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Acute psychological stress induces short-term variable immune response

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ABSTRACT

In spite of advances in understanding the cross-talk between the peripheral immune system and the brain, the molecular mechanisms underlying the rapid adaptation of the immune system to an acute psychological stressor remain largely unknown. Conventional approaches to classify molecular factors mediating these responses have targeted relatively few biological measurements or explored cross-sectional study designs, and therefore have restricted characterization of stress–immune interactions. This exploratory study analyzed transcriptional profiles and flow cytometric data of peripheral blood leukocytes with physiological (endocrine, autonomic) measurements collected throughout the sequence of events leading up to, during, and after short-term exposure to physical danger in humans. Immediate immunomodulation to acute psychological stress was defined as a short-term selective up-regulation of natural killer (NK) cell-associated cytotoxic and IL-12 mediated signaling genes that correlated with increased cortisol, catecholamines and NK cells into the periphery. In parallel, we observed down-regulation of innate immune toll-like receptor genes and genes of the MyD88-dependent signaling pathway. Correcting gene expression for an influx of NK cells revealed a molecular signature specific to the adrenal cortex. Subsequently, focusing analyses on discrete groups of coordinately expressed genes (modules) throughout the time-series revealed immune stress responses in modules associated to immune/defense response, response to wounding, cytokine production, TCR signaling and NK cell cytotoxicity which differed between males and females. These results offer a spring-board for future research towards improved treatment of stress-related disease including the impact of stress on cardiovascular and autoimmune disorders, and identifies an immune mechanism by which vulnerabilities to these diseases may be gender-specific.

1. Introduction

Chronic psychosocial and emotional distress impact immune function which leads to increased risk for disease. Current estimates forecast that by year 2030, stress-related pathologies will lead as the most debilitating and widespread health disorders (Mathers et al., 2008). At the same time, while chronic stress-related effects upon the immune system are uniformly deleterious, acute stress appears to have both protective and adverse effects. For example, acute stress can enhance the acquisition and expression of immunoprotection by activation of bodily defences prior to wounding or infection (Ackerman et al., 2002; Amkraut et al., 1971; Charney, 2004; Dhabhar, 2009), or alternatively induce immunopathology via exacerbating autoimmune inflammation, with respiratory and cardiovascular consequences (Al'Abadie et al., 1994; Black, 2006; Bosch et al., 2003; Dhabhar et al., 1995; Garg et al., 2001). The dissociation between excitatory and inhibitory molecular mechanisms remains incomplete. A more detailed

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understanding of immunomodulation throughout acute stress in humans is necessary not only to clinically reduce immunopathology, but also to harness stress-related immunoprotective effects.

One primary mechanism by which acute psychological stress induces immune response is through rapid changes in leukocyte distributions in the peripheral circulation (Bosch et al., 2005). Studies investigating acute short-term stressors in humans, such as public speaking, have reported brief increases of natural killer (NK) cell numbers and other leukocyte subtype cell numbers, a reduction in lymphocyte proliferation, an increase in pro-inflammatory cytokine production, and reduced healing capacity of the skin (Altemus et al., 2001; Segerstrom and Miller, 2004). Studies of acute (psychological) stress due to physical danger have used first-time tandem skydive (Mujica-Parodi et al., 2014; Schedlowski et al., 1993), as this challenge has the advantage of representing real risk, eliciting reliable effects, and yet permitting a high degree of experimental control. Studies using this paradigm report transient increases of T cells and NK cells in the blood, as well as a parallel increase in NK cell cytotoxic activity. This suggests that changes in leukocyte numbers may be an important mediator of apparent changes in leukocyte activity. Comparably, an equivalent study of bungee jumping reported increases in neutrophils, pro-inflammatory monocytes, and $CD8^+$ T cell numbers following the jump (van Westerloo et al., 2001).

While these studies are suggestive, one important limitation until recently has been the lack of computational and molecular approaches for large-scale immune system monitoring. Microarray analysis of blood transcriptional profiles offers a means to investigate immunological mechanisms relevant to acute psychological stress on a genome-wide scale. To complement these data, network analyses have been used in the field of immunology to identify the groups of coordinately expressed transcripts (modules) that are involved in the response of immune cells to immunomodulatory factors (i.e. acute stress). Indeed, the probability for multiple transcripts to follow a complex pattern of expression across dozens of participants throughout a time-series only by chance is low, and such sets of genes should therefore constitute coherent biologically meaningful transcriptional modules.

To exploit these capabilities, we performed a detailed molecular and cellular analysis upon two cohorts of participants undergoing their first-time tandem skydives. We first applied a comparative analysis of peripheral blood leukocyte (PBL) gene expression profiles between the four time-points (i) baseline, (ii) leading up to, (iii) during, and (iv) after each skydive to identify a unique panel of candidate stress responsive genes, which were validated by RT-qPCR assays. An unsupervised network analysis was then used to identify coordinately expressed genes (modules) involved in the short-term variable immune response to acute stress while considering gender-specific effects. Finally, the implications of gene expression analysis with respect to cell subset changes were validated by flow cytometry on a second cohort of participants.

2. Materials and methods

2.1. Ethical approval

State University of New York at Stony Brook and the University of California San Diego Institutional Review Boards approved this study. Thirty-nine skydivers participated in this study consisting of 13 subjects for RNA expression profiles (7 male, 6 female) and 26 subjects for flow cytometry (17 male, 9 female). All skydivers provided written consent prior to participation. Participants were recruited from individuals who independently contacted an area skydiving school (Skydive Long Island, Calverton, NY) to schedule their first-time tandem skydive. Skydivers were healthy adult subjects with no history of cardiac or mental illness, as determined by physical examination, medical history, and screening using the Structured Clinical Interview for DSM-IV.

2.2. Subjects and sample collection schedule

The study protocol adhered to a strict timeline for sample and data collection. Baseline blood samples were collected at 9:15 am within one week prior to or after the day of the skydive during a hospitalized testing that was time-locked to data collection during the skydive day and therefore served as a baseline and control. On the skydive day, all skydivers awoke at 6:30 am and arrived at Stony Brook University Hospital at 7:30 am. "Pre-boarding" samples were collected at 9:15 am, 1 h before take-off. Take-off occurred at 10:15 am, and the jump occurred at 10:30 am when the airplane reached an altitude of 11,500 feet (3,505.2 m). Skydivers landed at about 10:35 am and "post-landing" samples were collected at 10:45 am. Skydivers were immediately transported to Stony Brook University Hospital for a final blood draw at 11:30 am ("1 h post-landing" sample). Saliva was collected every 15 min from 9:15 am to 11:30 am on both the skydive and baseline hospital day.

2.3. RNA isolation and microarray gene expression analysis

Ten milliliters of blood were collected for each blood draw in an EDTA coated vacutainer blood collection tube and leukocytes were fractionated by passing the blood through LeukoLOCK filters. RNA isolation was performed using the LeukoLOCK Total RNA Isolation Kit and 100 ng of total RNA were used as starting material. RNA with a 260/280 ratio >1.7 and a RIN >6 was considered suitable for microarray analysis. Synthesis of cDNA and biotinylated cRNA and hybridization of cRNA to Illumina HumanHT12 v4 BeadChips (47,231 probes). Because the integrity of RNA was of low quality for three subjects, partially paired data was analyzed (Table S1).

2.4. Data pre-processing

Quality control of microarray data, variance-stabilizing transformation (vst), robust-spline normalization and removal of genes not expressed in any of the samples was performed in the R statistical computing environment version 2.8.0, using the Bioconductor package *lumi* (Du et al., 2008). Probes lacking gene symbol annotations were removed while probes with duplicate gene symbols were selected on the basis of having a higher average expression across all samples. This final filtering step left a total of 18,129 probes that passed into our subsequent analyses. We used two methods to identify outlier samples (2.5 standard deviations ± mean) for quality control: clustering analysis based on Pearson correlation and average distance metric and principal component analysis (PCA) using the first three components. This reduced our sample size from 50 subjects to a total 45 subjects (Table S1). The resulting quality-control treated data were used as input for differential expression and WGCNA analyses.

2.5. Differential gene expression analysis

We measured differential expression with respect to gene expression at baseline for each time point using 18,129 probes, correcting for gender differences. Differentially expressed genes were assessed using the moderated *t*-test in LIMMA (Smyth, 2005), and unless otherwise specified, a highly statistically significant threshold of p-value <0.01 was used. To ensure that genes found significantly differentially expressed post-landing were not solely a consequence of increased proportion of NK cells, we used a multivariate linear model to regress individual gene expression