Brain, Behavior, and Immunity 25 (2011) 302–313

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

A pilot study of immune network remodeling under challenge in Gulf War Illness

Gordon Broderick [a,*], Andrea Kreitz [a], Jim Fuite [a], Mary Ann Fletcher [b], Suzanne D. Vernon [c], Nancy Klimas [b,d]

[a] Department of Medicine, University of Alberta, Edmonton, Canada
[b] Department of Medicine, University of Miami, Miami, FL, USA
[c] The CFIDS Association of America, Charlotte, NC, USA
[d] Miami Veterans Affairs Medical Center, Miami, FL, USA

ARTICLE INFO

Article history:
Received 16 July 2010
Received in revised form 24 September 2010
Accepted 11 October 2010
Available online 16 October 2010

Keywords:
Cytokines
Network theory
Immune signaling
Gulf War
Sequential networks
Propagation of influence
Exercise challenge
Mathematical immunology

ABSTRACT

Gulf War Illness (GWI) is a complex disorder affecting nervous, endocrine and immune regulation. Accordingly, we propose that GWI presents with a distinct pattern of immune signaling. To explore this we compared interaction patterns linking immune markers and their evolution during exercise. Blood was collected from 9 GWI and 11 control subjects prior to a Graded eXercise Test (GXT) (t_0), at peak effort (t_1) and 4 h post-exercise (t_2). Salivary cortisol and plasma, serum or culture supernatants were analyzed for concentrations of neuropeptide Y (NPY), IL-1α, IL-5, IL-6, IL-10, TNF-α, IFN-γ and soluble CD26 (sCD26). Immune cell populations were surface stained for CD19, CD2, CD3, CD4, CD8, CD26, CD56, CD16, and CD11a. Mutual information (MI) networks linking these immune markers were generated in each group at each time point. Graph theory was used to describe the evolution of each network's structure and identify potential nucleating points. Distinct in topology, GWI networks had more abundant connections but were less organized. NPY, IL-1α, TNF-α and CD2+/CD26+ nodes were better integrated in the GWI network at rest. Under effort (t_1) these differences were replaced by significant restructuring around nodes for CD19+ B cell population, IL-5, IL-6 and soluble CD26 concentrations. This pattern subsided post-exercise. Further analysis indicated that IL-1α and CD2+/CD26+ nodes strongly influenced this characteristic modulation of B and T cell network motifs. This potentially heightened lymphocyte and HPA axis responsiveness to IL-1 stimulation in the context of a mixed Th1:Th2 immune signature supports an autoimmune component in GWI etiology.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Within months after their return from Operation Desert Storm an alarming number of Gulf War veterans began to report a variety of symptoms, including fatigue, musculoskeletal discomfort, skin rashes, and cognitive dysfunction (Haley, 1997; Fukuda et al., 1998; Wolfe et al., 1998). Several models have been proposed linking these symptoms to the hazardous conditions encountered by servicemen including infectious agents, medical prophylaxis, depleted uranium as well as chemical and biological warfare agents, and the psychological stressors of combat (Fukuda et al., 1998; Rijpkema et al., 2005; Gronseth, 2005). However these remain speculative and we still have no clear understanding of the pathophysiology of Gulf War Illness (GWI) nor do we have a characteristic biomarker for this illness. As the body is regulated by a number of highly integrated systems, consideration of the basic physiology of response to stress whether psychological, chemical or other may provide a useful starting point. Indeed clinical presentation of this illness shares dimensions with that of another stress-mediated illness namely Chronic Fatigue Syndrome (CFS) (Kang et al., 2003; Eisen et al., 2005).

Activation of the "fight or flight" response has a broad and significant impact on the body including modulation of the function and status of the immune system (Silverman et al., 2005). Not surprisingly several facets of immune dysregulation have been reported in these subjects by members of our team (Maher et al., 2003) including seminal work describing a significant expansion in activated CD2+/CD26+ T cell population (Klimas et al., 1990). A major contributor to the regulation of CD4+ T, NK and NK T cells, CD26 also plays a major role in T cell-dependent antibody production and immunoglobulin isotype switching in B cells. Abnormal expression is found in autoimmune diseases, HIV-related illness and cancer. Increased CD20+/CD25+ and decreased CD20+/CD23+ B cell populations were observed in CFS by Robertson et al. (2005) however these trends did not achieve statistical significance ($p < 0.01$). This was not the case for GWI patients where a significantly elevated CD19+ B cell population was reported along with

* Corresponding author. Address: Division of Pulmonary Medicine, Department of Medicine, University of Alberta, Suite 225B, College Plaza, 8215 112 Street NW, Edmonton, Alberta, Canada T6G 2C8. Fax: +1 780 407 3027.
E-mail addresses: gordon.broderick@ualberta.ca (G. Broderick), akreitz@ualberta.ca (A. Kreitz), jfuite@phys.ualberta.ca (J. Fuite), MFletche@med.miami.edu (M.A. Fletcher), sdvernon@cfids.org (S.D. Vernon), Nancy.Klimas@va.gov (N. Klimas).

0889-1591/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2010.10.011

increased concentration of auto-antibody directed against myelin basic protein (MBP) as well as striated and smooth muscle (Vojdani and Thrasher, 2004). In addition both CFS (Robertson et al., 2005) and GWI (Vojdani and Thrasher, 2004) patients also exhibited greater numbers of CD3−/CD16+ NK cells. CD25+/CD56+ and CD2−/CD56+ fractions also appeared higher in CFS but not with statistical significance. While NK cells may be greater in number we have found they display an impaired cytotoxicity (Fletcher et al., 2002; Siegel et al., 2006), a result confirmed by other groups (Vojdani and Thrasher, 2004). Indeed significantly reduced levels of intracellular perforin have been observed in CD3−/CD56+ NK and CD3+/CD8+ cytotoxic T cells in these veterans (Whistler et al., 2009).

Though frequently studied in isolation these immune cell subsets exist in the context of a larger and well-integrated community. It is reasonable to expect therefore that these anomalies in cell population would also manifest as altered patterns of cytokine signaling. Indeed veterans with CFS present with significantly higher levels of IL-2, IL-10, IFN-γ, and TNF-α than non-fatigued veterans (Zhang et al., 1999). Similarly Skowera et al. (2004) reported significantly elevated levels of IL-2, IFN-γ and IL-4 producing CD4+ cells in non-stimulated culture compared with asymptomatic veterans. Expression of IL-4 in these subjects was lost however after controlling for vaccination status, depression and atopic illness. In vitro polyclonal activation also revealed significantly increased levels of IL-10 producing memory CD4+ cells in symptomatic veterans. More recently members of our group reported concurrent expression of IFN-γ with Th2 cytokine IL-5 in PHA-stimulated culture during the course of a standardized exercise challenge (Whistler et al., 2009). This would suggest a concurrent Th2 component and argue against conventional Th1 polarization.

Though elements of immune involvement in GWI and CFS have been reported, many of these reports are conflicting and the nature of this involvement remains unclear. Unfortunately these studies typically focused on a narrow segment of the diverse cell types that compose the immune community and the signals that coordinate their interaction (Maher et al., 2003). By the same token no attempt is made to cast individual molecular messages in the greater context of concurrent immune signaling and changing cellular demographics. Indeed, analysis of these biological systems as coordinated networks has received relatively little attention. Efforts have focused largely on the visual inspection of small assemblies of known pathway elements (Kerr et al., 2008a,b; Whistler et al., 2009). Only recently has graph theory been used to quantitatively describe broad shifts in molecular interaction across phenotypes (Emmert-Streib, 2007). We have since extended this work by defining methods for identifying individual elements (Fuite et al., 2008) and sub-assemblies (Broderick et al., 2010) driving illness-mediated shifts in network topology.

In this work we apply these graph theoretical methods to the integration and analysis of a broad spectrum of cellular and molecular markers describing the immune status of Gulf War veterans. Once again our hypothesis is that immune abnormalities in GWI like CFS (Broderick et al., 2010; Fuite et al., 2008) are characterized not only by persistent low-grade inflammation but also more importantly by changes in the patterns of immune signaling suggestive of a change in functional mode. In an extension of previous analyses we now propose a methodology for integrating multiple networks constructed at sequential time points along the course of an exercise challenge. Our results indicate that immune networks not only differ significantly between groups but that network structure in GWI is bulkier and potentially less efficient in its response to exercise challenge. The latter is characterized by a shift in the balance of Th1 and Th2 signals under effort suggestive of an auto-antibody driven immune cascade. The propagation of interactions across time suggests that this characteristic evolution in network structure may be triggered or amplified by initial changes in IL-1α and CD2+/CD26+ cell abundance. This result would not have emerged from a conventional analysis of expression levels. We emphasize that because of the limited number of subjects involved this remains a preliminary analysis and these findings are presented as potential focal points for further investigation.

2. Materials and methods

2.1. Sample collection and processing

2.1.1. Cohort recruitment

As part of a larger ongoing study a subset of 10 GWI and 11 control subjects recruited from the Miami Veterans Administration Medical Center were used in the present analysis. Subjects were male and ranged in age between 30 and 55. Inclusion criteria was derived from Fukuda et al. (1998), and consisted in identifying veterans deployed to the theater of operations between August 8, 1990 and July 31, 1991, with one or more symptoms present after 6 months from at least 2 of the following: fatigue; mood and cognitive complaints; and musculoskeletal complaints. Subjects were in good health prior to 1990, and had no current exclusionary diagnoses (Reeves et al., 2003). Medications that could have impacted immune function were excluded. Use of the Fukuda definition in GWI is supported by Collins et al. (2002). Control subjects consisted of gulf war era sedentary veterans and were matched to GWI subjects by age, body mass index (BMI) and ethnicity.

Ethics statement: All subjects signed an informed consent approved by the Institutional Review Board of the University of Miami. Ethics review and approval for data analysis was also obtained by the IRB of the University of Alberta.

2.1.2. Subject assessment

All subjects received a physical examination and medical history including the GWI symptom checklist as per the case definition. Psychometric questionnaires included the Multidimensional Fatigue Inventory (MFI) (Smets et al., 1995), a 20-item self-report instrument designed to measure fatigue, and the Medical Outcomes Study 36-item short-form survey (SF-36) (Ware and Sherbourne, 1992) assessing health-related quality of life. Results of these psychometric surveys may be found in additional file Appendix A (Table S7). With only one exception (physical functioning index), SF-36 scores were significantly lower and MFI scores significantly higher in GWI patients than in healthy controls ($p < 0.05$).

Immune response was stimulated by administering a standard Graded eXercise Test (GXT) using a Vmax Spectra 29c Cardiopulmonary Exercise Testing Instrument, Sensor-Medics Ergoline 800 fully automated cycle ergometer, and SensorMedics Marquette MAX 1 Sress ECG. The McArdle protocol (McArdle et al., 2007) was used where subjects pedal at an initial output of 60 W for 2 min, followed by an increase of 30 W every 2 min until the subject reaches: (1) a plateau in maximal oxygen consumption (VO2); (2) a respiratory exchange ratio above 1.15; or (3) the subject stops the test. Prior to the exercise challenge a first blood draw was conducted subsequent to a 30-min rest. Second and third blood draws were conducted upon reaching peak effort (VO2 max) and at 4 h post-exercise, respectively. Importantly, these assessments were conducted at the same time of day for all subjects to control for diurnal variations in cortisol and other similarly affected indicators.

2.1.3. Laboratory analyses

At each blood draw five 8-mL tubes of blood were collected in CPT vacutainers (B-D-Biosciences, San Jose, CA). Heparinized whole blood was then cultured 48 h with phytohemagglutinin at 37 °C,

Download English Version:

https://daneshyari.com/en/article/922668

Download Persian Version:

https://daneshyari.com/article/922668

Daneshyari.com