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14. ABSTRACT
The primary goal of this study was to compare neuroimaging, immune-related genetics, and laboratory tests in veterans with GWI to those obtained from veterans with known autoimmune disorders, to determine the extent to which GWI reflects autoimmune abnormalities. Published findings from 193 completed acquisitions demonstrated the following: (1) brain function in GWI is indistinguishable from that of established autoimmune conditions, (2) specific human leukocyte antigen genes protect against brain structural alterations common in GWI; and (3) peripheral inflammatory markers are highly associated with GWI symptom domains involving the brain as well as with alterations in brain structure and axonal integrity.

15. SUBJECT TERMS
Gulf War Illness, Autoimmune, neuroimaging, genetics, biomarkers

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1. **INTRODUCTION:**

GWI has affected a large number of veterans of the 1990-1991 Persian Gulf War. GWI symptoms are characterized by chronic health problems, of unknown etiology. They resemble symptoms seen in various autoimmune disorders and are reflected in altered patterns of brain function. In this study, we comprehensively assessed the association of GWI to autoimmune disorders using cutting-edge measures of brain structure and function, genetic analysis, and laboratory tests. In preliminary studies, we had discovered that GWI possesses a distinct functional brain pattern that is very close to that observed in a well-known autoimmune disorder, Sjogren's syndrome. Hence, the main goal of this proposal was to test the hypothesis that GWI is an autoimmune disorder. For that purpose, we compared the results of brain, genetic and laboratory tests in subjects with GWI to those obtained from subjects with known autoimmune disorders, to determine the extent to which GWI reflects autoimmune abnormalities, with the ultimate goal of informing treatment.

2. **KEYWORDS:** Gulf War Illness, autoimmune, neuroimaging, genetics, biomarkers

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

The major goals of the project were to assess and compare 1) brain structure and function, 2) blood inflammatory and immune markers; 3) HLA genes; and 4) cognitive, mental health, neurological and general standardized clinical status in veterans with Gulf War Illness relative to veterans with autoimmune disorders.

- **What was accomplished under these goals?**

A total of 193 veterans (99 GWI) completed acquisitions, resulting in several published manuscripts. Overall, the findings indicate the following: (1) brain function in GWI is indistinguishable from that of established autoimmune conditions, (2) specific human leukocyte antigen genes protect against brain structural alterations common in GWI; and (3) peripheral inflammatory markers are highly associated with GWI symptom domains involving the brain as well as with alterations in brain structure and axonal integrity. Each of these findings is detailed below.

Brain function in GWI is indistinguishable from that of established autoimmune conditions.
Synchronous neural interactions, a measure of neural communication, have been shown to distinguish between conditions affecting the brain with a high degree of accuracy. Here we compared synchronous neural interactions derived from magnetoencephalography in veterans with GWI, healthy controls, and seven other diseases to determine which, if any, of the other diseases might have SNI that is similar to GWI. We found GWI SNIs differed significantly from controls and psychiatric conditions (schizophrenia, Alzheimer's disease, posttraumatic stress disorder, and major depressive disorder) but not from established autoimmune conditions (relapsing-remitting multiple sclerosis, Sjogren's syndrome, and rheumatoid arthritis). We also

found that brain correlates of immunogenetic protection conferred by human leukocyte antigen was did not differ between GWI and immune-related conditions but was highly different from controls and those with psychiatric conditions. The findings indicate that GWI brain synchronicity does not differ significantly from that of known immune-related diseases that this SNI similarity is present within the HLA-related SNIs. Consequently, we concluded that altered brain communication in GWI likely reflects immune-related processes. By extension, these findings also indicate that functional brain abnormalities in autoimmune conditions might be, in part, due to lack of protective HLA alleles as documented for GWI (Georgopoulos et al., EBioMedicine 3:79–85, 2015).

Georgopoulos AP, James LM, Carpenter AF, Engdahl BE, Leuthold AC, Lewis SM. Gulf War illness (GWI) as a neuroimmune disease. Experimental Brain Research. 2017 Oct 1;235(10):3217-25

Specific human leukocyte antigen genes protect against GWI-related brain structural alterations.

In separate prior publications we had documented the presence of subcortical brain atrophy in GWI and had identified human leukocyte antigen alleles that protect against GWI in a dose-dependent manner. Here we evaluated protective effects of a specific allele – HLA DRB1*13:02 – on brain volume using structural magnetic resonance imaging. We found that DRB1*13:02 spared subcortical brain, with the most significant differences between carriers and non-carriers found in the cerebellar gray matter. The findings document the protective effect of DRB1*13:02 on brain atrophy in Gulf War veterans. We hypothesized that the protection conferred by this allele is due to successful elimination of external foreign antigens to which Gulf War veterans were exposed.

James, L.M., Christova, P., Engdahl, B. E., Lewis, S. M., Carpenter, A. F., & Georgopoulos, A. P. Human Leukocyte Antigen (HLA) and Gulf War Illness (GWI): HLA-DRB1*13:02 spares subcortical atrophy in Gulf War veterans. Ebiomedicine 2017; 26: 126-131. doi: 10.1016/j.ebiom.2017.11.005

Taken together with prior studies in our lab, there is compelling evidence pointing to the role of human leukocyte antigens in moderating disease outcomes. To that end, we wrote a mini-review discussing the role of human leukocyte antigens in eliminating foreign antigens and describing how the breakdown of that process permits the foreign antigens to persist, thereby contributing to immune-related disease including GWI.

James LM, Georgopoulos AP. Persistent antigens hypothesis: the human leukocyte antigen (HLA) connection. J. Neurol. Neuromed. 2018;3:27-31.

Peripheral inflammatory markers are highly associated with GWI symptom domains involving the brain as well as with alterations in brain structure and axonal integrity.

Here we investigated the association between GWI symptoms and C-reactive protein (CRP), a marker of inflammation, in veterans with GWI. The results indicated a highly significant positive association between CRP and GWI symptom severity in several domains, most of which involve the brain - pain, neurocognitive/mood, fatigue, and respiratory. The results support the premise that GWI symptoms, particularly those implicating brain involvement, are a result of neuroinflammation. Though the cause for inflammation is uncertain, we have hypothesized harmful persistent antigens stemming from environmental exposures associated with service during the Gulf War in those lacking immunogenetic protection against them are at the root of it. that could not be successfully eliminated due to lack of specific

James LM, Engdahl BE, Johnson RA, Georgopoulos AP. Gulf War Illness and Inflammation: Association of symptom severity with C-reactive protein. J Neurol Neuromed. 2019;4(2):15-9.

Subsequent analyses demonstrated that C-reactive protein is also associated with brain atrophy and alterations in axonal integrity, particularly involving the fornix, in GWI. These analyses were presented at the Society for Neuroscience and manuscripts stemming from that presentation are currently under review for publication.
Christova PS, Engdahl BE, James LM, Johnson RA, Carpenter AF, Lewis SM, Georgopoulos AP. 2019. Gulf War illness brain and inflammation: Association of brain atrophy and axonal integrity with C-reactive protein. Poster presented at the annual meeting for the Society of Neuroscience; Oct 23 2019; Chicago, IL.

- **What opportunities for training and professional development has the project provided?**

The project provided professional development opportunities for study staff via presentation of research findings and networking opportunities at local and international conferences (i.e, Society for Neuroscience).

- **How were the results disseminated to communities of interest?**

Findings supported by this grant were disseminated via presentation at local (Minneapolis VA Medical Center) and international conferences, and via publication. In addition, findings supported by this grant were disseminated in the 2018 and 2020 CDMRP Gulf War Illness Research Program Booklets.

- **What do you plan to do during the next reporting period to accomplish the goals?**

Three manuscripts stemming from this project are currently under review with several additional manuscripts planned and/or in preparation.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**

The findings from the grant have demonstrated similar brain signatures in autoimmune disorders and GWI as well as a genetic lack of protection involving immune-related genes in the development of GWI-related brain effects. In particular, genes that have been shown to protect against various autoimmune disorders are also protective against GWI. These findings implicate immune system functioning in the development of GWI and point to exposure to external antigens in genetically vulnerable individuals as likely contributing to GWI. That is, GWI is thought to stem from exposure to foreign antigens that could not be successfully eliminated due to lack of specific immunity, causing the antigens to persist. The persistent antigens are presumed to underlie the inflammation that is observed in GWI. The findings have further demonstrated that peripheral inflammation (elevated C-reactive protein) is associated with GWI symptoms, including several involving the brain, along with evidence of brain alterations. Taken together, these findings implicating persistent antigens stemming from lack of immunogenetic protection against them, open avenues for potential treatment of GWI along the lines of

immunotherapy. Indeed, based in part on findings from this project, immunotherapy-based in vitro studies of GWI are currently underway in our lab.

- **What was the impact on other disciplines?**

The findings from this study led to investigations about immune-related genetic involvement in brain aging and, most recently, dementia. Two published papers have demonstrated that the same immune-related genes that are generally lacking in veterans with GWI and contribute to brain atrophy in GWI via inability to eradicate circulating antigens are associated with brain atrophy and neural network variability in healthy brain aging (James et al. 2018 EBioMedicine 29, 31-37; James et al., 2018 EBioMedicine 35, 288-294). Furthermore, two recent genetic epidemiology studies demonstrated that the frequency of those same genes are inversely related to dementia prevalence in 14 European countries (James & Georgopoulos, 2019 J Neurology Neuromed 4(5), 1-6; James & Georgopoulos 2020, J Neurol Neuromed 5(1), 12-17). Thus these findings suggest that, like GWI, brain aging and dementia may partially result from exposure to persistent antigens in those lacking immunogenetic (i.e,. HLA) protection against them.

- **What was the impact on technology transfer?**

Nothing to report.

- **What was the impact on society beyond science and technology?**

Despite 25 years of research, GWI has been poorly understood and even attributed to psychological distress, hampering efforts to effectively treat affected veterans. The findings from this project substantiate GWI as a medical condition, highlight genetic susceptibility to GWI, and offer insights regarding potential treatments. Taken together, these findings legitimize the difficulties of GWI veterans and offer hope for treatment to veterans who have suffered for decades.

5. **CHANGES/PROBLEMS:**

- **Changes in approach and reasons for change**

Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Though the project was delayed due to catastrophic failure of the magnetoencephalogram and equipment/technology updates to the MRI, the primary study goals were attained within the primary study period and subsequent no-cost extensions.

- **Changes that had a significant impact on expenditures**

Nothing to report.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

- **Significant changes in use or care of human subjects.**

Nothing to report.

- **Significant changes in use or care of vertebrate animals.**

Not applicable.

- **Significant changes in use of biohazards and/or select agents**

Not applicable.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**
  Journal publications.

Georgopoulos AP, James LM, Carpenter AF, Engdahl BE, Leuthold AC, Lewis SM. Gulf War illness (GWI) as a neuroimmune disease. Experimental Brain Research. 2017 Oct 1;235(10):3217-25. doi: 10.1007/s00221-017-5050-0

James, L.M., Christova, P., Engdahl, B. E., Lewis, S. M., Carpenter, A. F., & Georgopoulos, A. P. Human Leukocyte Antigen (HLA) and Gulf War Illness (GWI): HLA-DRB1*13:02 spares subcortical atrophy in Gulf War veterans. Ebiomedicine 2017; 26: 126-131. doi: 10.1016/j.ebiom.2017.11.005

James LM, Georgopoulos AP. Persistent antigens hypothesis: the human leukocyte antigen (HLA) connection. J. Neurol. Neuromed. 2018;3:27-31.

James LM, Engdahl BE, Johnson RA, Georgopoulos AP. Gulf War Illness and Inflammation: Association of symptom severity with C-reactive protein. J Neurol Neuromed. 2019;4(2):15-9.

- **Books or other non-periodical, one-time publications.**

Nothing to report.

- **Other publications, conference papers, and presentations.**

Christova PS, Engdahl BE, James LM, Johnson RA, Carpenter AF, Lewis SM, Georgopoulos AP. 2019. Gulf War illness brain and inflammation: Association of brain atrophy and axonal integrity with C-reactive protein. Poster presented at the annual meeting for the Society of Neuroscience; Oct 23 2019; Chicago, IL.

- **Website(s) or other Internet site(s)**

http://brain.umn.edu

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

We have developed a database in order to facilitate data analysis and dissemination of research findings.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Apostolos Georgopoulos
Brian Engdahl
Lisa James
Arthur Leuthold
Adam Carpenter

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report.

- **What other organizations were involved as partners?**

Nothing to report.

8. **SPECIAL REPORTING REQUIREMENTS**

Nothing to report.

9. **APPENDICES**

Publications related to this project attached.

Exp Brain Res (2017) 235:3217–3225
DOI 10.1007/s00221-017-5050-0

RESEARCH ARTICLE

Gulf War illness (GWI) as a neuroimmune disease

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Abstract Gulf War illness (GWI) is a chronic disease characterized by the involvement of several organs, including the brain (Christova et al., Exp Brain Res doi:10.1007/s00221-017-5010-8, 2017). In a previous study (Georgopoulos et al., J Neural Eng 4:349–355, 2015), we identified six protective alleles from Class II human leukocyte antigen (HLA) genes, and more recently, we investigated the brain correlates of this protection (James et al., EBioMedicine 13:72–79, 2016). Those and other studies (Israeli, Lupus, 21:190–194, 2012) suggested an involvement of the immune system in GWI. In a recent study (Engdahl et al., EBioMedicine doi:10.1016/j.ebiom.2016.08.030, 2016), we showed that the brain pattern of synchronous neural interactions (SNI; Georgopoulos et al., J Neural Eng 4:349–355, 2007) in GWI is distinctly different from that in healthy controls. Here we focused on the SNI itself, as a basic measure of neural communication (irrespective of specific connections) and compared it between GWI and seven other diseases that cover a broad spectrum of etiology and pathophysiology. Specifically, we sought to determine which, if any, of those diseases might resemble GWI SNI, overall and within the HLA protective domain, and thus gain further knowledge regarding the nature of GWI brain abnormality. We studied a total of 962 participants from a healthy control population ($N = 583$) and eight different diseases, including GWI ($N = 40$), schizophrenia (SZ; $N = 21$), Alzheimer's disease (AD; $N = 66$), posttraumatic stress disorder (PTSD; $N = 159$), major depressive disorder (MDD; $N = 10$), relapsing–remitting multiple sclerosis (RRMS; $N = 43$), Sjögren's syndrome (SS; $N = 32$), and rheumatoid arthritis (RA; $N = 8$). They all underwent a resting-state magnetoencephalographic (MEG) scan to calculate SNIs. Data were analyzed using analysis of covariance (ANCOVA) with disease as fixed factor, and sex and age as covariates. We found that GWI SNIs differed significantly from control SZ, AD, PTSD and MDD but not from RRMS, SS and RA. In addition, we compared GWI to RRMS, SS and RA with respect to SNIs of MEG sensor pairs that were related to the HLA alleles protective for GWI (James et al., EBioMedicine 13:72–79, 2016). We found that GWI SNIs did not differ significantly from any of these three diseases but they did so from control SZ, AD, PTSD and MDD. These findings indicate that (a) GWI brain synchronicity does not differ significantly from that of known immune-related diseases (RRMS, SS, RA), and (b) that this SNI similarity is present within the HLA-related SNIs. In contrast, GWI SNIs differed significantly from those of the other diseases. We conclude that altered brain communication in GWI likely reflects immune-related processes, as postulated previously (James et al., EBioMedicine 13:72–79, 2016). By extension, these findings also indicate

Adam F. Carpenter, Brian E. Engdahl, Arthur C. Leuthold, and Scott M. Lewis contributed equally and are listed in alphabetical order.

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that functional brain abnormalities in RRMS, SS and RA might be, in part, due to lack of protective HLA alleles as documented for GWI (Georgopoulos et al., EBioMedicine 3:79–85, 2015).

Keywords Gulf War illness (GWI) · Magnetoencephalography · Human leukocyte antigen (HLA) · Veterans · Schizophrenia · Alzheimer's disease · Posttraumatic stress disorder · Major depressive disorder · Relapsing–remitting multiple sclerosis · Sjögren's syndrome · Rheumatoid arthritis

Introduction

Gulf War illness (GWI)

Twenty-five years after the 1990–1991 Persian Gulf War, approximately 250,000 veterans continue to suffer from Gulf War illness (GWI), a condition characterized by chronic and diffuse physical and mental health symptoms that are not readily explained (White et al. 2016). Typical symptoms of GWI include widespread pain, fatigue, mood disruption, cognitive impairment and neurological abnormalities as well as skin rashes, respiratory complaints, and gastrointestinal problems (Fukuda et al. 1998; Steele 2000). The etiology of GWI remains unknown and definitive pathophysiological markers have not been identified. Recently, however, several lines of research suggest a clear explanation, specifically, that GWI involves immune system disruption (Georgopoulos et al. 2015; Parkitny et al. 2015; Skowera et al. 2004; Whistler et al. 2009) which is reflected (in part) in altered brain function (Engdahl et al. 2016; James et al. 2016) in genetically vulnerable individuals (Georgopoulos et al. 2015). Here we seek to extend that line of research and clarify GWI's relation to other immune-related conditions by comparing brain synchronicity in veterans with GWI to various immune- and non-immune-related diseases.

Synchronous neural interactions (SNI)

Several magnetic resonance imaging studies have identified brain abnormalities associated with GWI (White et al. 2016), although various methodological differences have hampered identification of definitive GWI-related brain biomarkers. We have taken a different approach, focusing on SNIs derived from task-free magnetoencephalography (MEG). Healthy brain functioning is characterized by patterns of synchronized neural communications that are conserved across individuals (Langheim et al. 2006). In contrast, diseases involving the brain manifest characteristic aberrations in neural synchrony. To that end, we have demonstrated that SNIs successfully discriminate various brain disorders including schizophrenia, chronic alcoholism, Sjögren's syndrome, multiple sclerosis, Alzheimer's disease temporomandibular joint disorder (Georgopoulos et al. 2007) and posttraumatic stress disorder (Georgopoulos et al. 2010; Engdahl et al. 2010) from each other and from healthy brain functioning. More recently, we demonstrated highly accurate discrimination of veterans with GWI from healthy controls based on regional SNI distributions (Engdahl et al. 2016), further substantiating the discriminatory power of SNI. In the current study, we compare SNI in GWI with that of healthy brain functioning and seven other diseases and to determine which, if any, resemble GWI.

Rationale of the study

In the present study, we test our hypothesis that GWI is a neuroimmune disorder by comparing GWI SNI, irrespective of its regional brain distribution, to seven other diseases with neurological-cognitive-mood (NCM) symptoms of diverse etiology: schizophrenia, Alzheimer's disease, posttraumatic stress disorder, major depressive disorder, relapsing–remitting multiple sclerosis, Sjögren's syndrome, and rheumatoid arthritis. We hypothesized that GWI SNI would be similar to the latter three known immune-related diseases but not to the other conditions. Based on our prior work demonstrating HLA- and non-HLA-related brain effects on GWI symptoms (James et al. 2016), we also compared SNI across diseases with regard to HLA status.

Materials and methods

Study participants

A total of 962 human subjects participated in this study as paid volunteers. The study protocol was approved by the relevant institutional review boards and informed consent was obtained prior to the study. Exclusionary criteria included cardiac pacemakers or implanted ferrous metal, central nervous system disorders (e.g., Parkinson's disease, cerebrovascular accidents, a history of traumatic brain injury, etc.), and current alcohol or drug dependence. There were eight groups, including healthy controls (HC), patients with GWI, schizophrenia (SZ), Alzheimer's disease (AD), posttraumatic stress disorder (PTSD), major depressive disorder (MDD), relapsing–remitting multiple sclerosis (RRMS), Sjögren's syndrome (SS), and rheumatoid arthritis (RA). Demographic information (age and sex) and counts per group of zero-lag partial cross-correlations (synchronous neural interactions, SNI) are given in Table 1. The diagnoses for each patient group were made by a specialist in the respective field of medicine at the time of the study, as follows. GWI patients met both Centers for Disease Control

Table 1 Demographic and SNI information for study groups

Group	Mean (years)	SD	*N* (participants)	*N* (men)	*N* (women)	*N* (SNI)	*N* (HLA-SNI)
Control	52.1	17.6	583	446	137	15531816	15012879
GWI	50.0	7.7	40	36	4	997227	961726
SZ	45.0	9.4	21	17	4	537775	520457
AD	78.3	7.4	66	61	5	1600581	1556639
PTSD	50.9	14.8	159	139	20	4109160	3973161
MDD	50.5	11.9	10	9	1	193048	186546
RRMS	41.3	10.3	43	12	31	1195130	1148449
SS	55.3	11.0	32	4	28	867689	838534
RA	63.2	15.5	8	6	2	215331	206581

SD standard deviation, *N* counts, *GWI* Gulf War illness, *SZ* schizophrenia, *AD* Alzheimer's disease, *PTSD* posttraumatic stress disorder, *MDD* major depressive disorder, *RRMS* relapsing–remitting multiple sclerosis, *SS* Sjögren's syndrome, *RA* rheumatoid arthritis

(Fukuda et al. 1998) and Kansas (Steele 2000) criteria. SZ patients were diagnosed based on DSM-IV criteria (APA 2000), had no history of electroconvulsive therapy, no past substance dependence, no current substance/alcohol dependence or abuse, and no medical conditions that effect the central nervous system (e.g., epilepsy). AD patients were diagnosed based on an interdisciplinary consensus diagnosis conference and determined to meet criteria for (1) a diagnosis of dementia according to DSM-IV (APA 2000) and (2) possible or probable AD according to NINCDS-ARDA criteria (McKhann et al. 1984). PTSD was diagnosed using the Clinician-Administered PTSD Scale for *DSM-IV* (CAPS; Blake et al. 1995). MDD was diagnosed using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID; First et al. 2002). RRMS patients met the modified McDonald criteria (Polman et al. 2005), had greater than or equal to 10 T2 cerebral lesions, were at least 30 days post relapse or steroid burst, and had a clear relapsing–remitting MS subtype. SS patients were diagnosed based on the classification criteria by the American-European consensus group for Sjögren's syndrome (Vitali et al. 2002). They complained of cognitive dysfunction verified clinically by their physicians and by neuropsychological measurements. RA patients had their diagnosis established at the rheumatology clinic. Finally, the control group comprised age-matched subjects to the patient groups, as well as additional healthy subjects. Patients were receiving medications relevant to their brain illness; some of these medications were psychotropic.

Data acquisition

All participants underwent a magnetoencephalographic (MEG) scan. As described previously (Georgopoulos et al. 2007, 2010), subjects lay supine within the electromagnetically shielded chamber and fixated their eyes on a spot ~65 cm in front of them, for 45–60 s. MEG data were acquired using a 248-channel axial gradiometer system (Magnes 3600WH, 4-D Neuroimaging, San Diego, CA), band-filtered between 0.1 and 400 Hz, and sampled at 1017.25 Hz. Data with artifacts (e.g., from non-removable metal or excessive subject motion) were eliminated from further analysis.

Data analysis

Standard statistical methods were used to analyze the data, including analysis of covariance (ANCOVA). The following packages were employed: IBM-SPSS statistical package, version 23, Matlab (version R2015b), and ad hoc Fortran computer programs employing the International Mathematics and Statistics Library (IMSL; Rogue Wave Software, Louisville, CO, USA) statistical and mathematical libraries. Prewhitening of the raw MEG series (see below) was performed using programs in Python (Mahan et al. 2015).

Single trial MEG time series from all sensors underwent 'prewhitening' (Box and Jenkins 1976; Priestley 1981) using a (50,1,3) ARIMA model (Mahan et al. 2015) to obtain innovations (i.e., residuals). All possible pairwise zero-lag cross-correlations ($N = 30{,}628$, given 248 sensors) were computed between the prewhitened MEG time series. Finally, the partial zero-lag cross-correlations PCC^0_{ij} (SNI) between i and j sensors were computed for all sensor pairs. PCC^0_{ij} was transformed to z^0_{ij} using Fisher's (Fisher 1958) z transformation to normalize its distribution:

$$\mathrm{SNI} = z^0_{ij} = \mathrm{atanh}(\mathrm{PCC}^0_{ij}) \quad (1)$$

An analysis of covariance (ANCOVA) was used to evaluate SNI differences between GWI and the remaining eight groups. For that purpose, SNIs were pooled from all subjects in each group; the number of SNIs per group are given in Table 1. Since age and sex differed among groups (Table 1), and since the objective was to test whether GWI SNIs differed significantly from those of the other groups, eight

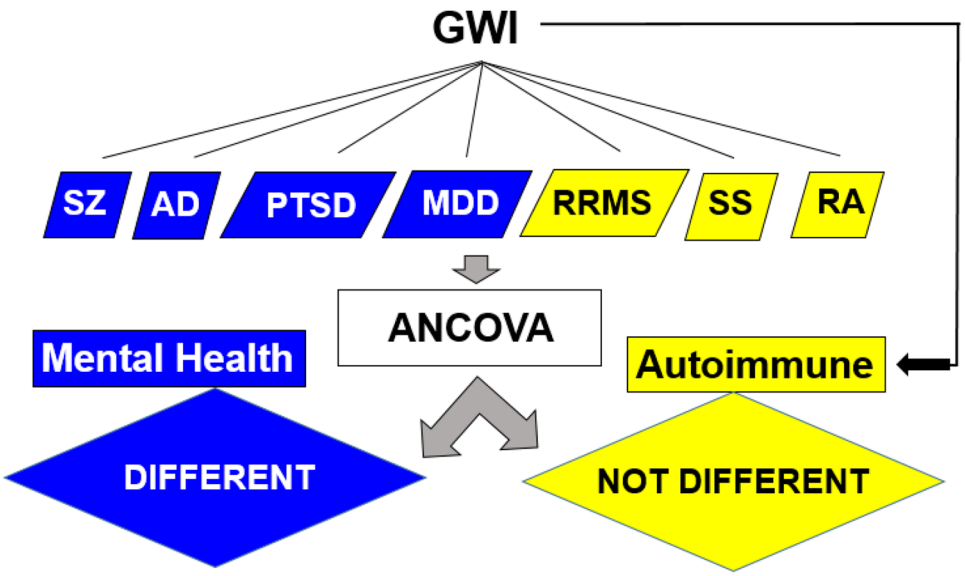

Fig. 1 Outline of study design and summary of outcomes of comparisons when all SNIs were used

ANCOVAs were carried out, one between GWI and each of the eight groups, where the SNI was the dependent variable, GWI and a specific disease were the Group fixed factor, and sex and age were covariates.

Additional analyses were performed to assess differences between GWI and other diseases in a subset of sensor pairs ($N = 29219$) the SNIs of which were found previously to possess a significant relation to the presence of any one (or more) HLA alleles protective for GWI (James et al. 2016; Georgopoulos et al. 2015) with respect to NCM symptom severity. Therefore, eight additional ANCOVAs as above were performed for this HLA-related SNI subset.

Table 2 Results of ANCOVA comparing GWI to other diseases using all SNIs

Group	*F*	*df* (denominator)	*P* value
Control	10.686	16529039	0.001
SZ	62.968	1534998	2.1×10^{-15}
AD	9.142	2597804	0.0025
PTSD	45.289	5106383	1.7×10^{-11}
MDD	93.328	1190271	4.4×10^{-22}
RRMS	2.157	2192353	0.142
SS	1.460	1864912	0.227
RA	1.707	1212554	0.191

Numerator *F* degrees of freedom = 1 for all ANCOVAs. Disease abbreviations are as in Table 1

F F test for the Group factor in the ANCOVA, *df* degrees of freedom

Results

All sensor pairs (Fig. 1)

GWI SNIs differed significantly from those in the control group ($P = 0.001$, *F* test in ANCOVA; Table 2). The results of the comparisons of GWI with the other seven disease groups are given in Table 2 and shown in Fig. 2. Of the seven diseases, GWI SNIs were highly significantly different from SZ, AD, PTSD, and MDD (the mental health disorders) but not so from RRMS, SS and RA, i.e., the three immune-related disorders.

HLA-related sensor pairs

The location of sensors related to HLA protection (James et al. 2016) is shown in Fig. 3. HLA-related GWI SNIs (i.e., SNIs of all sensor pairs in Fig. 3) differed significantly from those in the control group ($P < 0.001$, *F* test in ANCOVA; Table 3). The results of the comparisons of GWI with the other seven disease groups are given in Table 3 and shown in Fig. 4. Of the seven diseases, GWI SNIs were highly significantly different from SZ, AD, PTSD, and MDD (the mental health disorders) but not so from RRMS, SS and RA, i.e., the three immune-related disorders.

Adjustment for multiple comparisons

The experimental design was for planned two-group comparisons (GWI vs. another group); the number of the ANCOVAs (=16 in total) reflected the number of groups compared (eight: GWI vs. control and seven disease groups) × the two sets of sensor pairs (all and HLA-related). Thus, there were no multiple comparisons within each ANCOVA, and from this viewpoint, the probability values given in Tables 2 and 3 are valid at face value. However, it could be argued that an adjustment would be appropriate to account for the fact that 16 overall

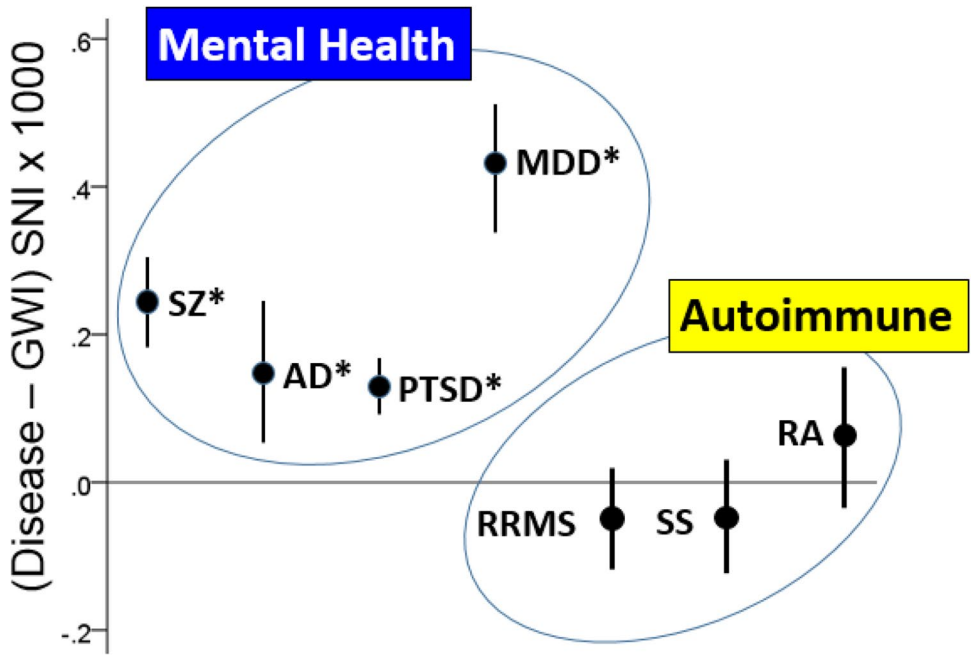

Fig. 2 Results for all sensor pairs to show means (±2SEM) of SNI differences between stated disease group and GWI, adjusted for age and sex (ANCOVA). An *asterisk* denotes a statistically significant result, as detailed in Table 2

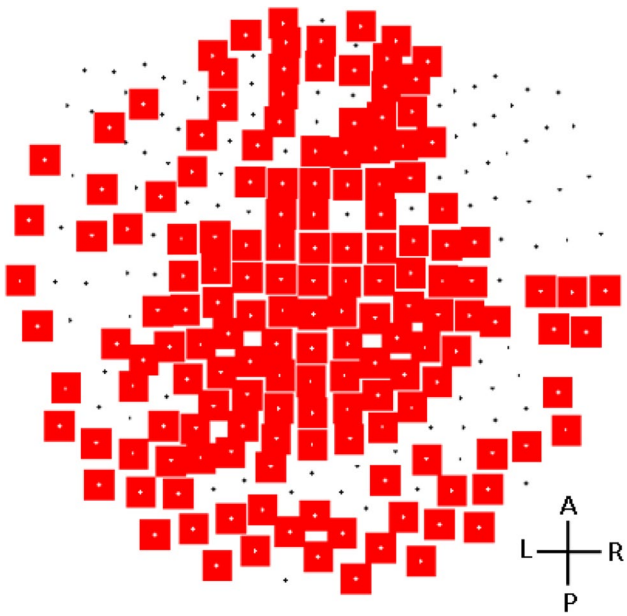

Fig. 3 Territory of HLA-related SNIs. Red squares indicate the MEG sensors contributing to SNIs related to HLA, with respect to severity of NCM symptoms in GWI (James et al. 2016). *A* anterior; *P* posterior; *L* left; *R* right

comparisons were performed. For that purpose, we computed an adjusted *P* value using the Bonferroni correction. We found that for control SZ, AD, PTSD and MDD, all corrected values were $P \leq 0.02$, whereas for RRMS, SS and RA they were $P = 1$. Therefore, the essence of the results regarding the comparison of GWI SNIs against

Table 3 Results of ANCOVA comparing GWI to other diseases using only HLA-related SNIs (see text)

Group	*F*	*df* (denominator)	*P* value
Control	18.351	15974601	0.000018
SZ	80.354	1482179	3.1×10^{-19}
AD	11.203	2518361	0.001
PTSD	53.519	4934883	2.6×10^{-13}
MDD	99.719	1148268	1.8×10^{-23}
RRMS	0.141	2110175	0.707
SS	0.380	1800260	0.537
RA	1.551	1168303	0.213

Numerator *F* degrees of freedom = 1 for all ANCOVAs. Disease abbreviations are as in Table 1

F F test for the Group factor in the ANCOVA, *df* degrees of freedom

control, mental health disorders and immune-related disorders remains the same with or without Bonferroni correction.

Discussion

Neural synchronicity: SNI

In the present study, we evaluated brain synchronicity in GWI relative to seven other diseases and healthy brain functioning to test the hypothesis that GWI is a neuroimmune disease. For that purpose, we focused on the SNI itself, the basic measure of neural synchronicity,

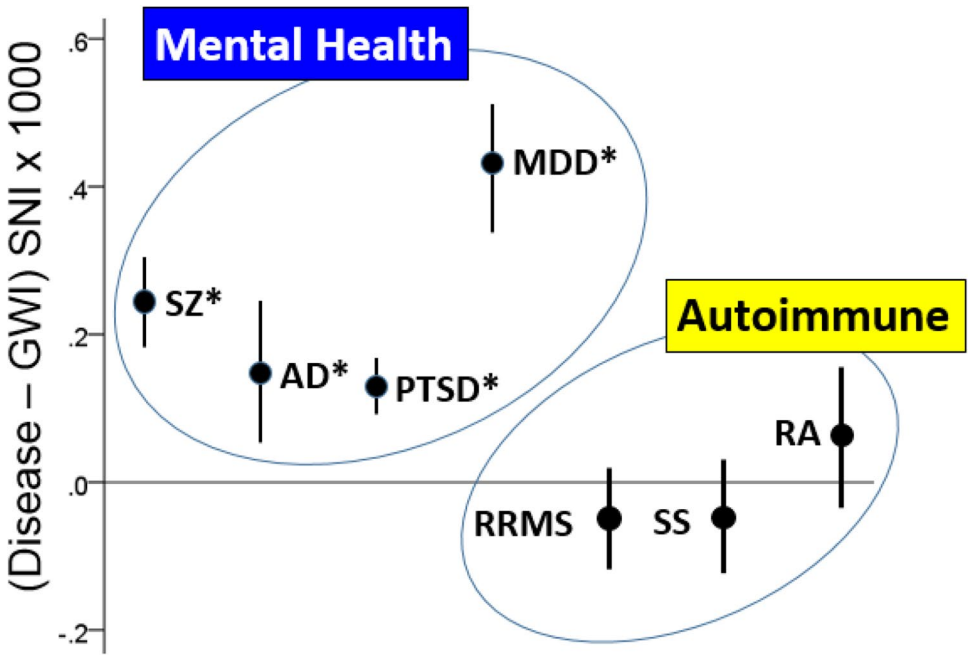

Fig. 4 Results for HLA-related sensor pairs to show means (±2SEM) of SNI differences between stated disease group and GWI, adjusted for age and sex (ANCOVA). An *asterisk* denotes a statistically significant result, as detailed in Table 3

irrespective of its regional brain distribution. This approach complements our previous one that focused on differences of brain patterns of SNI (Georgopoulos et al. 2007, 2010; Engdahl et al. 2010, 2016). As expected, the results highlight similarities in brain synchronicity between GWI and known immune-related conditions and point to genetically mediated mechanisms underlying similarities between GWI and other immune-related diseases.

An asset of this study is the use of a fine-rain physiological measure of neural synchronicity (SNI) derived from high-fidelity (MEG) measurements to compare GWI with other diseases. SNIs (~30628/brain) come from a dense MEG sensor array (248 sensors) and cover most of the brain, thus providing a detailed background dataset on which comparisons are made. A reduction of this rich dataset to single values (e.g., mean SNI/brain) would eliminate all meaningful information and be, therefore, unwise. Indeed, use of individual SNIs as predictors has proved very innovative during the past 10 years in discriminating various diseases (Georgopoulos et al. 2007, 2010; James et al. 2014; Engdahl et al. 2016); in fact, it is on the basis of subsets of such single SNIs that classification of subjects to various diseases has been made. Finally, it could be argued that use of SNIs would tend to yield "significant" results due to the large number of degrees of freedom. However, the results of this study show that using SNIs did not just find or amplify "significant" effects: the outcomes ("significant" or "nonsignificant") followed the nature of disease (immune-related or not) compared to GWI and did not just yield universally "significant" results.

A different issue concerns the neurobiological significance of neural synchronicity, which is measured by SNI. In general, neural synchronicity has been shown to be an important aspect of brain function in health and disease by many studies (see Singer 1999, and Uhlhaas and Singer 2006 for reviews). This is not surprising, since the essence of brain function as a massive communication network lies exactly in the interactions between neuronal populations. During the past decade, we have validated the clinical value of SNIs in several different ways. First, we showed that the brain pattern of SNI is very similar and robust across healthy subjects (Langheim et al. 2006). Second, we found that this pattern is distinctly different in brain disease, such as PTSD (Engdahl et al. 2010), GWI (Engdahl et al. 2016), and fetal alcohol syndrome (Lewis et al. 2016). Third, we showed that small subsets of SNIs can correctly classify with >90% accuracy healthy subjects and a number of brain diseases, including schizophrenia, Alzheimer's disease, multiple sclerosis, Sjögren's syndrome, temporomandibular joint disorder and chronic alcoholism (Georgopoulos et al. 2007), PTSD (Georgopoulos et al. 2010; James et al. 2014; Christova et al. 2015, using SNI from functional magnetic resonance data), and GWI (Engdahl et al. 2016). Finally, we have shown that neural synchronicity can be modulated in an orderly fashion by various, diverse factors, including trauma (James et al. 2012), pathological personality traits (James et al. 2015), posttraumatic growth (Anders et al. 2015), apolipoprotein E genotype (Leuthold et al. 2013), and HLA genes (James et al. 2016). Altogether, those studies have documented the importance of neural synchronicity as a fundamental aspect of brain network function and as an effective measure to

differentiate, quantify and evaluate the effects of disease and behavioral factors on integrative brain function.

Immune basis of GWI

A number of researchers have implicated immune system disruption in GWI (Hotopf et al. 2000; Israeli 2012; Moss 2013; Parkitny et al. 2015; Skowera et al. 2004; Toubi 2012; Whistler et al. 2009). To that end, we recently demonstrated genetic vulnerability involving human leukocyte antigen (HLA) genes in veterans with GWI (Georgopoulos et al. 2015). HLA genes, which are located in the Major Histocompatibility Complex of chromosome 6, play a central role in immune system functioning (Meuer et al. 1982). We reported that six Class II HLA alleles discriminate veterans with GWI from healthy controls and are inversely related to GWI symptom severity, suggesting a protective effect (Georgopoulos et al. 2015). That is, veterans with GWI lack protection, thereby increasing the likelihood of immune-related reactions and other aberrant immune responses when exposed to environmental triggers. We also demonstrated that these HLA alleles interact with brain function to influence symptoms of GWI including NCM (James et al. 2016). There, we concluded that in the absence of HLA protection, immune-related brain abnormalities develop in GWI, perhaps via the development of antibodies to brain antigens resulting in cellular abnormalities, anomalies in neural communication, and symptomatology.

Brain dysfunction in GWI and other disorders with immune involvement

GWI is associated with structural brain abnormalities, notably subcortical brain atrophy (Christova et al. 2017). Functionally, more than half of veterans with GWI report at least moderate neurological/cognitive/mood (NCM) impairment (Steele 2000). Typical symptoms include memory and concentration difficulty, word-finding trouble, headaches, blurred vision, tremors, numbness, and mood alterations among others. Similar cognitive and neuropsychiatric symptoms have been associated with various conditions characterized by disruptions in immune functioning including rheumatoid arthritis (Hanly et al. 2005; Shin et al. 2012, 2013; de Melo and Da-Silva 2012), systemic lupus erythematosus (Ainala et al. 2001; Antonchak et al. 2011; Carbotte et al. 1986; de Melo and Da-Silva 2012; Ginsburg et al. 1992; Hanly et al. 1994, 2005; Hay et al. 1992) Sjögren's syndrome (Alexander and Provost 1987; Lafitte et al. 2001; Martinez et al. 2010; Segal et al. 2012, 2014), and multiple sclerosis (Amato et al. 2006; Chiaravalloti and DeLuca 2008; Denney et al. 2005; Rao et al. 1991). Although estimates vary, some studies have found that two thirds of patients with these disorders exhibit cognitive impairment (Ainala et al. 2001; Hamed et al. 2012; Carbotte et al. 1986; Alexander and Provost 1987; Heaton et al. 1985). These deficits are observed in individuals with no prior cognitive or psychiatric history and have been shown to be associated with markers of inflammation or autoimmunity (Alexander and Provost 1987; Kozora et al. 2001; Hamed et al. 2012). Thus, like GWI, these conditions appear to exhibit interacting effects on the nervous system and immune system that result in both NCM impairment and immune system disruption.

GWI SNI differences from other diseases

We have previously demonstrated the power of SNI brain patterns derived from task-free MEG in successfully discriminating various brain diseases (Georgopoulos et al. 2007, 2010; Engdahl et al. 2010, 2016; James et al. 2014). In the present study, we compared average GWI SNI, irrespective of its brain distribution, to healthy brain functioning and other diseases of varied etiology, all of which involve NCM-related impairments. Results demonstrated that GWI SNI did not differ significantly from that of three immune-related diseases (SS, RRMS, and RA) but differed significantly from healthy brain functioning and from brain functioning in non-immune-related diseases (SZ, AD, PTSD, MDD), supporting our hypothesis that GWI is a neuroimmune disease. Although many researchers have recently surmised that GWI is an immune-related condition, this is the first study to empirically demonstrate brain-related similarities between GWI and known immune diseases.

GWI differences within protective HLA-related SNIs

In previous studies, we demonstrated HLA-involvement in GWI (Georgopoulos et al. 2015) as well as HLA-related neural influences on GWI symptoms (James et al. 2016). Here we sought to further evaluate SNI differences between GWI and the three immune-related diseases with regard to HLA status. The vast majority of SNIs (29219 out of 30628) were significantly related to HLA with respect to GWI NCM severity (James et al. 2016), highlighting robust interactions of neural and immune systems in GWI. The SNIs involved were widespread although entirely absent in the right temporal region (Fig. 3) and sparse in the right temporal region. Within this subset of HLA-related SNIs, there were no significant differences between GWI and the three immune-related diseases: RA, RRMS, and SS, in contrast to significant differences present between GWI and the four non-immune-related diseases (SZ, AD, PTSD, MDD).

Implications for possible HLA protective involvement in other diseases

The results of the present study highlight neuroimmune involvement in GWI and indicate brain-based similarities with other immune disorders, particularly with regard to HLA-related neural synchrony. Here, the focus is on disease and, with regard to HLA-related SNI, GWI is indistinguishable from RRMS, RA, and SS. However, in as much as the absence of certain HLA alleles has been linked to enhanced vulnerability for GWI, the presence of those alleles confers protection (Georgopoulos et al. 2015). This suggests the possibility that these same alleles may confer protection for brain involvement in other neuroimmune diseases as well. Interestingly, DRB1*13:02, one of our six GWI protective alleles (Georgopoulos et al. 2015), has been found to confer protection to a wide variety of immune-related disorders (Furukawa et al. 2017). This adds further support to the link between GWI and lack of HLA protection (Georgopoulos et al. 2015).

Limitation of the study

The main limitation of the study is the relatively small number of participants in the disease groups. Although the number of SNIs was large and allowed valid comparisons, the representation of adequate variety across participants with various diseases is important. Another possible limitation concerns the criteria used for diagnosis. In the present study, disease diagnosis was made by expert clinician at the time of study but such criteria may change over time. This limitation holds for many clinical studies and trials.

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Compliance with ethical standards

Conflict of interest The authors do not report any financial disclosures or conflicts of interest.

References

Ainala H, Loukkola J, Peltola J et al (2001) The prevalence of neuropsychiatric syndromes in systemic lupus erythematosus. Neurology 57:496–500

Alexander E, Provost TT (1987) Sjögren's syndrome: association of cutaneous vasculitis with central nervous system disease. Arch Dermatol 123:801–810

Amato MP, Zipoli V, Portaccio E (2006) Multiple sclerosis-related cognitive changes: a review of cross-sectional and longitudinal studies. J Neurol Sci 245:41–46

American Psychiatric Association (2000) Diagnostic and statistical manual of mental disorders, 4th edn. APA, Washington, DC

Anders SL, Peterson CK, James LM et al (2015) Neural communication in posttraumatic growth. Exp Brain Res 233:2013–2020

Antonchak MA, Saoudian M, Khan AR et al (2011) Cognitive dysfunction in patients with systemic lupus erythematosus: a controlled study. J Rheumatol 38:1020–1025

Blake D, Weathers F, Nagy LM et al (1995) Clinician-Administered PTSD Scale. National Center for PTSD, Boston

Box GEP, Jenkins GM (1976) Time series analysis: forecasting and control. Holden-Day, San Francisco

Carbotte RM, Denburg SD, Denburg JA (1986) Prevalence of cognitive impairment in systemic lupus erythematosus. J Nervous Mental Dis 174:357–364

Chiaravalloti ND, DeLuca J (2008) Cognitive impairment in multiple sclerosis. Lancet Neurol 7:1139–1151

Christova P, James LM, Engdahl BE, Lewis SM, Georgopoulos AP (2015) Diagnosis of posttraumatic stress disorder (PTSD) based on correlations of prewhitened fMRI data: outcomes and areas involved. Exp Brain Res 233:2695–2705

Christova P, James LM, Engdahl BE et al (2017) Subcortical brain atrophy in Gulf War Illness. Exp Brain Res. doi:10.1007/s00221-017-5010-8 **(Epub ahead of print)**

de Melo LF, Da-Silva SL (2012) Neuropsychological assessment of cognitive disorders in patients with fibromyalgia, rheumatoid arthritis, and systemic lupus erythematosus. Brazil J Rheumatol 52:175–188

Denney DR, Sworowski LA, Lynch SG (2005) Cognitive impairment in three subtypes of multiple sclerosis. Arch Clin Neuropsychol 20:967–981

Engdahl BE, Leuthold A, Tan HR et al (2010) Post-traumatic stress disorder: a right temporal lobe syndrome? J Neural Eng 7:066005

Engdahl BE, James LM, Miller RD et al (2016) A magnetoencephalographic (MEG) study of Gulf War Illness (GWI). EBioMedicine. doi:10.1016/j.ebiom.2016.08.030

First MB, Spitzer RL, Gibbon M et al (2002) Structural clinical interview for DSM-IV-TR axis I disorders, research version, nonpatient edition (SCID-I/NP). Biometrics Research, New York State Psychiatric Institute, New York

Fisher RA (1958) Statistical methods for research workers, 13th edn. Oliver and Boyd, Edinburgh

Fukuda K, Nisenbaum R, Stewart G et al (1998) Chronic multisymptom illness affecting Air Force veterans of the Gulf War. JAMA 280:981–988

Furukawa H, Oka S, Tsuchiya N et al (2017) The role of common protective alleles HLA-DRB1*13 among systemic autoimmune diseases. Genes Immun 18:1–7

Georgopoulos AP, Karageorgiou E, Leuthold A et al (2007) Synchronous neural interactions assessed by magnetoencephalography: a functional biomarker for brain disorders. J Neural Eng 4:349–355

Georgopoulos AP, Tan HM, Lewis SM et al (2010) The synchronous neural interactions test as a functional neuromarker for post-traumatic stress disorder (PTSD): a robust classification method based on the bootstrap. J Neural Engin 7:016011

Georgopoulos AP, James LM, Mahan MY et al (2015) Reduced Human Leukocyte Antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine 3:79–85

Ginsburg KS, Wright EA, Larson MG et al (1992) A controlled study of the prevalence of cognitive dysfunction in randomly selected patients with systemic lupus erythematosus. Arthritis Rheum 35:776–782

Hamed SA, Selim ZI, Elattar AM et al (2012) Assessment of biocorrelates for brain involvement in female patients with rheumatoid arthritis. Clin Rheumatol 31:123–132

Hanly JG, Fisk JD, Eastwood B (1994) Brain reactive autoantibodies and cognitive impairment in systemic lupus erythematosus. Lupus 3:193–199

Hanly JG, Fisk JD, McCurdy G et al (2005) Neuropsychiatric syndromes in patients with systemic lupus erythematosus and rheumatoid arthritis. J Rheumatol 32:1459–1466

Hay EM, Black D, Huddy A et al (1992) Psychiatric disorder and cognitive impairment in systemic lupus erythematosus. Arthritis Rheum 35:411–416

Heaton RK, Nelson LM, Thompson DS et al (1985) Neuropsychological findings in relapsing-remitting and chronic-progressive multiple sclerosis. J Consul Clin Psychol 53:103

Hotopf M, David A, Hull L et al (2000) Role of vaccinations as risk factors for ill health in veterans of the Gulf war: cross-sectional study. BMJ 320:1363–1367

Israeli E (2012) Gulf War Syndrome as a part of the autoimmune (autoinflammatory) syndrome induced by adjuvant (ASIA). Lupus 21:190–194

James LM, Engdahl BE, Leuthold AC et al (2012) Neural network modulation by trauma as a marker of resilience: differences between veterans with posttraumatic stress disorder and resilient controls. JAMA Psychiatry 70:410–418

James LM, Belitskaya-Levy I, Lu Y et al (2014) Development and application of a diagnostic algorithm for posttraumatic stress disorder. Psychiatry Res 231:1–7

James LM, Engdahl BE, Leuthold AC, Krueger RF, Georgopoulos AP (2015) Pathological personality traits modulate neural interactions. Exp Brain Res 233:3543–3552

James LM, Engdahl BE, Leuthold AC, Georgopoulos AP (2016) Brain correlates of human leukocyte antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine 13:72–79

Kozora E, Laudenslager M, Lemieux A, West SG (2001) Inflammatory and hormonal measures predict neuropsychological functioning in systemic lupus erythematosus and rheumatoid arthritis patients. J Int Neuropsychol Soc 7:745–754

Lafitte C, Amoura Z, Cacoub P et al (2001) Neurological complications of primary Sjögren's syndrome. J Neurol 248:577–584

Langheim FPJ, Leuthold AC, Georgopoulos AP (2006) Synchronous dynamic brain networks revealed by magnetoencephalography. Proc Natl Acad Sci USA 103:455–459

Leuthold AC, Mahan MY, Stanwyck JJ, Georgopoulos A, Georgopoulos AP (2013) The number of cysteine residues per mole in apolipoprotein E affects systematically synchronous neural interactions in women's healthy brains. Exp Brain Res 226:525–536

Lewis SM, Vydrová RR, Leuthold AC, Georgopoulos AP (2016) Cortical miscommunication after prenatal exposure to alcohol. Exp Brain Res 234:3347–3353

Mahan MY, Chorn CR, Georgopoulos AP (2015) White Noise Test: detecting autocorrelation and nonstationarities in long time series after ARIMA modeling. Proc 14th Python In Science Conference (Scipy 2015), Austin, TX

Martinez S, Caceres C, Mataro M et al (2010) Is there progressive cognitive dysfunction in Sjögren Syndrome? A preliminary study. Acta Neurol Scand 122:182–188

McKhann G, Drachman D, Folstein M et al (1984) Clinical diagnosis of Alzheimer's disease Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939–944

Meuer SC, Hussey RE, Hodgdon JC et al (1982) Surface structures involved in target recognition by human cytotoxic T lymphocytes. Science 218:471–473

Moss JI (2013) Gulf War illnesses are autoimmune illnesses caused by increased activity of the p38/MAPK pathway in CD4+ immune system cells, which was caused by nerve agent prophylaxis and adrenergic load. Med Hypotheses 81:1002–1003

Parkitny L, Middleton S, Baker K, Younger J (2015) Evidence for abnormal cytokine expression in Gulf War Illness: a preliminary analysis of daily immune monitoring data. BMC Immunol. doi:10.1186/s12865-015-0122-z

Polman CH, Reingold SC, Edan G et al (2005) Diagnostic criteria for multiple sclerosis: 2005 revisions to the "McDonald Criteria". Annals Neuro 58:840–846

Priestley MB (1981) Spectral analysis of time series. Academic, San Diego

Rao SM, Leo GJ, Bernardin L, Unverzagt F (1991) Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. Neurology 41:685–691

Segal BM, Pogatchnik B, Holker E et al (2012) Primary Sjogren's syndrome: cognitive symptoms, mood, and cognitive performance. Acta Neurol Scand 125:272–278

Segal BM, Rhodus N, Sivils KLM, Solid CA (2014) Validation of the brief cognitive symptoms index in sjögren syndrome. J Rheumatol 41:2027–2033

Shin SY, Katz P, Wallhagen M, Julian L (2012) Cognitive impairment in persons with rheumatoid arthritis. Arthritis Care Res 64:1144–1150

Shin SY, Julian L, Katz P (2013) The relationship between cognitive function and physical function in rheumatoid arthritis. J Rheumatol 40:236–243

Singer W (1999) Neuronal synchrony: a versatile code for the definition of relations? Neuron 24:49–65

Skowera A, Hotopf M, Sawicka E et al (2004) Cellular immune activation in Gulf War veterans. J Clin Immunol 24:66–73

Steele L (2000) Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. Am J Epidemiol 152:992–1002

Uhlhaas PJ, Singer W (2006) Neural synchrony in brain disorders: relevance for cognitive dysfunctions and pathophysiology. Neuron 52:155–168

Vitali C, Bombardieri S, Jonsson R (2002) Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Annals Rheum Dis 61:554–558

Whistler T, Fletcher MA, Lonergan W et al (2009) Impaired immune function in Gulf War illness. BMC Med Genom 2:1. doi:10.1186/1755-8794-2-12

White RF, Steele L, O'Callaghan JP et al (2016) Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: effects of toxicant exposures during deployment. Cortex 74:449–475

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Research Paper

Human Leukocyte Antigen (HLA) and Gulf War Illness (GWI): HLA-DRB1*13:02 Spares Subcortical Atrophy in Gulf War Veterans

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ABSTRACT

Background: Gulf War Illness (GWI) is a multisystem disorder that has affected a substantial number of veterans who served in the 1990–91 Gulf War. The brain is prominently affected, as manifested by the presence of neurological, cognitive and mood symptoms. We reported previously on the protective role of six Human Leukocyte Antigen (HLA) alleles in GWI (Georgopoulos et al., 2016) and their association with regional brain function (James et al., 2016). More recently, we reported on the presence of subcortical brain atrophy in GWI (Christova et al., 2017) and discussed its possible relation to immune mechanisms. Here we focused on one of the six HLA GWI-protective HLA alleles, DRB1*13:02, which has been found to have a protective role in a broad range of autoimmune diseases (Furukawa et al., 2017), and tested its effects on brain volumes.
Methods: Seventy-six Gulf War veterans (55 with GWI and 21 healthy controls) underwent a structural Magnetic Resonance Imaging (sMRI) scan to measure the volumes of 9 subcortical brain regions to assess differences between participants with (N = 11) and without (N = 65) HLA class II allele DRB1*13:02.
Findings: We found that DRB1*13:02 spared subcortical brain atrophy in Gulf War veterans; overall subcortical volume was 6.6% higher in carriers of DRB1*13:02 (P = 0.007). The strongest effect was observed in the volume of cerebellar gray matter which was 9.6% higher (P = 0.007) in carriers of DRB1*13:02 than in non-carriers. By contrast, DRB1*13:01 had no effect.
Interpretation: These findings document the protective effect of DRB1*13:02 on brain atrophy in Gulf War veterans and are in keeping with recent results documenting sharing of brain mechanisms between GWI and other immune-related diseases (Georgopoulos et al., 2017). We hypothesize that the protective role of DRB1*13:02 is due to its successful elimination of external antigens to which Gulf War veterans were exposed, antigens that otherwise would persist causing low-grade inflammation and possibly leading to autoimmunity.
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1. Introduction

1.1. Gulf War Illness

For over 25 years, veterans of the 1990–1991 Gulf War (GW) have been affected by chronic health problems, commonly referred to as Gulf War Illness (GWI), that are presumed to be sequelae of service-related exposures to toxins such as pyridostigmine bromide, pesticides, multiple vaccinations, and/or stress (White et al., 2016). Many symptoms of GWI involve the central nervous system; consequently, several studies have investigated brain structure and function as it relates to GWI, with mixed findings (White et al., 2016). We have recently identified functional (Engdahl et al., 2016) and structural (Christova et al., 2017) brain anomalies in GWI, both of which prominently involved subcortical regions. For example, compared to healthy control veterans, veterans with GWI showed an average of 10.4% reduction in cerebellar volume and 2× the rate of reduction of cerebellar gray matter volume with age (−14%/decade in GWI vs. −6.9%/decade in controls). We

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concluded that the marked subcortical volume reduction observed in veterans with GWI is likely attributable to direct exposure to toxins, akin to toxic encephalopathy (Valk and van der Knaap, 1992), in combination with lack of immunogenetic protection in GWI (Georgopoulos et al., 2016; James et al., 2016).

1.2. Immunogenetics and GWI

Although a quarter to one-third of GW veterans suffer from GWI (Research Advisory Committee on Gulf War Veterans' Illnesses, 2014), most GW veterans remain relatively healthy, suggesting that genetic variations likely play a role in determining their health outcomes. In fact, we have found robust evidence that genetic variations involving the Human Leukocyte Antigen (HLA) play a substantial role in promoting protection against or vulnerability to GWI (Georgopoulos et al. 2016). HLA genes are located in the Major Histocompatibility Complex (MHC) of chromosome 6 and play a central role in immune system functioning (Meuer et al., 1982). We previously demonstrated that six HLA class II alleles (DRB1*01:01, DRB1*08:11, DRB1*13:02, DQB1*02:02, DPB*01:01, DPB1*06:01) successfully discriminate veterans with GWI from controls (Georgopoulos et al. 2016) and interact with brain function to influence symptoms of GWI (James et al., 2016). We also found an inverse relation between GWI symptom severity and the number of copies of the 6 protective HLA alleles, and that the frequency of those 6 alleles in veterans with GWI is significantly lower than in unaffected veterans (Georgopoulos et al. 2016). These effects suggest that the presence of these HLA alleles confers protection against GWI.

Notably, all 6 of the protective HLA alleles identified in relation to GWI belong to HLA class II alleles. HLA class II alleles have been strongly associated with various immune-related conditions including multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, celiac disease, Crohn's disease, and Graves' disease, among others (Shiina et al., 2009; Gough and Simmonds, 2007). This overlap, in conjunction with several overlapping clinical signs and symptoms (Israeli, 2012), including similarities in brain synchronicity (Georgopoulos et al., 2017), places GWI squarely within the immune dysfunction realm.

1.3. Protective Effects of DRB1*13:02

Of the six HLA alleles previously identified as protective in terms of GWI (Georgopoulos et al. 2016), DRB1*13:02 has been found to be protective in various immune-related disorders (Bettencourt et al., 2015; Furukawa et al., 2017). Other HLA alleles have either received relatively minimal investigation in regards to their relation to autoimmune disorders, have been shown to promote susceptibility, or findings are mixed in terms of conferring susceptibility or resistance to various immune-related diseases. In a large study of associations between DRB1 alleles and six autoimmune disorders, DRB1*13 was found to be a protective factor for four autoimmune disorders (rheumatoid arthritis, systemic lupus erythematosus, psoriasis/psoriatic arthritis, and systemic sclerosis), whereas other DRB1 alleles were risk factors (Bettencourt et al., 2015). HLA DRB1*03, for instance, was strongly linked to 3 autoimmune disorders (systemic lupus erythematosus, multiple sclerosis, and myasthenia gravis). Thus, it appears that several autoimmune disorders share immunogenetic mechanisms, with DRB1*13 promoting protection, particularly for systemic and rheumatic diseases. Furthermore, the protective effects appear to be especially robust for the DRB1*13:02 allele. This protein contains 266 amino acids, of which amino acid residues at positions 30–266 form the beta chain. DRB1*13:02 contains a glycine residue at chain position 86, and differs by only one residue from the DRB1*13:01 protein which contains a valine residue at position 86. This single residue substitution makes a large difference in the electrostatic properties of pocket 9 (P9) of the peptide binding groove, i.e. the part of the HLA protein that binds to external antigens (Hov et al., 2011). DRB1*13:02 has been found to be protective against various systemic and organ-specific autoimmune disorders with gene-dosage effects conferring maximal protection in homozygous DRB1*13:02 carriers (for review, see Furukawa et al., 2017). DRB1*13:01 has also been found to protect against rheumatoid arthritis (van der Woude et al., 2010) but to be a risk factor for protracted hepatitis A infection (Pando et al., 1999) and associated pediatric autoimmune hepatitis (Fainboim et al., 2001), as well as primary sclerosing cholangitis (Hov et al., 2011). These mixed findings show that different alleles (DRB1*13:01, DRB1*13:02) can have very different disease associations, such that exploring such relations at the allele level (DRB1*13) can be misleading and uncertain. These considerations underscore the need to investigate HLA-disease associations at the protein (4-digit resolution) level, as pioneered by Todd et al. (1987) in the case of type 1 diabetes mellitus and further carried out following the publication of the crystal structures of the HLA class II molecule by Brown et al. (1993) (Jones et al., 2006).

1.4. The Present Study

Given the reported protective role of DRB1*13:02 for immune-related diseases and the evidence that GWI is closely related to such disorders (Georgopoulos et al., 2016, 217), we investigated the effect of DRB1*13:02 on the volumes of subcortical brain regions found to be reduced in GWI (Christova et al., 2017) to test the hypothesis that HLA DRB1*13:02 prevents subcortical brain atrophy in GW veterans, thus exerting a protective role in GWI too.

2. Materials and Methods

2.1. Participants

Seventy-six GW-era veterans (55 men, 21 women; mean age ± SEM, 53.87 ± 1.17 y) participated in the current study after providing informed consent, in adherence to the Declaration of Helsinki, and were financially compensated for their time. They included 55 veterans with GWI (52 men, 3 women) and 21 healthy controls (3 men, 18 women). All study protocols were approved by the appropriate Institutional Review Boards. GWI status was determined using a self-report symptom checklist that permits classification as GWI case or control according to the Center for Disease Control (Fukuda et al., 1998) and the Kansas criteria (Steele, 2000). All GWI veterans in the present study met both case definitions. Study participants completed diagnostic interviews including the Clinician-Administered PTSD Scale for *DSM-IV* (Blake et al., 1995) and the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (First et al., 2002) to evaluate mental health status. None of the participants in the present study met diagnostic criteria for any mental health condition.

2.2. HLA Genotyping

DNA isolation was carried out from 3 ml of whole blood drawn in EDTA tubes, using a commercially available kit (ArchivePure cat. 2300730) from 5Prime (distributed by Fisher Scientific or VWR) with an expected yield of 50-150 μg of DNA. The purified DNA samples were sent to Histogenetics (http://www.histogenetics.com/) for high-resolution HLA Sequence-based Typing (SBT; details are given in https://bioinformatics.bethematchclinical.org/HLA-Resources/HLA-Typing/High-Resolution-Typing-Procedures/ and https://bioinformatics.bethematchclinical.org/WorkArea/DownloadAsset.aspx?id=6482). Their sequencing DNA templates are produced by locus- and group-specific amplifications that include exon 2 and 3 for class I (A, B, C) and exon 2 for class II (DRB1, DRB3/4/5, DQB1, and DPB1) and reported as Antigen Recognition Site (ARS) alleles as per ASHI recommendation (Cano et al., 2007).

2.3. MRI Data Acquisition and Preprocessing

All data were acquired using a Phillips 3T MR scanner (Achieva, Philips Healthcare, Best, The Netherlands). In the initial phase of the study, data were acquired from 42 participants using a phased array SENSitivity Encoding (SENSE) 8-channel head coil for reception. For each participant a high resolution T1-weighted Turbo Field Echo (T1w TFE SENSE) was obtained (168 sagittal slices, TR = 8.1932 ms, TE = 3.7520 ms, Acquisition matrix 240 × 240, Flip angel 8 deg., voxel size 0.9375 × 0.9375 × 1 mm). A T2-weighted image (T2w VISTA HR SENSE) was also obtained (180 slices, TR = 2500 ms, TE = 363.072 ms, Acquisition matrix 252 × 252, voxel size = 0.7813 × 0.7813 × 1 mm). Subsequently, upgrades were applied to the system and data were acquired from the remainder 34 participants using a phased array SENSitivity Encoding (SENSE) 15-channel head coil for reception. For each participant a high resolution T1-weighted Turbo Field Echo (T1w TFE SENSE) was obtained (168 sagittal slices, TR = 8.0928 ms, TE = 3.698 ms, Acquisition matrix 240 × 240, Flip angel 8 deg., voxel size 0.7500 × 0.7500 × 1 mm). The T2-weighted (T2w VISTA HR SENSE) was also obtained (168 slices, TR = 2500 ms, TE = 370.346 ms, Acquisition matrix 240 × 240, voxel size = 0.7500 × 0.7500 × 1 mm).

A 704-core High Performance Computing system (CentOS 6.5 Linux, Rocks 6.1.1) with Matlab R2012 (64 bit), Human Connectome Project (HCP humanconnectome.org) pipeline with FreeSurfer (FS; http://surfer.nmr.mgh.harvard.edu) HCP version (freesurfer-hpc) was used for data processing. MRI data with high contrast between gray matter, white matter, and cerebrospinal fluid as well as high spatial resolution are necessary for accurate results. We acquired T1w and T2w images with high spatial resolution (≤1 mm^2) to achieve precise surface reconstruction. Standard FS software requires only T1w images as input. However, we used a modified version of FS, implemented in the structural HCP pipeline, which utilizes both T1w and T2w images to eliminate uncertainty due to the fact that dura and blood vessels are isointense to gray matter in the T1w image alone. In addition, T2w allows improved pial surface reconstruction (Glasser et al., 2013). Specifically, we used the first 2 structural HCP pipelines, namely *PreFreeSurfer* and *FreeSurfer*. One goal of the *PreFreesurfer* pipeline is to align the T1w and T2w images. *PreFreeSurfer* pipeline processing was followed by *FreeSurfer* pipeline processing which is based on FS version 5.2 with improvements. From the segmentation statistics output we obtained estimated total intracranial volume (eTIV), and the volumes of left and right cerebellar gray matter, brainstem, thalamus, caudate, putamen, pallidum, accumbens, amygdala and diencephalon. We calculated the sum of the left and right volumes for each region and used them as dependent variables in the ANCOVA. Finally, the sum of these subcortical volumes was the "subcortical" brain volume.

2.4. Data Analysis

Standard statistical methods were employed to analyze the data using the IBM-SPSS statistical package (version 23). More specifically, we carried out a univariate and a multivariate analysis of covariance (ANCOVA) to assess the effect of DRB1*13:02, DRB1*13:01 and DRB1*13 on brain volumes. In an initial analysis, we explored the possibility that the acquisition systems during the two phases of the study might have an effect on the results. For that purpose, we added a categorical "Acquisition" factor in the ANCOVAs, taking the values of 0 and 1 for the first and second phase of the study, respectively, and assessed its effect. In the univariate ANCOVA, the total subcortical volume was the dependent variable, the presence (or absence) of DRB1*13:02 was a fixed factor, and sex, age, and eTIV were covariates. Since all carriers of this HLA allele were heterozygotes in our sample, the DRB1*13:02 factor took values of zero and 1in the ANCOVA. In repeated measures ANCOVAs, the Regions (N = 9 subcortical regions) were the Within-Subjects factor (since they came from the same subject), the presence (or absence) of DRB1*13:02 was the Between-Subjects factor, and sex, age, and eTIV were covariates. The same analyses were carried out for allele DRB1*13:01.

3. Results

No participant carried both DRB1*13:02 and DRB1*13:01.

3.1. DRB1*13:02

3.1.1. Frequencies

Of the total of 76 participants, DRB1*13:02 was present in 11 and absent in 65. The relative frequency of occurrence of this allele was ~7× higher in controls (8/21 = 0.38) than in GWI (3/55 = 0.054), indicating a protective effect of DRB1*13:02 (Pearson chi-square = 13.08, P = 0.003; estimated odds ratio ($\hat{\omega}$) = 0.094, ln($\hat{\omega}$) = −2.367, P = 0.001) (Table 1).

3.1.2. Volumes

The Acquisition factor did not have any statistically significant effect in any of the DRB1*13:02-related ANCOVAs performed (P = 0.737 for Acquisition Main Effect; P = 0.805 for Acquisition X DRB1*13:02 Interaction).

All statements on volumes below refer to volumes adjusted for sex, age and eTIV. Overall, mean volumes of the 9 subcortical regions (Table 2) were significantly higher in the presence than in the absence of DRB1*13:02 (P = 0.028, Wilcoxon Signed Rank test). In addition, the overall subcortical volume (i.e. the sum of the volumes of all 9 subcortical regions) was significantly higher by 6.6% (P = 0.007, F-test in univariate ANCOVA) (Fig. 1). A more detailed analysis was carried out using a repeated measures ANCOVA (see Methods) which revealed that the effect of the Between-Subjects DRB1*13:02 was highly significant (P = 0.007, F-test in repeated measures ANCOVA), as was the Region x DRB1*13:02 interaction (P = 0.007, Greenhouse-Geisser test), reflecting the differential effect of DRB1*13:02 on individual regions. Indeed, the strongest effect (9.6% higher in DRB1*13:02) was observed in the cerebellar gray matter (Fig. 2).

3.2. DRB1*13:01

3.2.1. Frequencies

Of the total of 76 participants, DRB1*13:01 was present in 10 and absent in 66. All DRB1*13:01 carriers belonged to the GWI group. This higher frequency of occurrence of DRB1*13:01 in GWI (18.2% vs zero) indicated an increased risk for GWI in carriers of DRB1*13:01

Table 1
Results of two-way table analysis for DRB1*13:02 and GWI.

A. Two-way table

		Group Control	GWI	Total
DRB1*13:02	Absent	13	52	65
	Present	8	3	11
Total		21	55	76

B. Analysis of the two-way table

Test	Value	DF	Significance (2-sided)
Pearson Chi-Square	13.08	1	P = 0.0003

C. Mantel-Haenszel common odds ratio estimate

Estimated odds ratio ($\hat{\omega}$)	ln($\hat{\omega}$)	SE of ln($\hat{\omega}$)	Asymptotic significance (2-sided)
0.094	−2.367	0.745	P = 0.001
95% lower bound: 0.022			
95% upper bound: 0.403			

Table 2
Brain region volumes (mm^3) (adjusted for sex, age, and eTIV) in the absence and presence of DRB1*13:02.

Brain region	DRB1*13:02 Absent		DRB1*13:02 Present	
	Mean	SEM	Mean	SEM
Cerebellum Gray Matter[a]	78,116.5	1000.8	85,657.3	2497.6
Brainstem[a]	21,540.1	230.7	22,537.6	575.8
Thalamus[a]	14,198.0	160.4	14,554.4	400.3
Caudate[a]	7003.6	112.5	7411.2	280.8
Putamen[a]	9839.5	139.6	9811.5	348.4
Accumbens[a]	1088.4	19.5	1122.4	48.7
Pallidum[a]	2750.9	45.9	2851.1	114.7
Amygdala[a]	3278.0	49.1	3239.9	122.6
Diencephalon[a]	7421.6	80.2	7552.5	200.1
Total Subcortical[b]	145,236.5	1270.9	154,738.0	3171.7

[a] Statistics from a repeated measures ANCOVA where the 9 regions were the Within-Subjects factors, the absence or presence of DRB1*13:02 was the Between-Subjects factor, and sex, age and eTIV were covariates.
[b] Statistics from a univariate ANCOVA where the subcortical volume was the dependent variable, the absence or presence of DRB1*13:02 was a fixed factor, and sex, age and eTIV were covariates.

(Pearson chi-square = 4.397, P = 0.036; estimated odds ratio ($\hat{\omega}$) = 9.923, $\ln(\hat{\omega}) = 2.29$) (Table 3).

3.2.2. Volumes

The Acquisition factor did not have any statistically significant effect in any of the DRB1*13:01-related ANCOVAs performed (P = 0.780 for Acquisition Main Effect; P = 0.975 for Acquisition X DRB1*13:01 Interaction).

Overall, mean volumes of the 9 subcortical regions (adjusted for age, sex and eTIV) did not differ significantly between carriers and non-carriers of DRB1*13:01 (P = 0.953, Wilcoxon Signed Rank test). The mean overall subcortical volume was 0.8% smaller in DRB1*13:01 carriers but not significantly different (P = 0.756, F-test in univariate ANCOVA), and similarly for the volume of cerebellar gray matter (2.0% smaller in DRB1*13:01 carriers; P = 0.592, F-test in univariate ANCOVA).

3.3. DRB1*13

In this analysis, the fixed factor was the allele group DRB1*13, which was deemed present when either DRB1*13:01 or DRB1*13:02 were present. No statistically significant results were yielded by any analysis.

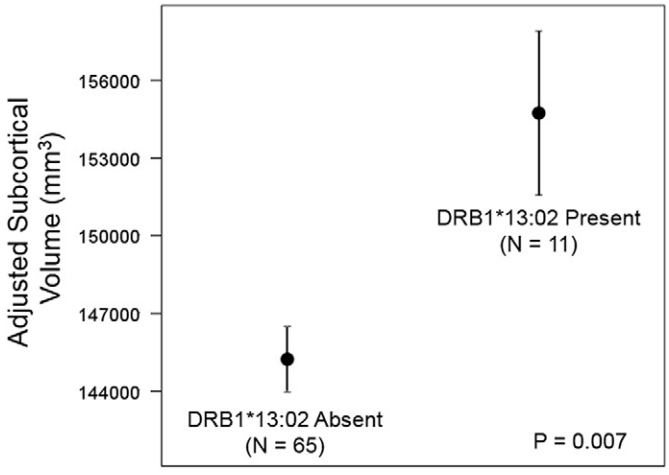

Fig. 1. Mean (±SEM) subcortical volumes in the absence and presence of DRB1*13:02. Statistics are from a univariate ANCOVA where the Subcortical volume was the dependent variables, the absence or presence of DRB1*13:02 was a fixed factor, and sex, age and eTIV were covariates.

4. Discussion

4.1. Protective Role of DRB1*13:02

In this study we investigated possible protection conferred by HLA DRB1*13:02 in GW veterans based on the facts that (a) DRB1*13:02 is protective for GWI (Georgopoulos et al., 2016), (b) DRB1*13:02 is broadly protective for immune-related disorders (Bettencourt et al., 2015; Furukawa et al., 2017; Hov et al., 2011), and (c) GWI is a neuroimmune disorder (James et al., 2016; Georgopoulos et al., 2017). Unlike typical studies based on analysis of relative frequencies of occurrence of DRB1*13:02 in various healthy and disease populations (Bettencourt et al., 2015; Furukawa et al., 2017), we, additionally, assessed its effect on subcortical brain volumes found previously to be reduced in GWI (Christova et al., 2017); indeed, we found here that DRB1*13:02 exerted a protective effect on these volumes and spared their atrophy. Specifically, the subcortical volume was significantly higher in carriers of DRB1*13:02 than in non-carriers (Fig. 1); the strongest effect was observed in the cerebellar gray matter (Fig. 2). These findings are in keeping with the overall protective role of DRB1*13:02 in immune-related disorders and in GWI, as reviewed above.

In contrast to DRB1*13:02, DRB1*13:01 had no significant effect on brain volumes in any analysis, although it was significantly more frequent in GWI. Although DRB1*13:01 has been reported to have a protective role in various immune-related diseases (Furukawa et al., 2017), it has also been reported as risk factor for autoimmune hepatitis (Duarte-Rey et al., 2009) and primary sclerosing cholangitis (Hov et al., 2011).

4.2. The Importance of HLA-coded Proteins

Our findings above highlight the importance of working at the HLA-protein (β-chain) level, which is given by the 4-digit, high-resolution HLA genotyping, as advocated by Jones et al. (2006). Most studies of HLA-disease associations in general (too many to cite), have been focused at the gene level (e.g. DRB1, DQB1, etc.) or at the allele group level (e.g. DRB1*01, DQB1*02, etc.). However, the specificity of action of a HLA allele resides on the specific HLA protein (β-chain) coded by it, as specified by the second set of digits in the 4-digit resolution HLA genotyping (e.g. DRB1*01:02, DPP1*06:15, etc.). Given that different HLA proteins have different properties, it follows that the proper level of analysis is at this HLA-specific protein level. Looking for HLA-disease associations at the gene or allele group levels can be misleading,

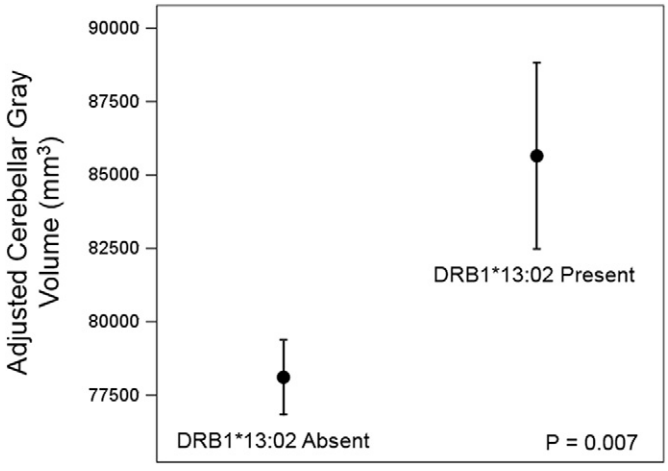

Fig. 2. Mean (± SEM) volumes of cerebellar gray matter in the absence and presence of DRB1*13:02. Statistics are from a multivariate ANCOVA where the cerebellar gray matter volume (one of 9 subcortical regions; see Table 2) was a dependent variable, the absence or presence of DRB1*13:02 was a fixed factor, and sex, age and eTIV were covariates.

Table 3
Results of two-way table analysis for DRB1*13:01 and GWI. The odds ratio was estimated after adding 0.5 to all counts to avoid taking the logarithm of zero. This procedure underestimates the true effect; statistics for the odds ratio cannot be calculated.

A. Two-way table				
		Group Control	GWI	Total
DRB1*13:01	Absent	21	45	66
	Present	0	10	10
Total		21	55	76

B. Analysis of the two-way table			
Test	Value	DF	Significance (2-sided)
Pearson Chi-Square	4.397	1	P = 0.036

C. Mantel-Haenszel common odds ratio estimate	
Estimated odds ratio ($\hat{\omega}$)	$\ln(\hat{\omega})$
9.923	2.295

yielding mixed (risk/protective) or uncertain (i.e. statistically nonsignificant) results. This problem is compounded in studies of frequencies of occurrence of various HLA alleles in different populations (e.g. healthy or suffering from a specific disease) because of the large sample sizes needed and, therefore, the increased diversity expected of HLA-specific proteins in the sample. The findings of the present study illustrate these considerations clearly because the target of the study was a concrete biological variable (i.e. volume of a brain region) and not frequency of occurrence. This afforded a clear-cut evaluation of the effect of individual HLA proteins and a contrast between the effects of either HLA protein as well as the effect of the allele group DRB1*13.

The importance of working at the HLA protein level was first demonstrated by Todd et al. (1987) in their pioneering study of the role of residue 57 of the HLA-DQβ polypeptide in type 1 diabetes mellitus. Recent advances in HLA protein sequencing and 3-D conformation have opened new vistas in investigating HLA-disease relations (Brown et al., 1993; Jones et al., 2006). As succinctly expressed by Donaldson, "This changed the way in which HLA associations were perceived. No longer were they seen as unexplainable genetic anomalies; it was now possible to put these associations into a functional context." (Donaldson, 2011, p 1798). Our study rests firmly on this approach. Actually, the study by Hov et al. (2011) on the relations between HLA proteins and primary sclerosing cholangitis (PSC) is directly relevant in discussing the results of our study. Hov et al. (2011) performed a 3-D modeling of the HLA-DRβ molecule to explore the effect of key residues on the 3-D configuration at the β-chain peptide binding groove. The charge of Pocket 9 (P9) of the peptide binding groove was differentially associated with PSC, such that a positive or negative charge is associated with PSC risk or protection, respectively. Specifically, Hov et al. (2011) found that in DRB1*13:01 (a risk factor for PSC; Spurkland et al., 1999) a positive P9 charge was induced by a remote action of Valine at residue 86, whereas in DRB1*13:02 (protective for PSC; Hov et al., 2011) a negative one was induced by glycine at that residue position. Extending the implications of this discovery to our study, it is reasonable to suppose that the sparing of subcortical brain atrophy we found to be associated with DRB1*13:02 is due in part to the negative charge in P9, whereas a positive charge in P9 is neutral, since DRB1*13:01 had no effect.

4.3. The "Persistent Antigen" Hypothesis for GWI

All of the considerations above regarding the structural biological and physicochemical properties of the HLA-DRβ peptide binding groove ultimately relate to the family of external antigens that can bind to it, to be presented to CD4 + T lymphocytes for subsequent antibody production by B cells (Fig. 3). The ultimate goal of this HLA class II-mediated specific immunity is to eliminate pathogens by producing antibodies against them. The process of successful antibody production can be

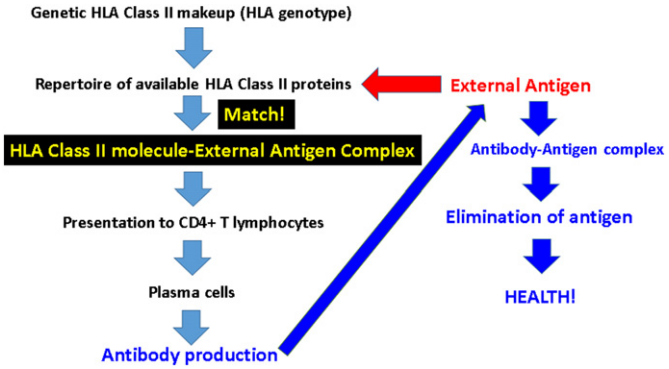

Fig. 3. Schematic diagram illustrating the steps of antibody production in health.

disrupted at different stages, from the absence of a match between antigen and HLA class II protein (due to genetic factors) to problems with CD4 + T cells and/or plasma cell function (due to disease and/or drugs) (Fig. 4). In such cases, the external antigen/pathogen is not eliminated and can persist in the body causing inflammation and ultimately cell damage, and potentially autoimmunity through molecular mimicry (Institute of Medicine, 2012). Assuming that GW veterans were healthy when activated (in 1990–91) with respect to lymphocyte function, and given that GWI is associated with genetic lack of HLA protection (Georgopoulos et al., 2016), the most likely scenario in GWI involves a lack of antigen match with HLA class II protein, resulting in persistent, pathogenic antigen, as illustrated in Fig. 4. We call this the "Persistent Antigen Hypothesis" for GWI. Although we do not know which specific pathogens were involved in GWI, an insight can be gained from the case of pediatric autoimmune hepatitis, for which DRB1*13:01 is a risk (Fainboim et al., 2001) and DRB1*13:02 a protective factor (Pando et al., 1999). Pediatric autoimmune hepatitis frequently follows a protracted course of infection with hepatitis A virus (Fainboim et al., 2001). These authors suggested that the protracted (but not acute) hepatitis A infection leads to a sustained release of liver self-antigens, which, in turn, lead to autoimmunity (Fainboim et al., 2001). Now, DRB1*13:01 (but not DRB1*13:02) was found to be strongly associated with the protracted forms of this infection and resulting autoimmune hepatitis. Thus a connection is made between a protracted, chronic infection and a developing autoimmune disease.

4.4. Concluding Remarks

This line of evidence is in keeping with our "persistent antigen" hypothesis above for GWI pathogenesis. Such antigens could sustain low-grade inflammation and also lead to autoimmunity, both of which could underlie chronic inflammatory processes reported in GWI (Johnson et

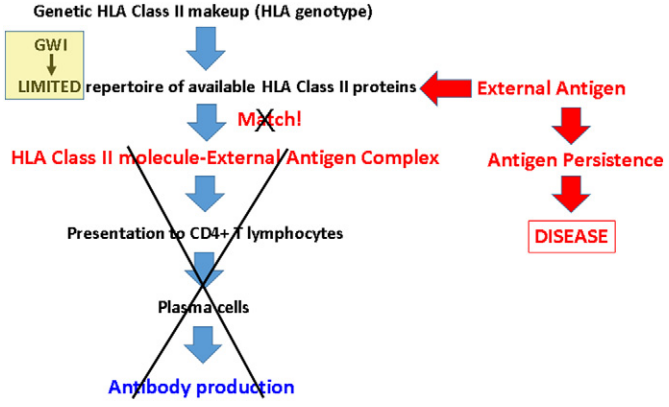

Fig. 4. Schematic diagram illustrating the disruption at various possible stages of antibody production leading to disease.

al., 2013). Either or both of these mechanisms (i.e. protracted low-grade inflammation and/or autoimmunity) could be involved in subcortical brain atrophy observed in GWI (Christova et al., 2017), as discussed in detail in that publication. Given the considerations above, it is possible that the protective role of DRB1*13:02 may be primarily due to preventing infection by providing "matches" (Fig. 3) for many external antigens, leading to successful production of antibodies, eliminating pathogens and thus, in the long run, preventing autoimmunity. In other words, the DRB1*13:02 protein would be a "pluripotent" HLA class II molecule. The reported protective role of DRB1*13:02 against severe malaria (Hill et al., 1991) is in keeping with this notion.

Finally, a challenge for the future is the identification of persistent antigens in GWI and their elimination. Such antigens could come from the many antigens administered to GW veterans as vaccines (Institute of Medicine National Research Council, 2000, page 295) or from other exposures, and could be at the root of the involvement of several organs systems in GWI. If identified, they could be eliminated by administering specific antibodies, e.g. as an antiserum. These possibilities are currently under investigation in our laboratory.

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The authors do not report any financial disclosures.

Author Contributions

Contributed to data collection and clinical evaluation: LMJ, PC, BEE, SML, AFC. Contributed to study design: APG, LMJ, PC, BEE, SML, AFC. Contributed to data analysis: LMJ, PC, APG. Wrote the paper: LMJ, APG. Contributed to editing the paper: All.

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References

Bettencourt, A., Carvalho, C., Leal, B., et al., 2015. The protective role of HLA-DRB1*13 in autoimmune diseases. J Immunol Res 948723:15. https://doi.org/10.1155/2015/948723.

Blake, D.D., Weathers, F.W., Nagy, L.M., et al., 1995. The development of a clinician-administered PTSD scale. J. Trauma. Stress. 8, 75–90.

Brown, J.H., Jardetzky, T.S., Gorga, J.C., et al., 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. Nature 364, 33–39.

Cano, P., Klitz, W., Mack, S.J., et al., 2007. Common and well-documented HLA alleles: report of the Ad-Hoc committee of the American society for histocompatiblity and immunogenetics. Hum. Immunol. 68, 392–417.

Christova, P., James, L.M., Engdahl, B.E., et al., 2017. Subcortical brain atrophy in Gulf War Illness. Exp. Brain Res. 235:2777–2786. https://doi.org/10.1007/s00221-017-5010-8.

Donaldson, P.T., 2011. Electrostatic modifications of the human leukocyte antigen dr p9 peptide-binding pocket in primary sclerosing cholangitis: back to the future with Human Leukocyte Antigen DRβ. Hepatology 53, 1798–1800.

Duarte-Rey, C., Pardo, A.L., Rodriguez-Velosa, Y., et al., 2009. HLA class II association with autoimmune hepatitis in Latin America: a meta-analysis. Autoimmun. Rev. 8, 325–331.

Engdahl, B.E., James, L.M., Miller, R.D., et al., 2016. A magnetoencephalographic (MEG) study of Gulf War Illness (GWI). EBioMedicine 12, 127–132.

Fainboim, L., Canero Velasco, M.C., et al., 2001. Protracted, but not acute, hepatitis A virus infection is strongly associated with HLA-DRB*1301, a marker for pediatric autoimmune hepatitis. Hepatology 33, 1512–1517.

First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., 2002. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition (SCID-I/NP). Biometrics Research New York State Psychiatric Institute, New York.

Fukuda, K., Nisenbaum, R., Stewart, G., et al., 1998. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. JAMA 280, 981–988.

Furukawa, H., Oka, S., Tsuchiya, N., et al., 2017. The role of common protective alleles HLA-DRB1*13 among systemic autoimmune diseases. Genes Immun. 18:1–7. https://doi.org/10.1038/gene.

Georgopoulos, A.P., James, L.M., Mahan, M.Y., et al., 2016. Reduced human leukocyte antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine 3, 79–85.

Georgopoulos, A.P., James, L.M., Carpenter, A.F., et al., 2017. Gulf War Illness (GWI) as a neuroimmune disease. Exp. Brain Res. https://doi.org/10.1007/s00221-017-5050-0.

Glasser, M.F., Sotiropoulos, S.N., Wilson, J.A., et al., 2013. The minimal preprocessing pipelines for the human connectome project. NeuroImage 80, 105–124.

Gough, S.C., Simmonds, M.J., 2007. The HLA region and autoimmune disease: associations and mechanisms of action. Curr. Genomics 8:453–465. https://doi.org/10.2174/138920207783591690.

Hill, A., Allsopp, C., Kwiatkowski, D., et al., 1991. Common West African HLA antigens are associated with protection from severe malaria. Nature 352, 595–600.

Hov, J.R., Kosmoliaptsis, V., Traherne, J.A., et al., 2011. Electrostatic modifications of the HLA-DR P9 peptide-binding pocket and susceptibility to primary sclerosing cholangitis. Hepatology 53:1967–1976. https://doi.org/10.1002/hep.24299.

Institute of Medicine, 2012. Adverse Effects of Vaccines: Evidence and Causality. National Academies Press, Washington, DC.

Institute of Medicine National Research Council (2000) Gulf War and health: volume 1. Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines. Washington, DC: National Academies Press.

Israeli, E., 2012. Gulf War Syndrome as a part of the autoimmune (autoinflammatory) syndrome induced by adjuvant (ASIA). Lupus 21, 190–194.

James, L.M., Engdahl, B.E., Leuthold, A.C., Georgopoulos, A.P., 2016. Brain correlates of human leukocyte antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine 13:72–79. https://doi.org/10.1016/j.ebiom.2016.10.019.

Johnson, G.J., Leis, L.A., Slater, B.C., Bach, R.R., 2013. Elevated platelet count, C-reactive protein and thromboxane analog-induced platelet aggregation in patients with Gulf War veterans' illnesses: evidence of a chronic inflammatory state? Blood Coagul. Fibrinolysis 24, 736–741.

Jones, E.Y., Fugger, L., Strominger, J.L., Siebold, C., 2006. MHC class II proteins and disease: a structural perspective. Nat. Rev. Immunol. 6, 271–282.

Meuer, S.C., Hussey, R.E., Hodgdon, J.C., et al., 1982. Surface structures involved in target recognition by human cytotoxic T lymphocytes. Science 218, 471–473.

Pando, M., Larriba, J., Fernandez, G.C., et al., 1999. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. Hepatology 20, 1374–1380.

Research Advisory Committee on Gulf War Veterans' Illnesses, 2014. Gulf War Illness and the Health of Gulf War Veterans: Research Update and Recommendations. U.S. Government Printing Office, Washington, D.C, pp. 2009–2013.

Shiina, T., Hosomichi, K., Inoko, H., Kulski, J.K., 2009. The HLA genomic loci map: expression, interaction, diversity and disease. J. Hum. Genet. 54:15–39. https://doi.org/10.1038/jhg.2008.5.

Spurkland, A., Saarinen, S., Boberg, K.M., et al., 1999. HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. Tissue Antigens 53, 459–469.

Steele, L., 2000. Prevalence and patterns of Gulf War Illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. Am. J. Epidemiol. 152, 992–1002.

Todd, J.A., Bell, J.I., McDevitt, H.O., 1987. HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329, 599–604.

Valk, J., van der Knaap, M.S., 1992. Toxic encephalopathy. Am. J. Neuroradiol. 13, 747–760.

White, R.F., Steele, L., O'Callaghan, J.P., et al., 2016. Recent research on gulf war illness and other health problems in veterans of the 1991 gulf war: effects of toxicant exposures during deployment. Cortex 74, 449–475.

van der Woude, D., Lie, B.A., Lundström, E., et al., 2010. Protection against anti-citrullinated protein antibody-positive rheumatoid arthritis is predominantly associated with HLA-DRB1*1301: a meta-analysis of HLA-DRB1 associations with anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis in four European populations. Arthritis Rheum. 62: 1236–1245. https://doi.org/10.1002/art.27366.

James LA, Engdahl BE, Johnson RA, Georgopoulos AP. Gulf War Illness and Inflammation: Association of symptom severity with C-reactive protein. J Neurol Neuromed (2019) 4(2): 15-19

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Gulf War Illness and Inflammation: Association of symptom severity with C-reactive protein

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Abstract

Gulf War Illness (GWI) is a chronic multi-system condition that has affected one-third of U.S. veterans who served in the Persian Gulf. Although GWI etiology remains unclear, mounting evidence points to immune system involvement and inflammation, in particular, as underlying the host of symptoms associated with the condition. Here we investigated the association between GWI symptoms and C-reactive protein (CRP), a marker of inflammation, in 76 veterans with GWI. Results indicated a highly significant positive association between CRP and mean GWI symptom severity. At the symptom domain level, CRP was significantly and positively associated with Pain, Neurocognitive/Mood, Fatigue, and Respiratory symptom severity but not with Skin or Gastrointestinal symptom severity. These results support the premise that GWI symptoms, particularly those implicating brain involvement, are a result of neuroinflammation. The cause for inflammation is not known. We have hypothesized that at the root of GWI are harmful persistent antigens stemming from environmental exposures associated with service during the Gulf War that could not be successfully eliminated due to lack of specific immunity[1,2]. Work is underway in our laboratory to identify and eliminate persistent antigens in veterans with GWI which we anticipate will result in reduced inflammation and reduced GWI symptoms.

Introduction

Gulf War Illness is a chronic disease of unclear etiology that has affected a large number of veterans of the 1990-1991 Persian Gulf War. Symptoms affect several systems and include fatigue, musculoskeletal pain, neurological and cognitive impairment, and mood disruptions[3] in addition to respiratory, gastrointestinal, and dermatological complaints[4]. Burgeoning evidence suggests that genetic vulnerability related to immune system functioning coupled with environmental insults may underlie the host of symptoms observed in GWI[1,2].

Increasingly, immune system disruption has been recognized in relation to GWI[1,5-14]. Consistent with evidence of immune-mediated loss of blood brain barrier integrity[15], alterations in brain structure and function have been associated with GWI[16-19]. Furthermore, brain function in GWI has been shown to be indistinguishable from that of known immune-related conditions[20]. Robust evidence of immune involvement in conjunction with brain alterations suggests GWI is best characterized as a neuroimmune condition[7,20] resulting from persistent antigens that contribute to immune system disruption and inflammation[2].

Evidence of inflammation has been reported in veterans with GWI[10,21,22]. For example, C-reactive protein (CRP), a non-specific acute-

phase biomarker of inflammation, has been shown to be elevated in GWI[10,21]. In fact, of 61 plasma proteins evaluated in one study of GWI[10], CRP was one of only 6 that significantly differed between veterans with and without GWI and the only protein identified from a stepwise multivariate logistic regression model that contributed to a diagnostic model of GWI (along with lymphocytes, and monocytes). CRP is synthesized primarily in the liver hepatocytes; however, other cell types including smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes have also been shown to synthesize CRP[23]. Notably, emerging evidence also indicates local CRP production in human neuronal cells of patients with Alzheimer's disease and upregulation of CRP in Alzheimer's-affected brain areas[24]. Finally, CRP has historically been viewed as a marker of inflammation that arises in response to inflammatory cytokines such as interleukin 6; yet, mounting evidence suggests CRP may also play a causal role in inflammation[23]. Thus, CRP may both signal and potentiate inflammation in various cell types and tissues, perhaps underlying the diffuse symptoms involving multiple systems as seen in GWI. To date, however, relatively little is known about CRP as it relates to GWI. Here we aim to evaluate the association between GWI symptoms and CRP levels to further assess the link between CRP and GWI symptomatology.

Materials and Methods

Participants: A total of 76 veterans with GWI and no comorbidities were studied (70 men, age 56.3 ± 8.1 y [mean ± SD], 6 women, age 50.5 ± 5.2 y). GWI status was determined using a self-report symptom checklist that evaluates the presence and severity of various symptoms comprising 6 domains characteristic of GWI: fatigue, pain, neurological/mood/cognitive, gastrointestinal, skin rashes, and respiratory. Items were rated on a scale from 0 (absent) to 3 (severe). Veterans who met either Center for Disease Control[1] or Kansas criteria[2] for GWI were included in the present analyses. All study protocols were approved by the appropriate Institutional Review Boards. Study participants provided informed consent, in adherence to the Declaration of Helsinki, and were financially compensated for their time.

CRP: Non-fasting peripheral venous blood samples were collected for evaluation of high sensitivity C reactive protein and analyzed using standard procedures by the Minneapolis VAHCS Clinical Laboratory.

Other variables: The Body Mass Index (BMI) was 31.65 ± 5.21 (mean ± SD, N = 76). No participant reported illegal drug use or alcohol abuse. Twenty-one participants were receiving medications (15 on antidepressants, 4 on pain relievers, 2 on beta blockers); five were receiving opioids for pain relief (3 tramadol, 2 hydrocodone).

Data analysis: The IBM-SPSS statistical package (version 23) was used to analyze the data. The average GWI symptom severity across all domains was computed as well as the average symptom severity within each of the 6 GWI symptom domains. The correspondence between GWI symptoms and CRP were evaluated using stepwise linear regressions., where GWI symptom severity was the dependent variable, CRP was the independent variable, and medication status and BMI were covariates.

Results

CRP. CRP values were distributed in a non-normal fashion (Figures 1 and 2). Therefore, they were transformed to their logarithms to normalize their distribution (Figures 3 and 4). The log-transformed CRP values, CRP', were used in all subsequent analyses:

$$CRP' = \ln(CRP) \quad (1)$$

Association of GWI symptom severity with CRP. The mean of GWI symptom severity (across the 6 GWI symptom

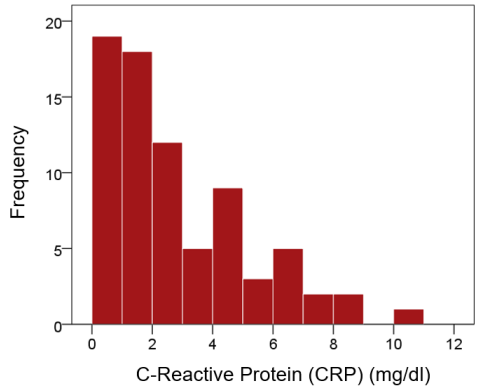

Figure 1: Exponential distribution of CRP values.

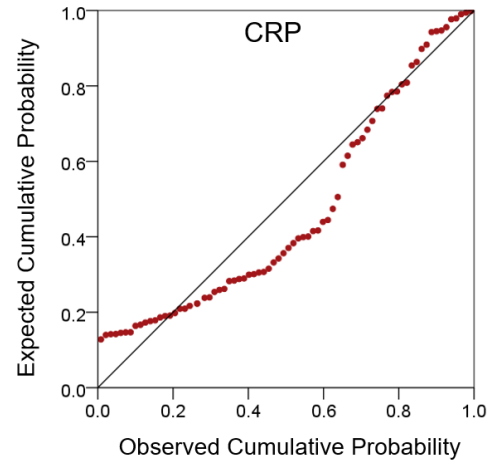

Figure 2: Probability plot of CRP values, under the assumption of a normal distribution. A substantial deviation from normality is indicated by the deviation of the plotted values from the midline.

domains) was significantly and positively associated with ln(CRP) [CRP', equation 1] (Figure 5; r = 0.353, P = 0.002). The results of the stepwise regression analyses are shown in Table 1.

It can be seen that CRP' had a significant effect in all but the Gastrointestinal and Skin domains. BMI did not have a significant effect in any analysis, whereas medication status had a significant effect in all but the Respiratory domain.

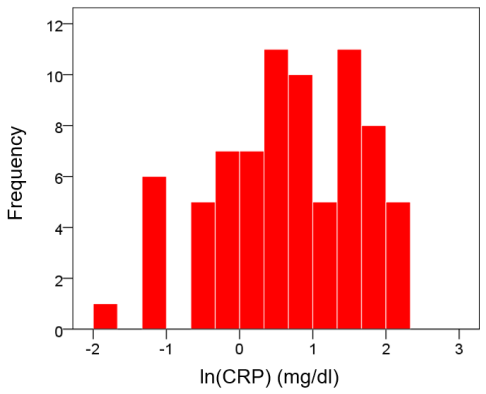

Figure 3: Log-transformed distribution of CRP values.

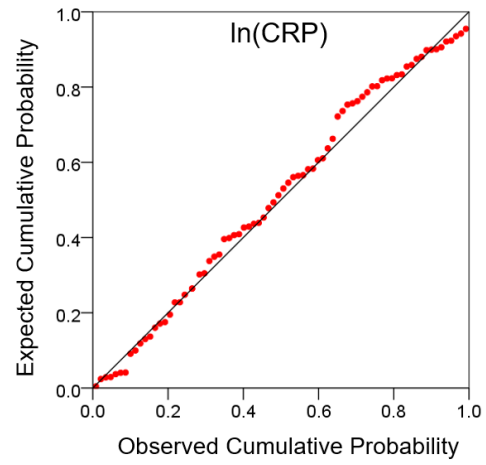

Figure 4: Probability plot of log-transformed CRP values, under the assumption of normal distribution. Notice the closeness of the plotted values to the midline.

Discussion

Here we investigated the association between inflammation and GWI symptoms in a sample of GW veterans and found a highly significant positive association between CRP, a marker of inflammation, and GWI symptom severity. The results add to the literature highlighting the role of inflammation in GWI[10] and point to the potential benefit of interventions for GWI aimed at reducing inflammation.

GWI is a chronic condition characterized by widespread symptoms spanning several systems including the central nervous system, respiratory, dermatologic, and gastrointestinal system. Of these, the brain is prominently involved with three of the six symptom domains - fatigue, pain, and neurocognitive/mood symptoms - implicating brain involvement. Notably, all three of these domains were highly significantly associated with CRP in the present study, further cementing GWI as a neuroimmune condition[20]. Respiratory symptoms were the only other domain that was significantly associated with inflammation.

Based on a series of recent findings in our lab we have proposed that GWI is a result of persistent antigens stemming from environmental exposures associated with service during the Gulf War that could not be successfully eliminated due to lack of specific immunity[25]. Initial support

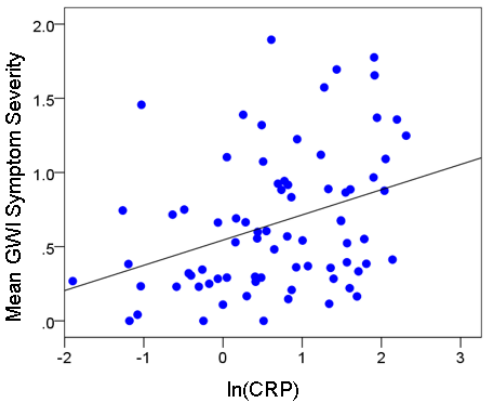

Figure 5: Association of mean GWI symptom severity with log-transformed CRP.

Table 1. Result of the stepwise regression analyses. P-values are those obtained for the final-step model; "excluded" denotes absence of the variable from this model.

Dependent variable (symptom score)	Independent variable	Covariates	
GWI symptom domain	CRP'	Medication status	BMI
Mean symptom severity	P < 0.001	P < 0.001	P = 0.242 (excluded)
Fatigue	P < 0.001	P < 0.001	P = 0.838 (excluded)
Pain	P = 0.013	P = 0.024	P = 0.163 (excluded)
Neurocognitive	P = 0.004	P < 0.001	P = 0.977 (excluded)
Respiratory	P = 0.018	P = 0.317 (excluded)	P = 0.618 (excluded)
Gastrointestinal	P = 0.097 (excluded)	P = 0.012	P = 0.112 (excluded)
Skin	P = 0.603 (excluded)	P = 0.045	P = 0.323 (excluded)

of the "Persistent Antigen" hypothesis was established on findings demonstrating that 6 Class II human leukocyte antigens (HLA) distinguish healthy Gulf War veterans from veterans with GWI[1]. Specifically, the 6 alleles were significantly more common among healthy veterans suggesting that their presence is protective against GWI; conversely, the absence of these HLA alleles and consequent lack of protection results in GWI. Subsequently, we demonstrated that one of the 6 protective alleles, in particular – HLADRB1*13:02 – is highly protective against brain atrophy[2,26] that has been observed in GWI veterans[16]. The protection provided by the presence of these class II alleles is inherent in their function which is elimination of foreign antigens via antibody production; however, the ability to stimulate antibody production hinges on a match between HLA proteins and epitopes derived from foreign antigens. In the absence of a match, the antigen persists, resulting in inflammation (reflected here by elevated CRP) and other detrimental effects including cell damage and atrophy. Thus, we suspect that prominent GWI symptoms, particularly those implicating brain involvement, are a result of neuroinflammation due to the persistence of foreign antigens resulting from lack of HLA protection.

Although the specific cause of inflammation in GWI veterans remains to be elucidated, we hypothesize that it is the result of the existence of harmful persistent antigens in GWI; indeed, we have studies underway aimed at identifying persistent antigens in veterans with GWI with the goal of ultimately eliminating them (and thereby reducing inflammation) via personalized immunotherapy. Two recent in vitro studies in our lab have provided initial evidence supporting immunotherapy as a promising intervention for GWI. Specifically, we have demonstrated that serum from veterans with GWI results in detrimental changes to cell morphology in neural cultures; however, the addition of serum from healthy Gulf War veterans[27] or human immunoglobulin G (IgG)[28] neutralize those damaging effects. The neutralizing effects are presumed to result from the ability of antibodies present in serum from healthy Gulf War veterans and in pooled IgG to eliminate persistent antigens in veterans with GWI. We anticipate that elimination of persistent antigens would result in reduced inflammation and reduced GWI symptoms. Inflammatory response regulation via monoclonal antibodies targeting specific cytokines or neuroendocrine control of the cytokine network, as is under investigation in other diseases[29] may prove to be useful alternative therapeutic strategies for reducing GWI-related inflammation and symptoms.

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Author Contributions

Contributed to data collection: LMJ, RAJ. Contributed to participant recruitment and evaluation: BEE, LMJ, RAJ. Contributed to study design: APG, BEE, LMJ. Contributed to data analysis: APG, LMJ. Wrote the paper: LMJ, APG. Contributed to editing the paper: All.

References

1. Georgopoulos AP, James LM, Mahan MY, et al. Reduced Human Leukocyte Antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine. 2016; 3: 79-85
2. James LM, Christova P, Engdahl BE, et al. Human leukocyte antigen (HLA) and Gulf War Illness (GWI): HLA-DRB1*13:02 spares subcortical atrophy in Gulf War veterans. EBioMedicine. 2017; 26: 126-131.
3. Fukuda K, Nisenbaum R, Stewart G, et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. JAMA. 1998; 280: 981-988.
4. Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. Am J Epidemiol. 2000; 152: 992-1002.
5. Broderick G, Ben-Hamo R, Vashishtha S, et al. Altered immune pathway activity under exercise challenge in Gulf War Illness: an exploratory analysis. Brain Behav Immun. 2013; 28: 159–169.
6. Broderick G, Fletcher MA, Gallagher et al. Exploring the diagnostic potential of immune biomarker coexpression in Gulf War Illness. Methods Mol Biol. 2012; 934: 145–164.
7. Coughlin SS. A neuroimmune model of Gulf War Illness. J Environ Health Sci. 2017; 3: 106.
8. Hotopf M, David A, Hull L, et al. Role of vaccinations as risk factors for ill health in veterans of the Gulf war: cross-sectional study. BMJ. 2000; 320: 1363–1367.
9. Israeli E. Gulf War Syndrome as a part of the autoimmune (autoinflammatory) syndrome induced by adjuvant (ASIA). Lupus. 2012; 21: 190–194.
10. Johnson GJ, Slater BC, Leis LA, et al. Blood biomarkers of chronic inflammation in Gulf War Illness. PLoS One. 2016; 11(6): e0157855
11. Moss JI. Gulf War illnesses are autoimmune illnesses caused by increased activity of the p38/MAPK pathway in CD4+ immune system cells, which was caused by nerve agent prophylaxis and adrenergic load. Med. Hypotheses. 2013; 81: 1002–1003
12. Parkitny L, Middleton S, Baker K, et al. Evidence for abnormal cytokine expression in Gulf War Illness: A preliminary analysis of daily immune monitoring data. BMC Immunol. 2015; 16: 57.
13. Skowera A, Hotopf M, Sawicka E, et al. Cellular immune activation in Gulf War veterans. J Clin Immunol. 2004; 24: 66-73.
14. Whistler T, Fletcher MA, Lonergan W, et al. Impaired immune function in Gulf War Illness. BMC Med Genomics. 2009; 2: 12 (8794–2-12).
15. Esposito P, Gheorghe D, Kandere K, et al. Acute stress increases permeability of the blood–brain-barrier through activation of brain mast cells. Brain Res. 2001; 888: 117–127
16. Christova P, James LM, Engdahl BE, et al. Subcortical brain atrophy in Gulf War Illness. Exp Brain Res. 2017; 235: 2777-2786.

17. James LM, Engdahl BE, Leuthold AC, et al. Brain correlates of human leukocyte antigen (HLA) protection in Gulf War Illness (GWI). EbioMedicine. 2016; 13: 72-79.

18. Engdahl BE, James LM, Miller RD, et al. A magnetoencephalographic (MEG) study of Gulf War Illness (GWI). EBioMedicine. 2016 Oct 1; 12: 127-32.

19. White RF, Steele L, O'Callaghan JP, et al. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: effects of toxicant exposures during deployment. Cortex. 2016; 74: 449-475.

20. Georgopoulos AP, James LM, Carpenter AF, et al. Gulf War illness (GWI) as a neuroimmune disease. Exp Brain Res. 2017; 235(10): 3217-3225.

21. Johnson GJ, Leis LA, Slater BC, et al. Elevated platelet count, C-reactive protein and thromboxane analog-induced platelet aggregation in patients with Gulf War veterans' illnesses: evidence of a chronic inflammatory state? Blood Coagul. Fibrinolysis. 2013; 24: 736–741.

22. Kelsall HL, McKenzie DP, Sim MR, et al. Physical, psychological, and functional comorbidities of multisymptom illness in Australian male veterans of the 1991 Gulf War. Am J Epidemiol. 2009; 170: 1048–1056

23. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Frontiers Immunol. 2018; 9.

24. Yasojima K, Schwab C, McGeer EG, et al. Human neurons generate C-reactive protein and amyloid P: upregulation in Alzheimer's disease. **Brain Res**. 2000; 887: 80–89.

25. James LM, Georgopoulos AP. Persistent antigen hypothesis: The human leukocyte antigen connection. J Neurol Neuromed, in press.

26. James LM, Christova P, Lewis SM, et al. Protective Effect of Human Leukocyte Antigen (HLA) Allele DRB1* 13: 02 on Age-Related Brain Gray Matter Volume Reduction in Healthy Women. EBioMedicine. 2018; 29: 31-7.

27. Georgopoulos AP, Tsilibary EP, Souto EP, et al. Adverse effects of Gulf War Illness (GWI) serum on neural cultures and their prevention by healthy serum. J Neurol Neuromed. 2018; 3(2): 19-27.

28. Tsilibary CEP, Souto EP, James LM, et al. Human immunoglobulin G (IgG) neutralizes adverse effects of Gulf War Illness (GWI) serum in Neural cultures: Paving the way to immunotherapy for GWI. J Neurol Neuromed. 2018; 3(5): 23-28.

29. Lissoni P, Messina G, Cenaj V, et al. The role of IL-17 secretion in mediating the influence of stress on cancner and other human systemic diseases. MOJ Immunol. 2018; 6(5): 180–183.

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Opinion Article Open Access

Persistent Antigens Hypothesis: The Human Leukocyte Antigen (HLA) Connection

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Human Leukocyte Antigen (HLA) Overview

HLA genes play a critical role in immune protection from foreign antigens including viruses, bacteria, and parasites[1]. Located in the Major Histocompatibility Complex (MHC) of chromosome 6, HLA genes code for glycoproteins that exist on the surface of most cells in order to facilitate immune surveillance and initiate an immune response to eliminate foreign antigens. There are two main classes of HLA (Class I and Class II) that support the elimination of cytosolic or extracellular foreign antigens through cell destruction and antibody production, respectively. HLA genes have evolved to be the most highly polymorphic in the human genome, thereby maximizing species resistance to foreign antigens and promoting survival. Nonetheless, successful elimination of foreign antigens is predicated on a match between one's HLA and epitopes derived from foreign antigen proteins. Each person has a limited repertoire of HLA proteins inherited in a Mendelian fashion for each class. Fortunately, each HLA protein can match with various epitopes and, since everyone has one or two alleles at each of the classical loci (Class I HLA-A, B, and C and Class II HLA-DP, DQ, and DR), a large number of antigens can be effectively eliminated.

The Match

The structure of the HLA molecule determines the specific epitopes that can bind and, therefore, be eliminated. Although the overall structure of Class I and Class II HLA molecules differ, they are similar in that for both classes polypeptide chains form a binding groove. It is variation within the binding groove that contributes to high polymorphism. For instance, within the Class II HLA DRB1*13 allele family alone there are 330 known variants[2], although some alleles such as DRB1*13:01 and DRB1*13:02 are much more common than others forms of the allele. DRB1*13:01 and DRB1*13:02 differ by a single amino acid in the β-chain; yet that difference has been shown to significantly alter the electrostatic properties of the binding groove[3]. Indeed, seemingly small differences conferred by single amino acid substitutions result in variations in charge, stability, and binding affinity[4] that translate into the differential ability to effectively eliminate foreign antigens. Those differences are amplified across different genes and even more so across HLA classes. Class I HLA molecules, which are expressed on nucleated cells, present small peptides (8-10 amino acid residues) from proteolytically degraded intracellular viruses, bacteria, and tumors to cytotoxic T cells for degradation. The process is facilitated by transport of the cytosolic peptides to the endoplasmic reticulum where they

are loaded onto Class I molecules and exported to the cell surface for presentation to CD8+ cytotoxic T cells for cell destruction. Class II HLA, which are expressed on lymphocytes and antigen presenting cells including macrophages, dendritic cells, and monocytes, present larger peptides (12-20 amino acid residues) derived from endocytosed exogenous antigens including viruses and bacteria to CD4+ T cells which stimulate the production of antibodies. For Class II molecules, antigen binding is facilitated by Cathepsin S which removes an invariant chain that blocks binding. Despite all of these differences, Class I and Class II HLA share the same overarching goal and work in concert to maximize elimination of foreign antigens.

HLA-Disease Associations

The HLA region of the genome is associated with the greatest number of human diseases[5]. Given its role in immune system functioning it is not surprising that HLA has been implicated in a host of autoimmune and infectious diseases[6]; however, more recent studies have also implicated HLA in diseases ranging from schizophrenia[7] and autism[8] to neurodegenerative diseases such as Alzheimer's disease[9,10] and Parkinson's disease[11]. Reports of HLA-disease associations have typically been derived from population studies in which certain genes are found to be more prevalent in specific disease groups. This particular approach of identifying risk-related genes is complicated by high linkage disequilibrium. Due to high linkage disequilibrium within the HLA region, some combinations of genes, referred to as haplotypes, are inherited together thereby leading to potential "mistaken identity" in terms of disease risk[12].

Etiopathological models underlying HLA-disease associations have been summarized elsewhere[5,6]. Briefly, prevailing models implicate non-mutually exclusive processes including alterations in T cell repertoire, molecular mimicry, aberrant antigen recognition, and ineffective interactions of the antigen-HLA complex with T cells as promoting disease susceptibility. A multitude of viral escape mechanisms may also contribute to diseases via disruption of immune system functioning[13,14]. Despite extensive study of mechanisms underlying HLA-disease associations, the fact remains that in most cases the mechanisms remain poorly understood and likely represent genetic vulnerability coupled with environmental insults[6,15]. Further, as compellingly discussed elsewhere[12], most prevailing theories of HLA-disease association are inconsistent with the biology, epidemiology, and evolution of HLA molecules.

HLA Protective Effects

Given the biological and evolutionarily adaptive role of HLA in the clearance of foreign antigens and maintenance of immune system functioning, the concept of HLA-disease associations is counterintuitive. That is, HLA's primary function is host protection. Accordingly, there also exists robust evidence of HLA protective effects. To that end, protective HLA alleles have been observed in conditions including autoimmune disorders[6,16], HIV[17,18], Hepatitis B and C[19], malaria[20], and Gulf War Illness[15,21], among others. Furthermore, certain HLA alleles have been shown to exert protective effects in healthy individuals, minimizing age-related brain changes typically attributed to "normal" aging[22,23](see below).

In many cases, protective effects have been shown to vary by population[19]. HLA is known to vary by ethnicity and/or locale, presumably reflecting evolutionary adaptations related to population differences in pathogen exposure. Nonetheless, some alleles appear to more broadly promote protection across a wide variety of diseases and populations. For example, HLA-DRB1*13 has been shown to be protective against Hepatitis B and C[19] and various autoimmune conditions[16,24] across several populations. Similarly, variants of the DRB1*13 allele group have been shown to protect against the development of Gulf War Illness[15], and protect against brain atrophy in Gulf War veterans[20]. Furthermore, the protective effects of DRB1*13 have been observed in cognitively healthy women with carriers evidencing minimal age-related brain atrophy and functional brain changes relative to non-carriers[22,23]. Of note, within the DRB1*13 allele family, variations at the protein level confer differential protection. For example, while DRB1*13:02 has been associated with broad protective effects, DRB1*13:01, which differs by only a single amino acid residue, has shown both protective[23,25] and risk effects[3,26]. The differential effects are presumably associated with changes in the binding groove that affect antigen binding and subsequent elimination.

An alternative: Persistent Antigen Hypothesis

In light of the biological role of HLA in immune system surveillance and regulation coupled with observed HLA-associated disease protection, we have proposed an alternative model of disease susceptibility referred to as the Persistent Antigen hypothesis. Essentially, maintenance of immune system functioning rests on successful elimination of foreign antigens, a process that partially depends on a match between the antigen and an individual's HLA composition. In contrast, the absence of an antigen-HLA match prevents the elimination of the antigen. Consequently, a persistent antigen may lead to inflammation, cell damage, autoimmunity[27], and atrophy[21,22]. The Persistent Antigen hypothesis contends that the HLA-antigen match is a critical step for the elimination of foreign antigens and health maintenance; in the absence of an HLA-antigen match, any number of downstream processes involved in foreign antigen recognition, cell

destruction, and antibody production would be disrupted including those specifically implicated in HLA-disease associations[5,6]. Thus, the Persistent Antigen hypothesis, which highlights the fundamental role of the HLA-antigen match, is complementary to other etiopathological models of HLA-disease associations.

Our research on Gulf War Illness (GWI) provides an illustrative example. GWI is a neuroimmune condition[28] of uncertain etiology that has affected one-third of US veterans deployed to the 1990-1991 Gulf War[29]. GWI is characterized by diffuse, chronic symptoms including fatigue, joint and muscle pain, and neurocognitive and mood disruptions, as well as gastrointestinal, respiratory, and skin problems[30,31]. Given the overlap of GWI symptoms with known autoimmune conditions and evidence of abnormal immune responses in veterans with GWI[32-34], we investigated HLA involvement with GWI. We identified 6 HLA alleles that were present in healthy GW veterans but were absent or significantly less frequent in those with GWI - that is, GWI was associated with genetic lack of HLA protection[15]. We hypothesized that this genetic vulnerability coupled with environmental hits including multiple, often concurrent vaccinations and/or chemical or other toxic exposures that have been implicated in GWI[27] underlie the development of GWI and explain why one-third of veterans were affected whereas the remainder were not. That is, the former may not have been able to effectively eliminate pathogens due to an insufficient match between the pathogenic epitopes and HLA proteins whereas the latter was afforded genetic protection that presumably facilitated effective elimination of pathogens derived from the various environmental exposures that were associated with service during the Gulf War[27].

Notably, HLA proteins are expressed on most cells (Class I on nucleated cells and Class II on professional antigen presenting cells), providing widespread protection in the case of HLA-antigen matching; however, the absence of HLA-antigen matching in GWI may inflict widespread damage. Indeed, multiple systems and organs are involved in GWI including the brain, lungs, gastrointestinal tract, and musculoskeletal systems. Regarding GWI effects on the brain, infection, autoimmunity, and stress have been shown to result in loss of the blood-brain barrier integrity[35], which may permit entry of circulating pathogens into the brain, ultimately leading to several GWI brain-related symptoms including fatigue, pain, and neurocognitive mood symptoms. We have also shown that GWI is associated with significant brain atrophy, particularly involving subcortical structures[36]. Remarkably, the presence of protective HLA has been shown to protect against brain atrophy in Gulf War veterans[21].

The implications of the Persistent Antigen hypothesis extend well beyond Gulf War Illness. In fact, we have recently demonstrated HLA protective effects on maintaining healthy cognitive function across the lifespan. Age-related changes in brain structure and function have been widely investigated and reviewed elsewhere[37]. For example, age-related brain atrophy at the rate of 2-5% volume loss per decade beginning around age 40 has been firmly established[38]. However, we recently showed HLA protection against age-related brain atrophy in cognitively healthy women[22]. Remarkably, we also demonstrated HLA protection against changes in brain functioning in cognitively healthy women, even in the presence of apolipoprotein E4, a well-known Alzheimer's disease risk gene[23]. Thus, we hypothesize that efficient HLA-antigen matching protects the brain (and other organs) from what has typically been referred to as "normal" age-related deterioration. In contrast, the absence of an HLA-antigen match may hinder elimination of foreign antigens that might otherwise result in subtle, accumulative damage such as that ascribed to cellular senescence[39,40] and ultimately neurodegenerative diseases including Alzheimer's disease.

Conclusion

The Persistent Antigen hypothesis provides a novel perspective on HLA-disease associations that is firmly rooted in the biological role of HLA in eliminating foreign antigens via HLA-antigen matching. Thus, the Persistent Antigen hypothesis suggests a plausible mechanism underlying a multitude of diseases in which immune and inflammatory processes are implicated – that is, lack of HLA-antigen matching contributes to antigen persistence which ultimately leads to disease. This, of course, suggests that both exposures to foreign antigens and genetic vulnerability that precludes their elimination are integral in disease development. On the other hand, limited exposure to foreign antigens and/or genetic protection may limit disease and enhance longevity. Indeed, we suspect that these very factors contribute to the enhanced longevity observed in several isolated communities referred to as the "blue zones"[41]. Finally, the Persistent Antigen hypothesis also implies potential treatment avenues along the lines of personalized precision immunotherapy aimed at facilitating the elimination of circulating persistent antigens which we are currently pursuing[42,43].

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References

1. Meuer SC, Hussey RE, Hodgdon JC, et al. Surface structures involved in target recognition by human cytotoxic T lymphocytes. Science. 1982; 218: 471-473.

2. Robinson J, Halliwell JA, Hayhurst JH, et al. The IPD and IPD-IMGT/

HLA Database: allele variant databases. Nucleic Acids Research. 2015; 43: D423-431, version 3.34.0, release date 10-18-18. Retrieved November 15, 2018.

3. Hov JR, Kosmoliaptsis V, Traherne JA, et al. Electrostatic modifications of the HLA-DR P9 peptide-binding pocket and susceptibility to primary sclerosing cholangitis. Hepatology. 2011; 53: 1967-1976.
4. Stern LJ, Brown JH, Jardetzky TS, et al., Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. Nature. 1994; 368: 215–221.
5. Trowsdale J, Knight JC. Major histocompatibility complex genomics and human disease. Ann Rev Genom Hum Genet. 2013; 14: 301–323.
6. Gough SC, Simmonds MJ. The HLA region and autoimmune disease: Associations and mechanisms of action. Curr Genomics. 2007; 8(7): 453-65.
7. Brucato N, Guadalupe T, Franke B, et al. A schizophrenia-associated HLA locus affects thalamus volume and asymmetry. Brain Behav Immun. 2015; 46: 311-8.
8. Torres AR, Maciulis A, Stubbs EG, et al. The transmission disequilibrium test suggests that HLA-DR4 and DR13 are linked to autism spectrum disorder. Hum Immunol. 2002; 63(4): 311-6.
9. Lambert JC, Ibrahim-Verbaas CA, Harold D, et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet. 2013; 45: 1452-1458.
10. Steele NZ, Carr JS, Bonham LW, et al. Fine-mapping of the human leukocyte antigen locus as a risk factor for Alzheimer disease: a case–control study. PLOS 2017, 10.1371/journal.pmed.1002272.
11. Nalls MA, Plagnol V, Hernandez DG, et al. International Parkinson Disease Genomics Consortium Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet. 2011; 377: 641–649.
12. Holoshitz J. The quest for better understanding of HLA-disease association: scenes from a road less travelled by. Discov Med. 2013; 16(87): 93-101.
13. Lucas M, Karrer U, Lucas A, et al. Viral escape mechanisms--escapology taught by viruses. Int J Exp Pathol. 2001; 82(5): 269-86.
14. Alcami A, Koszinowski UH. Viral mechanisms of immune evasion. Trends Microbiol. 2000; 8(9): 410-8.
15. Georgopoulos AP, James LM, Mahan MY, et al. Reduced Human Leukocyte Antigen (HLA) protection in Gulf War Illness (GWI). EBioMedicine. 2016; 3: 79-85.
16. Bettencourt A, Carvalho C, Leal B, et al. The protective role of HLA-DRB1*13 in autoimmune dis eases. J Immunol Res. 2015; 948723. http://dx.doi.org/10.1155/2015/948723.
17. Pereyra F, Jia X, et al. International HIV Controllers Study, The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. Science. 2010; 330: 1551-7.
18. Goulder PJ, Walker BD. HIV and HLA class I: an evolving relationship. Immunity. 2012; 37: 426-40.
19. Singh R, Kaul R, Kaul A, et al. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. World J Gastroenterol. 2007; 13(12): 1770-87.
20. Hill AV, Allsopp CE, Kwiatkowski D, et al. Common West African HLA antigens are associated with protection from severe malaria. Nature. 1991; 352: 595-600.
21. James LM, Christova P, Engdahl BE, et al. Human leukocyte antigen (HLA) and Gulf War Illness (GWI): HLA-DRB1*13:02 spares subcortical atrophy in Gulf War veterans. EBioMedicine. 2017; 26: 126-131.
22. James LM, Christova P, Lewis SM, et al. Protective Effect of Human Leukocyte Antigen (HLA) Allele DRB1* 13: 02 on Age-Related Brain Gray Matter Volume Reduction in Healthy Women. EBioMedicine. 2018; 29: 31-7.
23. James LM, Dolan S, Leuthold AC, et al. The effects of human leukocyte antigen DRB1* 13 and apolipoprotein E on age-related variability of synchronous neural interactions in healthy women. EBioMedicine. 2018; 35: 288-94.
24. Furukawa H, Oka S, Tsuchiya N, et al. The role of common protective alleles HLA-DRB1*13 among systemic autoimmune diseases. Genes Immun. 2017; 18: 1–7.
25. van der Woude D, Lie BA, Lundström E, et al. Protection against anti-citrullinated protein antibody-positive rheumatoid arthritis is predominantly associated with HLA-DRB1*1301: a meta-analysis of HLA-DRB1 associations with anti-citrullinated protein antibody-positive and anti-citrullinated protein antibody-negative rheumatoid arthritis in four European populations. Arthritis Rheum. 2010; 62: 1236-1245.
26. Fainboim L, Canero VMC, Marcos CY, et al. Protracted, but not acute, hepatitis a virus infection is strongly associated with HLA-DRB*1301, a marker for pediatric autoimmune hepatitis. Hepatology. 2001; 33: 1512-1517.
27. Institute of Medicine National Research Council. Gulf War and Health: Volume 1. Depleted Uranium, Pyridostigmine Bromide, Sarin, and Vaccines. Washington, DC: National Academies Press, 2000.
28. Georgopoulos AP, James LM, Carpenter AF, et al. Gulf War illness (GWI) as a neuroimmune disease. Exp Brain Res. 2017; 235(10): 3217-3225.
29. White RF, Steele L, O'Callaghan JP, et al. Recent research on Gulf War illness and other health problems in veterans of the 1991 Gulf War: effects of toxicant exposures during deployment. Cortex. 2016; 74: 449-475.
30. Fukuda K, Nisenbaum R, Stewart G, et al. Chronic multisymptom illness affecting Air Force veterans of the Gulf War. JAMA. 1998; 280: 981-988.
31. Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service Am J Epidemiol. 2000; 152: 992-1002.
32. Broderick G, Ben-Hamo R, Vashishtha S, et al. Altered immune pathway activity under exercise challenge in Gulf War Illness: an exploratory analysis. Brain Behav Immun. 2013; 28: 159–169.
33. Hotopf M, David A, Hull L, et al. Role of vaccinations as risk factors for ill health in veterans of the Gulf war: cross-sectional study. BMJ. 2000; 320: 1363–1367.
34. Johnson GJ, Slater BC, Leis LA, et al. Blood biomarkers of chronic inflammation in Gulf War Illness. PLoS One. 2016; 11(6): e0157855
35. Esposito P, Gheorghe D, Kandere K, et al. Acute stress increases permeability of the blood–brain-barrier through activation of brain mast cells. Brain Res. 2001; 888: 117–127.
36. Christova P, James LM, Engdahl BE, et al. Subcortical brain atrophy in Gulf War Illness. Exp Brain Res. 2017; 235: 2777-2786.
37. Raz N, Rodrigue KM. Differential aging of the brain: patterns, cognitive correlates and modifiers Neurosci Biobehav Rev. 2006; 30: 730-748.
38. Enzinger C, Fazekas F, Matthews PM, et al. Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects. Neurology. 2005; 64(10): 1704-11.
39. Baker DJ, Petersen RC. Cellular senescence in brain aging and neurodegenerative diseases: evidence and perspectives. J Clin Invest. 2018; 128(4): 1208-1216.

40. Kirkland JL, Tchkonia T. Cellular senescence: a translational perspective. EBioMedicine. 2017; 21: 21-8.

41. Poulain M, Herm A, Pes G. The Blue Zones: areas of exceptional longevity around the world. Vienna Yearbook of Population Research. 2013 Jan 1; 87-108.

42. Georgopoulos AP, Tsilibary EP, Souto EP, et al. Adverse effects of Gulf War Illness (GWI) serum on neural cultures and their prevention by healthy serum. J Neurol Neuromed. 2018; 3(2): 19-27.

43. Tsilibary CEP, Souto EP, James LM, et al. Human immunoglobulin G (IgG) neutralizes adverse effects of Gulf War Illness (GWI) serum in Neural cultures: Paving the way to immunotherapy for GWI. J Neurol Neuromed. 2018; 3(5): 23-28.