Defining the neurotoxin derived illness chronic ciguatera using markers of chronic systemic inflammatory disturbances: A case/control study

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ARTICLE INFO

Article history:
Received 25 February 2010
Received in revised form 25 May 2010
Accepted 26 May 2010
Available online xxxx

Keywords:
Ciguatera fish poisoning
Ciguatoxin
Inflammation
Immune
Biotoxin
Chronic inflammatory response syndrome

ABSTRACT

Background: Ciguatoxins are extremely potent neurotoxins, produced by tropical marine dinoflagellates, that persistently enter into our food web. Over 100,000 people annually experience acute ciguatera poisoning from consuming toxic fish. Roughly 5% of these victims will develop chronic ciguatera (CC), a widespread, multisystem, multisystem, chronic illness that can last tens of years. CC is marked by disproportionate disability and non-specific refractory symptoms such as fatigue, cognitive deficits and pain, and is suggestive of other illnesses. Its unknown pathophysiology makes both diagnosis and treatment difficult.

Objectives: We wanted to compare objective parameters of visual contrast sensitivity testing, measures of innate immune response and genetic markers in cases to controls to assess the potential for the presence of persistent inflammatory parameters that are demonstrated in other biotoxin associated illnesses at a single specialty clinic.

Methods: Using 59 CC cases and 59 controls we present in retrospective review, in all cases, abnormalities in immune responses paralleling the chronic systemic inflammatory response syndrome seen in several other chronic diseases.

Results: This study defines a preliminary case definition using medical history, total symptoms, visual contrast sensitivity, HLA DR genotype analysis, reduction of regulatory neuropeptides VIP and MSH, and multiple measures of inflammatory immune response, especially C4 and TGFβ, thereby providing a basis for identification and targeted therapy.

Conclusion: CC provides a model for chronic human illness associated with initiation of inflammatory responses by biologically produced neurotoxins.

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1. Introduction

Ciguatera, the most common marine poisoning worldwide, is acquired after ingestion of toxins produced by the tropical marine dinoflagellate Gambierdiscus spp. The most incriminated toxins in this illness are ciguatoxins (CTX), a suite of colorless, odorless, heat stable, cyclic polyether neurotoxins that are potent activators of voltage-gated sodium channels. Ciguatoxins are biotransformed and biomagnified through trophic transfer in multiple fish species, and by recent estimates result in more than 100,000 cases annually of ciguatera fish poisoning, while considerable under-reporting still exists [17]. Ciguatoxin congeners from the Pacific and Indian Oceans, and the Caribbean Sea differ slightly in their structures and toxicities, which may underlie the variability of symptoms following exposure in these regions [27]. The predominant congener found in Pacific and Caribbean ciguatoxin 1 (P-CTX-1), can cause human illness at 0.1 ppb and is roughly 10 times more potent than the most common Caribbean congener, Caribbean ciguatoxin 1 (C-CTX-1) [26]. This extreme potency makes detection of ciguotoxins in fish difficult even in the most advanced research labs. Species of Gambierdiscus prefer to...
live as epiphytes on macroalgae, which can now be found dominating newly bleached coral reefs. Given the acceleration of global coral bleaching, there are reasonable public health concerns regarding expansion of habitat for this toxic dinoflagellate. Elimination of ciguatoxins from fish is reported to be slow [26], which may serve as a reservoir for toxin accumulation in the food web.

Recognition of acute ciguatera poisoning is based on a history of near-immediate onset of a multisystem, multisymptom illness acquired after eating piscivorous reef fish, although many herbivorous fish are also toxic. Acute symptoms usually are (i) gastrointestinal, especially nausea, vomiting and diarrhea; (ii) neurologic, with numbness, tingling, paresthesias and dysesthesias; (iii) general symptoms of fatigue, weakness and peripheral pain [31]. Most patients recover without treatment. However, approximately 5% of patients develop a chronic illness, often lasting years, termed chronic ciguatera (CC) [37]. CC is characterized by persistent symptoms including fatigue, cognitive deficits, chronic pain and respiratory restriction, in addition to symptoms that may not resolve from the acute stage. The exact prevalence of CC cases is unknown due to the lack of a case definition, the inability to routinely associate the chronic illness with a distant point source exposure, and the potential for misdiagnosis as there are no diagnostic markers that would separate CC from other chronic disorders. The occurrence of ciguatera cases both in the tropics, where fresh fish may be consumed, and distant areas where ciguatoxich fish may be exported further confounds diagnosis. Without biomarkers or understanding the chronic syndrome’s pathophysiology, chances for therapies targeted to specific mechanisms in CC are remote.

Currently, treatment of ciguatera is limited. Native islanders commonly turn to herbal remedies whose characterization is ongoing [7], but the efficacy of such therapy still remains to be determined. Although a double blinded trial showed no significant difference in outcome between saline and the osmolyte mannitol [44], use of mannitol in acute cases reportedly provides benefit if administered shortly after exposure. Likewise, little therapy exists for the chronic syndrome. Since 1999, the Center for Research on Biotoxin Associated Illnesses (author affiliation, RS) has treated over 200 CC patients using cholestramine (CSM), an orally administered, non-absorbable anion-binding resin as the first step in sequential therapies. Use of CSM consistently reduced symptoms when used as the initial therapy, although less benefit was seen in patients with illness of longer duration. Such persistent illness underscored the need for newer diagnostic modalities to achieve enhanced therapies.

As the mechanism of action for ciguatoxins is well characterized, the acute presentation is understood. However, the chronic syndrome appears more complex than the result of transient nervous injury and peripheral neuropathies. As literature on chronic inflammatory response syndromes emerged [18,16,25], many similarities to CC were noted. The current study employed newly available laboratory blood tests, especially the regulatory neuropeptides alpha melanocyte stimulating hormone (MSH) and vasoactive intestinal polypeptide (VIP); the split product of complement component C4, C4a; and transforming growth factor beta (TGFβ)1 to determine if CC results from a complex dysregulation of innate and adaptive immune responses. We additionally sought to identify if HLA DR haplotypes, recently found predictive for other chronic illnesses of similar characteristics, were predictive of CC. A two tiered approach of (i) medical history, symptom rotors and visual contrast sensitivity (VCS) testing, coupled with (ii) lab testing of HLA DR, and multiple measures of immune response successfully identified cases of CC as markedly different from controls and other non-biotoxin-associated, chronic illnesses such as asthma, somatoform disorder, allergy and depression.

Aspects of inflammatory pathways critical to the data were evaluated to advance understanding of how these pathways may contribute to the illness. This is the first paper to describe any underlying pathophysiology in the CC syndrome and is meant to expose its elements for discussion, diagnosis, treatment and future work. The work is exploratory; it is a building block and not an absolute presentation of endpoints.

2. Methods

2.1. Patients

For all cases and controls, medical history was obtained concerning possible confounders, including, but not limited to other biotoxin exposure (other dinoflagellates, fungi, actinomycetes, mycobacteria, endotoxin-producing bacteria, cyanobacteria, apicomplexans and spirochetes), undiagnosed neurologic disease, alcoholism, occupational exposure to solvents, petroleum products, known neurotoxicants and metal fumes. For cases, differential diagnosis techniques were used to determine whether or not a cause of illness other than ciguatera could be identified. Patients were included as CC cases (N = 59) if they were considered to have (1) developed an acute illness typical of ciguatera following piscivorous reef fish consumption, (2) no confounding illnesses and (3) symptoms that persisted beyond three months. Confirmation of the presence of ciguatoxin by testing in fish was not required for diagnosis as such testing (i) is rarely readily available in all locations (ii) more often than not, there is no remaining fish after the meal or remaining fish has been discarded. Patients coming to the clinic for well-physicals were included as controls (N = 59) if they had (i) no illness of any kind requiring acute intervention during that office visit; (ii) no history of acute multisystem illness after consumption of fish, or multisystem, multisymptom illness following exposure to environmentally produced biotoxins as described above; (3) any untreated chronic illness. Patients meeting inclusion criteria received a physical examination and blood analyses. Pregnant or nursing patients were excluded from study participation. All participants signed a HIPAA waiver permitting use of their clinical data. Internal review board (IRB) approval for retrospective analysis was obtained from the Copernicus Group IRB, Cary, NC. Participants were not remunerated for study participation.

2.2. Vision testing

Visual contrast sensitivity (VCS) testing measures the eye’s ability to resolve patterns and was performed by an experienced physician using a previously published protocol [45]. Visual acuity and VCS testing were administered monocularly, with patients wearing necessary corrective lenses, under a “daylight” illuminator (exceeding 70 FL) in a clinical unit with normal background lighting. A test card holder was used to position the acuity and VCS test cards at a constant, standardized distance (acuity — 36 cm, contrast sensitivity — 46 cm). Visual acuity using Snellen score (e.g. 20/20) was determined for each eye using the acuity test card (MIS Pocket Vision Guide, © 1997 MIS, Inc.). To avoid confounding the VCS results, a visual acuity of 20/50 or better was required for each eye to be included in analysis. All participants had at least one eye included in analysis (N = 112 in cases, N = 113 in controls). Two-tailed Student t-tests were performed, using the mean score ± s.e.m. of each participant’s two eyes, to determine if acuity scores differed significantly (p < 0.05) between cohorts.

The contrast sensitivity test card (Functional Acuity Contrast Test (FACT), Stereo Optical Co., Chicago, IL) contained a matrix (5 × 9) of circles filled with sinusoidal gratings (dark and light bars) with spatial frequencies of 1.5, 3, 6, 12 and 18 cycles/° of visual arc. The gratings were oriented either vertically, or tilted 15° to the left or right. Subjects identified the orientation of the grating by saying either: vertical, left, right or blank. The contrast sensitivity score for each row (spatial frequency) was recorded as the contrast of the last circle correctly identified on that row following verification by repeated testing of that circle. The procedure was repeated for each row in
2.3. Blood tests

Laboratory measurements were performed by CLIA licensed facilities, LabCorp, Quest Diagnostics, National Jewish Center and Cambridge Biomedical. Testing included HLA DR by PCR, alpha melanocyte stimulating hormone (MSH), vasoactive intestinal peptide (VIP), leptin, matrix metalloproteinase 9 (MMP9), split product of complement component 3 (C3a) and complement 4 (C4a), transforming growth factor beta-1 (TGFβ1), IgG for gliadin (AGA), and IgM for cardioliphin (ACLA), vascular endothelial growth factor (VEGF), plasminogen activator inhibitor (PAI-1), cortisol, erythrocyte sedimentation rate, C-reactive protein (CRP), lipid profile, complete blood count (CBC), comprehensive metabolic panel (CMP), gamma-glutamyl transpeptidase (GGTP), thyroid stimulating hormone (TSH), lipid profile, and von Willebrand’s profile. Patients were classified abnormal for von Willebrand’s antigen for results either <50 or >150 IU. Dysregulation of simultaneously measured ACTH/cortisol and ADH/osmolality was determined by adding (i) the number of cases with absolute high (ACTH > 45 or cortisol > 21; ADH > 13 or osmolality > 300) or low (ACTH < 5 or cortisol < 4; ADH < 1.3 or osmolality < 275) values for the two paired tests; to the cases (ii) in which ACTH was below 10 when cortisol was below 7; or ADH was below 2.2 when osmolality was 292–300; to the cases (iii) in which ACTH was > 15 when cortisol was > 16; and ADH > 4.0 when osmolality was 275–278.

2.4. Statistical methods

There were 37 symptoms and 22 blood parameters measured in this study for a total of 59 variables not including VCS. Because of this multiplicity problem, the Bonferroni correction was applied to symptom and blood variables which resulted in a single variable p-value being considered statistically significant if $p < 0.001 (.05/59$ rounded) in order to have an experiment wise $p < 0.05$. The units of analysis for the VCS test were the mean scores of the participant’s two eyes at each spatial frequency. The VCS data were analyzed using multivariate analyses of variance (MANOVA, with the Wilks’ lambda statistic) procedures suitable for repeated measures with an $\alpha = 0.05$. The factors in this model were group, spatial frequency, age, and their interaction terms. A factor for gender was not included, as no gender differences in susceptibility to ciguatoxin-induced effects had been indicated, and no gender differences in VCS have been reported. Results further showed that a significant group-by-spatial-frequency interaction were further analyzed in step down, two-tailed Student’s t-tests ($\alpha = 0.05$), the equivalent of a univariate ANOVA, to determine which spatial frequencies accounted for the overall effect.

2.4.1. Symptoms

The prevalence of each symptom in the illness and control groups was compared for statistical significance ($p < 0.001$) using Fisher’s exact test.

2.4.2. Blood testing parameters

For each blood parameter, the difference between the two groups was tested for statistical significance ($p < 0.001$) using the two-tailed two-sample Student t-test.

2.4.3. Statistical program

JMB of SAS was used for data analysis.

2.4.4. VCS

The VCS data were analyzed using multivariate analyses of variance procedures suitable for repeated measures. The factors in the model were group, spatial frequency, and their interaction. A significant ($p < 0.05$) overall group-by-spatial-frequency interaction was further analyzed by a two-tailed Student t-test at each spatial frequency to determine which frequencies accounted for the effect.

2.4.5. HLA haplotype relative risk

Differences in relative risk were assessed using incidence in cases to incidence in an established control population ($N=111$) [46]. Results were considered significant if the ratio exceeded 2.0.

3. Results

3.1. Patient demographics

The 118 patients were predominately Caucasian with five African Americans (2 cases, 3 controls) and two Asian Americans (1 case, 1 control). Putative cases were selected from patients seeking therapy for a chronic illness and meeting inclusion criteria described in Methods. Based on location for acquisition of illness (Supp. Table 1) we feel the majority of cases were exposed to the less potent Caribbean ciguatoxin. Mean age was 51 years for the 39 female and 20 male controls, and 50 years for the 17 female and 42 male cases. Although gender was not evenly matched, diagnostic facilities (LabCorp, etc.) directly communicated there are no known gender differences in normative values for blood tests presented here. Additionally, comparisons were broken out by gender (Supp. Table 2) with similar results.

3.2. Symptom roster

Patients identified the presence or absence of 37 different symptoms (Table 1). For all symptoms except sinus congestion and joint pain the prevalence among cases was significantly higher than controls ($p < 0.001$). The greatest separation (occurrence of symptom in cases minus occurrence in controls) was seen for light sensitivity, memory impairment, and fatigue; while the greatest sensitivity (occurrence in cases divided by occurrence in controls) was seen for unusual pain, cramping, ice pick pain, and confusion. Controls had few of the queried symptoms, but paralleled those seen in control groups for similar studies [47,48].

3.3. Visual testing

Visual contrast deficits have been shown after a number of neurologic insults such as mercury exposure [12], Parkinson’s disease [9], and organic solvent exposure [19] among others. No significant differences were noted between cases and controls for visual acuity. However, cases showed a significant ($p < 0.005$) pattern of depressed VCS at all frequencies with a maximal shift from 6 cycles/° of visual arc to 3 cycles/° of visual arc (Fig. 1).

3.4. Blood tests

Not all patients were subjected to the complete battery of diagnostic blood tests as severity of immune dysfunction was only apparent after analysis of the first 30 cases. Increased relative risk ($>2.0$) was seen for three haplotypes of HLA DR: 1) DRB1–4, DQ-3 and DRB4–53; 2) DRB1–4, DQ-7/8 and DRB4–53; and 3) DRB1–11, DQ-3, DRB3–52B (Supp. Table 3). Statistical differences ($p < 0.001$) were seen between cases and controls for (i) serum protein measures of the immune parameters; alpha melanocyte stimulating hormone (MSH), vasoactive intestinal peptide (VIP), matrix metalloproteinase 9 (MMP9), split product of complement component 4 (C4a), transforming growth factor beta-1 (TGFβ1); (ii) autoimmune parameters of IgG for gliadin (AGA), and IgM for cardioliphin (ACLA); (iii) clotting parameters of von Willebrand’s profile and (iv) hormone
relationships of ACTH compared to simultaneously measured cortisol (ACTH/cortisol), and comparison of ADH to simultaneously measured osmolality (ADH/osmolality) as described in Methods (Table 2). No significant differences between cases and controls were seen for C3a, VEGF, PAI-1, ACTH, cortisol, erythrocyte sedimentation rate, CRP, lipid profile, CBC, CMP, GCTP, TSH or leptin. Of note is that cases presented with a bimodal distribution of VEGF with deficiency (<31) or elevation (>86) seen in 31 of 43 patients compared to 10 of 49 controls. These laboratory abnormalities identify a complex syndrome marked by host responses of inflammation, autoimmunity and coagulopathy.

Use of a two tiered structure for case definition served to separate all CC cases from all controls. All cases and no controls had the presence of a multisymptom illness from at least four organ systems, without confounders, persisting following consumption of a fish meal. Analysis of testing showed all 59 cases but no control subject presented with at least 4 of nine objective parameters including VCS deficits, HLA DR from a roster with a relative risk that exceeded 2.0, MSH, VIP, C4a, TGFβ1, MMP9, ACTH/cortisol and ADH/osmolality abnormalities (Table 3a). Not all patients were subjected to the complete battery of diagnostic blood tests as severity of immune dysfunction was only apparent after analysis of the first 30 cases. Although we could only include roughly half the study subjects for markers of TGFβ1, C4a and VIP, adding these tests aided case definition in mean number and distribution of abnormalities (Table 3b). The values given for case definition threshold in Table 3a are upper limit normative ranges defined by the testing facility (Quest, LabCorp, etc.).

4. Discussion

The results of this study identify, for the first time, that a series of immune abnormalities are present in chronic ciguatera cases. Objective identification of these abnormalities can now be routinely performed and should speed the advent of treatments for victims who may suffer with this syndrome for years. The discussion of these data and abnormalities involves various biochemical pathways and mechanisms, not always in a confluent format, but valuable to understanding the pathophysiological backdrop.

Table 1
Symptom roster. The number of cases and controls reporting symptoms by group.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Group</th>
<th>Ciguatera (n=59)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>Control (n=59)</td>
<td>56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Weak</td>
<td></td>
<td>44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ache</td>
<td></td>
<td>45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cramp</td>
<td></td>
<td>39</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unusual pain</td>
<td></td>
<td>35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ice pick pain</td>
<td></td>
<td>37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Headache</td>
<td></td>
<td>47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Light sensitivity</td>
<td></td>
<td>51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Red eyes</td>
<td></td>
<td>51</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blurred vision</td>
<td></td>
<td>37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tearing</td>
<td></td>
<td>29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sinus</td>
<td></td>
<td>32</td>
<td>0.008</td>
</tr>
<tr>
<td>Cough</td>
<td></td>
<td>30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td></td>
<td>45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td></td>
<td>41</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
<td>42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Joint pain</td>
<td></td>
<td>31</td>
<td>0.003</td>
</tr>
<tr>
<td>Morning stiffness</td>
<td></td>
<td>24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td>52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Focus/concentration</td>
<td></td>
<td>45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Word recall</td>
<td></td>
<td>40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Decrease assimilation</td>
<td></td>
<td>35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Confusion</td>
<td></td>
<td>37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Disorientation</td>
<td></td>
<td>22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Skin sensitivity</td>
<td></td>
<td>27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mood swings</td>
<td></td>
<td>43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Appetite</td>
<td></td>
<td>29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sweats</td>
<td></td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temp regulation</td>
<td></td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thirst</td>
<td></td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increased urination</td>
<td></td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Static shocks</td>
<td></td>
<td>28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Numbness</td>
<td></td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tingling</td>
<td></td>
<td>43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vertigo</td>
<td></td>
<td>29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Metallic taste</td>
<td></td>
<td>31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tremor</td>
<td></td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* From testing hypothesis of no difference between groups.

Fig. 1. Mean visual contrast sensitivity by spatial frequency show a comparison of visual contrast sensitivity between age matched controls and chronic ciguatera cases. The values of the spatial frequencies score are plotted as means of right and left eyes. Error bars indicate standard error. Significant (p < 0.005) differences were noted at all frequencies.
Ciguatoxins are extremely potent voltage gated sodium channel (VGSC) activators, exerting their acute effects predominantly on the peripheral nervous system. Although this mechanism of action explains the acute neuropathies acquired after exposure, the physiologic basis for the chronic syndrome was largely unapparent. VGSCs have now been characterized in several types of non-excitable cells and studies have shown that these channels contribute to activation of inflammatory pathways in many immune cells [42]. In a study of microglial and macrophage activation, Craner et al. [13] demonstrated correlation between up-regulation of VGSC 1.6 and transition from resting state to activated phenotypes in MS and experimental autoimmune encephalitis (EAE). Moreover, they demonstrated the utility of phenytoin, a VGSC blocker, in mitigation of experimental autoimmune encephalitis (EAE). Moreover, in vivo studies have shown interaction between the complement and coagulation systems with evidence of shared inhibitors and activators [1]. In particular, platelets and platelet microparticles can activate C4 in the absence of immune complexes [38].

4.2. C3a, C4a

The complement system is a component of both innate and adaptive immune responses. Patients in this cohort had four times the upper limit normative value for the anaphylatoxin C4a, although near normal levels of C3a, a product just downstream of C4 activation. Complement activation through both the classical pathway and mannose binding lectin system will generate increased levels of C4a. Autoactivation of the C4 protease MASP2 has been reported, which could lead to persistent elevation of C4a [54]. The diversity of C4 genotypes can also influence disease progression [39]. The C4 gene locus resides in the HLA class III region and aside from simple polymorphic bases, this gene typically varies in diploid copy number (2-6, although >6 in rare instances), size (long and short forms), and isoforms (A and B, for acidic and basic [5]). In the Caucasian population the maximal gene dosage of 6 has a frequency of 3.3% [5], a proportion not dissimilar to progression of acute to chronic ciguatera. Recent studies have shown interaction between the complement and coagulation systems with evidence of shared inhibitors and activators [1]. In particular, platelets and platelet microparticles can activate C4 in the absence of immune complexes [38].

4.3. Von Willebrand’s profile

Results of von Willebrand’s profile for CC patients show that the acute phase reactant Factor VIII remains abnormal, as does ristocetin associated cofactor and von Willebrand’s antigen itself. Although unexplained bleeding is rare, disturbances in coagulation pathways are commonly seen in CC patients, just as in other chronic inflammatory response syndromes [41]. These protein level abnormalities would most likely result in abnormal coagulation times, although this theory has never been clinically tested for CC.

4.4. Neuropeptides VIP and MSH

The regularly observed deficits in two neuropeptides, vasoactive intestinal peptide (VIP) and melanocyte stimulation hormone (MSH), both neuroendocrine regulators of inflammatory responses, suggests an absence of regulation of inflammation in the development and persistence of CC. These two neuropeptides have profound anti-inflammatory effects both in vivo and in vitro; each shows great promise for treatment of inflammatory disease progression (for excellent reviews see [8,16]). Deficiency in these neuropeptides can be acquired either acutely or delayed, as well as through diverse mechanisms such as acute brain injuries [28] or persistent viral infection [51]. Although both VIP and MSH have specific receptors in immune cells, MSH is also thought to directly antagonize the classical inflammatory interleukin-1β receptor [34]. Receptor density and affinity for these peptides have been proven crucial to function. VIP can enhance newly defined inflammatory Th17 differentiation pathways through VIP receptor type 1 (VPAC1) [55] while VPAC2 levels are critical in maintaining Th1 and Th2 states in CD4+ T cells of MS.

Please cite this article as: R.C. Shoemaker, et al., Defining the neurotoxin derived illness chronic ciguatera using markers of chronic systemic inflammatory disturbances: A case/control study, Neurotoxicol. Teratol. (2010), doi:10.1016/j.ntt.2010.05.007
This cytokine has wide ranging effects on the immune system, including important roles in autoimmunity and inflammatory disease. However, timing, duration and target tissue are important aspects for its protection or pathological activity, so the effects of TGFβ1 elevation seen in CC have yet to be fully understood. Similar to VIP and MSH, TGFβ1 can regulate T-cell differentiation pathways and is considered anti-inflammatory [25]. Matrix metalloproteinase-9 (MMP9), whose expression is up-regulated by TGFβ1, can influence disease progression by both tissue destruction and cytokine processing and its elevation is also characteristic of many inflammatory and autoimmune conditions [53]. Elevated levels of both MMP9 and TGFβ1 have been reported in systemic sclerosis, a generalized disorder of the microvasculature characterized by excessive fibrosis [40]. Further, the role of TGFβ1 as a stimulant to pro-fibrotic effects in lung parenchyma, including epithelial to mesenchymal transformation, may support an explanation of restrictive pulmonary function seen in these current cases [29] (data not shown).

The incidence of developing CC from acute ciguatera remains relatively low (5%) and it is unknown as to what generates this transition. Curiously, the incidence of chronic ciguatera parallels the incidence of particular HLA haplotypes (Supp. Table 3). Although a relatively small cohort size for HLA allele analysis, cases showed an increased relative risk for certain immune haplotypes. Findings of increased risk associated with HLA DRBI–4 in chronic disease, as seen in this cohort, are not uncommon and were also seen in patients with persistent illness from Lyme disease [49], water damaged building cases [46], autoimmune hepatitis [32], severe malaria [36], pulmonary tuberculosis [22] and rheumatoid arthritis [20], among other illnesses. Additionally, expression of HLA DR genes in antigen presenting cells can be regulated by cytokine and Th1/Th2 ratio [24], parameters that are influenced by the immunomodulators already discussed.

Studies of chronic ciguatera (CC) are largely anecdotal case reports, with recording of symptoms but without laboratory testing. Given that many chronic medical conditions may present with similar symptoms, especially those for which objective diagnostic laboratory parameters have not been defined, greater accuracy in diagnosis of CC would be provided by a case definition that includes objective laboratory testing. Use of symptom recording in a medical history followed by parameters have not been defined, greater accuracy in diagnosis of CC would be provided by a case definition that includes objective laboratory testing. Use of symptom recording in a medical history followed by cinematic disturbances: A case/control study, Neurotoxicol. Teratol. (2010), doi:10.1016/j.ntt.2010.05.007.

References


433 patients [50]. Furthermore, VIP receptor agonists in rats showed
434 efficacy in protection against Alzheimers related learning impairment
435 [21] while deficiency was shown to cause cognitive deficits in mice
436 [11], a common symptom of cases in this cohort. Another critical
437 role for these neuropeptides is the induction of tolerogenic dendritic
438 cells and suppression of T regulatory cells (Tregs), which suppress
439 autoreactive T cells and autoimmune progression [16]. Even in
440 healthy individuals autoreactive T cells can escape clonal deletion
441 and must be policed in the periphery by Tregs to prevent pathologic
442 autoimmunity [14]. Of note, these CC study patients with deficiency
443 of VIP and MSH show evidence of autoimmunity findings in elevated
444 anti-gliadin and anti-cardiolipin antibodies.

4.5. TGFβ1

5. Conclusions

These data support a complex interaction of environmental exposure, genetics, innate and adaptive immunity, and neuropeptide regulatory mechanisms in patients with CC. Along with abnormal neuropeptide regulation, increased autoimmune findings and genetic susceptibility we document increased levels of the inflammatory mediators TGFβ1, MMP9 and C4a. These findings are consistent with the hypothesis that CC is an illness characterized by immune dysregulation based on genetic control of host responses. Further research is required. This immune dysregulation seen in CC parallels findings that seen in other chronic inflammatory response syndromes (CIRS) initiated in diverse diseases such as sepsis [41], acute liver failure [2] and acute multiple trauma [52]. The differences in self-limited acute illness versus development of chronic illness may be related to HLA genotype, dysregulation of antigen presentation or policing of autoreactive T cells as seen in some of the above diseases. Now that such abnormalities are known to be routinely found in CC cases, these markers not only help identify the illness but also provide a basis for targeted therapies and monitoring of sequential intervention. As research of the chronic illness caused by ciguatoxins expands, additional delineation of the physiologic basis of fatigue, cognitive, neurologic, rheumatologic, respiratory, and other symptoms may permit sub-typing of cases, leading to improved therapies.