EBioMedicine 26 (2017) 126–131

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

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

Lisa M. James [a,b,c], Peka Christova [a,b], Brian E. Engdahl [a,b,d], Scott M. Lewis [a,e], Adam F. Carpenter [a,e], Apostolos P. Georgopoulos [a,b,c,e,*]

[a] Brain Sciences Center, Department of Veterans Affairs Health Care System, Minneapolis, MN 5541, USA
[b] Department of Neuroscience, University of Minnesota Medical School, Minneapolis, MN 55455, USA
[c] Department of Psychiatry, University of Minnesota Medical School, Minneapolis, MN 55455, USA
[d] Department of Psychology, University of Minnesota, Minneapolis, MN 55455, USA
[e] Department of Neurology, University of Minnesota Medical School, Minneapolis, MN 55455, USA

ARTICLE INFO

Article history:
Received 8 October 2017
Received in revised form 31 October 2017
Accepted 6 November 2017
Available online 9 November 2017

Keywords:
Gulf War Illness
Human Leukocyte Antigen
DRB1*13:02
DRB1*13:01
Subcortical brain atrophy
Cerebellum

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.
Funding source: U.S. Department of Defense (W81XWH-15-1-0520), Department of Veterans Affairs, American Legion Brain Sciences Chair, and University of Minnesota.

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

* Corresponding author at: Brain Sciences Center (11B), Minneapolis VAHCS, One Veterans Drive, Minneapolis, MN 55417, USA.
E-mail address: omega@umn.edu (A.P. Georgopoulos).

https://doi.org/10.1016/j.ebiom.2017.11.005

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).