Innate immunity, MR spectroscopy, HLA DR, TGF beta-1, VIP and capillary hypoperfusion define acute and chronic human illness acquired following exposure to water-damaged buildings

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SUMMARY

Progress in understanding host innate immune inflammatory responses has led to advances in diagnosis and treatment of patients with illness acquired following exposure to the interior environment of water-damaged buildings (WDB). Profiling cases compared to controls based on innate immunity abnormalities that are correlated with measures of capillary hypoperfusion resulted in accurate classification. WDB-illness is a complex syndrome of absent or deficient regulatory neuropeptides MSH and VIP, elevated C4a, TGF beta-1 cytokines and hormonal dysregulation. Use of symptom rosters, visual contrast sensitivity testing and genetic markers adds to diagnostic certainty. Treatment modalities that correct these abnormalities lead to symptom reduction and clinical improvement.

KEYWORDS

Water-damaged buildings, inflammatory innate immune responses, regulatory neuropeptides

INTRODUCTION

Understanding the pathophysiology of illness acquired following exposure to the interior environment of water-damaged buildings (WDB-illness) has markedly improved with the advent of newer diagnostic modalities that illuminate the illness as a chronic systemic inflammatory response syndrome. Sources of innate immune responses are toxins and inflammagens (Rao, 2007). Earlier published work from our group has confirmed that innate immune responses are commonly seen in cases of WDB-illness, but not controls (Shoemaker, 2005 a, b, Shoemaker 2006 a, b). Classification of patients using a two-tiered case definition previously (Shoemaker, 2005 a) was based on exposure parameters, symptoms and differential diagnosis; as well as presence of genetic susceptibility, evidence of loss of the regulatory neuropeptide alpha melanocyte stimulating hormone (MSH), hormonal dysregulation, elevated pro-inflammatory cytokine responses and deficits in visual contrast sensitivity (VCS). New diagnostic methods that demonstrate the illness beyond the earlier definition include use of (1) magnetic resonance spectroscopy (MRS), a tool that provides metabolic evidence of neural dysfunction following hypoperfusion caused by elevated levels of C4a that are in turn correlated with cognitive dysfunction (2) TGF beta-1 that correlates with both auto-immunity and Th-17 inflammation (3) correlation of low levels of VEGF with low VO2 max (4) reduced levels of VIP that correlate with acquired pulmonary hypertension. Here we look at baseline data on 850 patients with WDB-illness and 150 controls to (i) document ability of newer innate immune markers to classify patients as cases or not (ii) identify potential treatment modalities. Our first major hypothesis was (1) abnormalities in symptoms and VCS as part of the 2-tiered case definition established previously (Shoemaker, 2005 a) would be replicated, with differences between cases and controls classifying 100 % of known cases as cases and 100% of known controls as controls, using exposure, symptoms, differential diagnosis; and finding 3 of 6 objective parameters (VCS, HLA, MSH,

ADH/osmolality, ACTH/cortisol, MMP9); our second major hypothesis was (2) elements of activated innate immune response known to reduce capillary perfusion, i.e. low VEGF, high C4a and high TGF beta-1 would be enhanced in cases compared to controls. Our minor hypothesis predicts (2a) differences between cases and controls would be highly associated with HLA DR haplotypes. Given (2b) elevated TGF beta-1, we would see evidence of abnormal T regulatory cell function as manifested by increased presence of autoimmunity. Finally, minor hypothesis (2c), given elevated C4a , we would see the same elevation of lactate in frontal lobes and hippocampus and reduction of G/G ratios seen on MR spectroscopy previously that would correlate with symptoms of cognitive impairment.

METHODS

1000 consecutive adult patients seen at a single medical clinic site specializing in diagnosis and treatment of patients made ill by exposure to WDB signed an IRB-approved HIPAA waiver (Copernicus IRB, Cary, NC) that permits use of baseline data in research. Patients were diagnosed with WDB-illness if (1) they developed illness following exposure to a building with water intrusion and amplified microbial growth as shown by environmental testing (2) their illness was multi-system, multisymptom (3) differential diagnosis showed no confounders (4) they improved with cholestyramine therapy. Controls were identified as patients coming to the site for well adult physical exam without known untreated acute or chronic illness. Data was extracted from charts retrospectively including symptoms (from a roster of 37); visual contrast sensitivity testing (VCS); lab studies: HLA DR by PCR, MSH, VIP, leptin, ADH/osmolality, ACTH/cortisol, MMP9, PAI-1, CBC, CMP, CRP, ESR, lipid profile, testosterone, DHEAS, androstenedione, GGTP, VEGF, erythropoietin, ACLA (IgA, IgM, IgG), AGA (IgG, IgA), TGF beta-1, C3a, C4a, IgE, TSH, von Willebrand's (vWF) profile were compared for cases to controls as were results of deep aerobic nasal cultures looking for multiply antibiotic resistant coagulase negative staphylococci (MARCoNS). MR spectroscopy data on N-acetyl aspartate, choline, creatinine, myoinositol, lactate and ratio of glutamate to glutamine (G/G), measured in left and right frontal lobes and hippocampus, were analyzed. Test results were compared using two-sample T-test.

RESULTS

There was no difference between groups in gender, age or ethnicity. Mean total symptoms were 21.4 in cases and 2.6 in controls. 37 individual symptoms were assessed in all patients (N= 1000); results for all symptoms were all different in cases ($p < 0.001$) compared to controls except for sinus congestion ($p=.137$) and tremor ($p=0.006$). VCS, a neurotoxicologic test of the neurologic function of vision, showed deficits in cases from controls; visual acuity was no different. Six HLA DR haplotypes were present in cases compared to controls with relative risk > 2.0 for 4-3-53; 7-2-53; 11-3-52B; 13-6-52A; 14-5-52B; and 17-2-52A. Labs with no differences ($p > 0.05$) between cases and controls were leptin, PAI-1, CBC, CMP, CRP, ESR, lipid profile, testosterone, DHEAS, androstenedione, GGTP, erythropoietin, C3a, IgE, TSH. Labs with differences ($p <0.001$) were MSH, VIP, ADH/osmolality, ACTH/cortisol, MMP9, VEGF, ACLA, AGA, TGF beta-1, C4a, vWF. Controls never had MARCoNS, yet over 80% of cases did. There were statistically significant differences between cases (N=759) and controls (N=86) for lactate and G/G ratio, mean 5.2 abnormalities in cases and 0.9 in controls out of 8 measurements.

DISCUSSION

Our major and minor hypotheses were confirmed:

(1) Total and 35/37 individual symptoms, and VCS are again shown to be markedly different in cases compared to controls, with results essentially identical to prior published findings

(Shoemaker 2005 a, b; Shoemaker 2006 a, b, Shoemaker 2007 a); all cases met the two-tiered case definition. (2) Markers of capillary hypoperfusion (C4a, TGF beta-1 and VEGF) from innate immune activation are statistically more prevalent in cases compared to controls. (2a) Finding relative risk > 2.0 for HLA haplotypes of patients, found in a total of 24% of well patients, replicates earlier published relative risks. (2b) TGF beta-1 elevation was associated with autoimmunity (AGA and ACLA). (2c) C4a elevation was associated with impairment of executive cognitive function; with elevated lactate and reduced G/G ratios on MRS.

The mechanism of illness seen in patients made ill by exposure to WDB is dysregulated, chronic innate immune activation (Janeway, 1989) evidenced by high levels of C4a, TGF beta-1, MMP9; low levels of VEGF; dysregulated ADH/osmolality and ACTH/cortisol; presence of increased autoimmunity, particularly antigliadin antibodies; presence of coagulation disorders seen in von Willebrand's profile; and deficiency of regulatory neuropeptides VIP and MSH. Taken together, these findings are consistent with a chronic, systemic inflammatory response syndrome (CIRS). Part of the results of this combination of metabolic abnormalities is systemic capillary hypoperfusion that can be measured directly in brain, using CNS lactate, and indirectly in lung, using VO2 max and anaerobic threshold. It remains likely that the underlying reason for ongoing CIRS with dysregulated innate immune response is deficiency of regulatory neuropeptides MSH and VIP, a finding seen in > 90% of cases (10). In control patients, who invariably have normal MSH and normal VIP, increased TGF beta, high C4a or low VEGF were seen in <5%. Concordant deficiency of both MSH and VIP was not seen in controls but was present in >90% in cases. Given the not-infrequent history of epistaxis and hemoptysis in this cohort of WDB-illness patients the abnormal vWF findings are consistent with an acquired coagulopathy seen in systemic inflammatory illness due to endotoxemia (Rittirsch, 2008).

Persistent elevation of C4a, an otherwise short-lived anaphylatoxin, suggests ongoing activation of mannose binding lectin pathway of complement activation, thought to be due to ongoing autoactivation of the enzyme MASP2 (Wallis, 2007), continuing despite absence of an environmental source of antigenic stimulus of the MBL pathway.

Studies of WDB-illness patients have noted neurologic symptoms (GAO, 2008), but other than hyperacute trials of re-exposure, in which rising C4a correlates with increasing cognitive dysfunction (AIHA, 2008, Shoemaker, 11), no studies have been published that document a mechanism of acquisition of cognitive impairment. Finding the clear link between peripheral inflammation (i.e. rising C4a) and central metabolic disturbances (elevated lactate) provides a plausible mechanism of hypoperfusion to explain cognitive impairment. Previous studies confirm that reduction of C4a, using low dose erythropoietin, simultaneously resolves the CNS hypoperfusion and cognitive symptoms (Shoemaker, 2007(b).

CONCLUSIONS

These results are consistent with the hypothesis that WDB-illness is a CIRS with ongoing capillary hypoperfusion. Symptoms and VCS taken as a whole create distinct clusters that classify cases accurately. Lab results show a dense, dysregulated innate immune inflammatory response. Linking labs and symptoms, especially when associated with deficits in VCS, a neurotoxicologic measure, provides a landscape approach to a definable illness invariably seen in WDB-illness patients. Particular HLA haplotypes, ones with increased relative risk in cases/controls, are found in nearly 100% of cases, suggesting defective antigen presentation. Affected patients lose control of inflammatory pathways as levels of regulatory neuropeptides VIP and MSH decline, further adding to the inflammatory burden. Abnormal activation of MBL pathway of complement (Wallis, 2008) and elevated TGF beta-1 (Vignali, 2008) add to systemic inflammation and loss of normal regulatory T-cell function,

respectively. Dysregulated responses of hypoxia inducible factor to capillary hypoperfusion lead to low levels of VEGF.

Treatment of this complex syndrome to date involves sequential (1) removal from exposure; (2) correction of toxin carriage, using VCS monitoring to assess endpoints; (3) eradication of biofilm-forming MARCoNS; (4) correction of elevated MMP9; (5) correction of ADH/osmolality; (6) correction of low VEGF; (7) correction of elevated C4a (8) reduction of elevated TGF beta-1 and (9) replacement of low VIP.

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