [42–45].

Since immune mediators can drive metabolic and nervous system disturbances, a focus on their role in AN is conceptually and practically important as it suggests potential interventions with immunomodulatory agents; such agents could interrupt an injurious cycle of metabolic and psychological disturbance. To begin to explore the expression of immune mediators in AN, we analyzed a panel of immune chemokines and cytokines in a cohort of well-characterized AN patients undergoing inpatient treatment. Results of these studies indicate that patients with AN show few abnormalities in the expression of cytokines and chemokines compared to laboratory values for healthy populations. These findings are consistent with the hypothesis that patients with AN may differ from patients with other psychiatric disorders where immune disturbance is more prominent.

2. Methods

2.1. Participants

Participants were 30 females ages 15–45 who met *DSM-IV* criteria for AN (any subtype), who were admitted for inpatient treatment at the University of North Carolina Center of Excellence for Eating Disorders. Participants were assessed at the time of admission, typically at <75% ideal body weight (IBW). For participants who were unable to complete the assessment at admission, testing occurred within 1 week of admission with one exception (participant ID = 16). Prior to the assessment, participants completed a semi-structured screening interview designed specifically for this investigation to assess recent illness, health history and current medications that could affect cytokine levels. This investigation was approved by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill and all participants provided informed consent.

Blood sampling was unsuccessful in 5 participants, who were therefore omitted from the analyses. From the remaining 25 participants, we were unable to assess the full battery of psychological or immunological measures on all patients related in part to availability of material and patient-related issues.

2.2. Measures

2.2.1. Body composition

Height and weight were assessed using a stadiometer and a calibrated digital scale. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Body fat percent was determined by dual X-ray absorptiometry (DXA).

2.2.2. Eating disorder and psychopathology assessment

2.2.2.1. Structured clinical interview for DSM-IV Axis I disorders – patient edition module H (SCID-I/P) [46]. The SCID-I/P, a well-studied and frequently employed semi-structured interview for Axis I disorders was used to assesses eating pathology.

2.2.2.2. The Eating Disorders Examination-Questionnaire (EDE-Q) [47]. The EDE-Q is a 38-item self-report measure of eating disorder psychopathology over the past 28 days. The EDE-Q is based on the EDE [48], a valid and reliable investigator–administered interview for assessing current eating disorder symptoms. The EDE-Q yields a global score and four subscale scores (restraint, eating concerns, weight concerns and shape concerns), each with a range of 0–6.

2.2.2.3. Beck Depression Inventory-II (BDI-II) [49]. The BDI is a 21-item self-report questionnaire that is used to assess the severity of current depressive symptoms. Each answer is scored on a 4-point scale (0–3), with total scores indicating minimal (0–13), mild (14–19), moderate (20–28), and severe (29–63) depression.

2.2.2.4. Spielberger state-trait anxiety inventory (STAI) [50]. The STAI is a 40-item self-report questionnaire that assesses both state (current, event-related) and trait (characterological) anxiety using two 20-item scales. In this study, the STAI was used to assess state anxiety. Participants completed a 20-item self-report questionnaire based on a 4-point scale ranging from 1 (“not at all”) to 4 (“very much”). Example items include statements such as, “I am calm”, “I am worried”. Low, median, and high scores indicate mild, moderate, and severe forms of anxiety, respectively. A score >40 is considered high.

2.3. Laboratory

A venous blood sample was obtained from each participant and analyzed for complete blood count as well as a comprehensive metabolic panel to assess current kidney and liver function and electrolyte and acid/base balance. Additional blood samples were frozen at −80 °C, stored, and later assayed in batch to measure levels of cytokines.

Immune assays (R&D Systems, Minneapolis, MN, quantitative ELISA) included high-sensitivity interleukin (IL) 6 and 8 (IL-6, IL-8), IL-1 receptor antagonist (IL1-ra), C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and soluble TNF receptor (sTNFR75), and monocyte chemotactic protein-1 (MCP-1). sTNFR75 provides another index of the function of TNF-α. CRP was assessed as a general measure of inflammation and acute phase reactant production. Assays were performed at the Cytokine Analysis Facility of the Bioanalytical Core Laboratory at the University of North Carolina at Chapel Hill (IL-6, CRP, TNF-a, sTNFR75) and University Hospital of Geneva, Switzerland (IL-8, IL1-Ra, MCP-1, RANTES).

For some determinants, IL-8, IL-1Ra, MCP-1 and CCL5/RANTES were measured by a commercially available multiplex beads immunoassay, based on the Luminex platform (Fluorokine MAP