

Main Identity

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Sent: Monday, May 19, 2003 6:36 AM
Subject: HLA abstract, just getting started

Linkage disequilibrium in alleles of HLA DR: differential association with susceptibility to chronic illness following exposure to biologically produced neurotoxins

Background: Chronic illness acquired following exposure to toxigenic invertebrate species, including dinoflagellates, *Pfiesteria* (PEAS), ciguatera, *Chattonella*; indoor resident fungi, *Stachybotrys*, *Aspergillus*, *Acremonium*, *Penicillium* and *Chaetomium*; and spirochetes, *Borrelia burgdorferi*, is characterized by multisystem, multisymptom illnesses, with fatigue, executive cognitive deficits, musculoskeletal complaints, gastrointestinal and respiratory problems the most common. Not all patients with the same exposure will acquire chronic illnesses, or remain ill following definitive treatment, as in *Borrelia* infections. Risk factors for persistent illness do not include age, gender, race, presence of diabetes, cardiac abnormalities, medications, including prednisone, non-steroidal anti-inflammatory drugs, duration of exposure or location of exposure.

HLA DR genotypes have been investigated as important immune response genes involved with antigen presentation from dendrites to naïve T cells. The structures of the HLA DR molecules are felt to be critical for the initial steps in peptide/antigen recognition. Recent advances in commercial availability of assays for alleles of HLA DR, including DRB1, DQ, DRB3, DRB4 and DRB5 prompted a search for possible association of unique HLA DR genotypes with acquisition of chronic illness. The correlation of expression of HLA DR is influenced by pro-inflammatory cytokine responses, raising the question as to whether susceptibility to biotoxin-associated illness was unveiled by a prior cytokine-dominant illness.

A search of Pub Med revealed 640 articles regarding linkage of HLA DR and illness. Linkage disequilibrium was noted in various studies for haplotypes DRB1-15, DQ-6, DRB5-51; DRB1-7, DQ-2, DRB3-53; DRB1-11, DQ-3, DRB3-52B; DRB1-1, DQ-5; DRB1-8, DQ-4; DRB1-4, DQ-3, DRB4-53, DRB1-12, DQ-3, DRB3-52B, DRB1-13, DQ6, 52A. Marked variation in nomenclature for recording genotypes was noted. No articles assigned genotypes as risk factors for acquired biotoxic illnesses and only 6 articles listed linkages extended to more than two alleles of HLA DR. Prior studies have focused on prevalence of given HLA DR genes in populations, looking for disproportionate representation of a given genotype in the ill population compared to controls. Little attempt has been made to associate an individual given genotype in a population with presence of illness.

Methods: After providing informed consent, 580 patients coming to a single site for treatment of chronic fatiguing illnesses following exposure to biotoxins and 100 control patients underwent HLA DR profiling by PCR. Illnesses were treated and definitive cause of illness was assigned by exposure, response to treatment and relapse following discontinuance of treatment with re-exposure. 26 unique haplotypes were identified, based on the known linkages, using standard statistical methods. Prevalence of unique genotypes in the population of known well patients was compared to prevalence of genotypes in known ill, by illness. Population studies of genotypes were then compared to individual genotypes associated with illness. Family studies were done in 25 families to evaluate the genetic basis of susceptibility in offspring, correlated with parental genotypes.

Results: Populations of dinoflagellate illness patients had a disproportionate representation of DRB1-4, DQ-7, DRB4-53 and DRB1-4, DQ-8, DRB4-53. Populations of *Borrelia* patients had disproportionate representation of DRB1-15, DQ-6, DRB5-51 and DRB1-16, DQ-5, DRB5-51. Populations of symptomatic patients with fungal toxin exposure included five genotypes with slightly higher representation than normal populations, but only one, DRB1-7, DQ-2, DRB4-53 had a significant increased risk compared to all other genotypes. Comparing individual genotypes of those with illness showed a significant correlation of individual genotypes with illness in all three groups, with five fungal toxin susceptible genotypes present. Each of the individual dinoflagellate, fungal and *Borrelia* genotypes was

associated with a >90% association with that illness in the ill population. The multiple fungal genotypes obscured the significance of each in the entire ill fungal population. Individual genotypes DRB1-1, DQ-5; DRB1-4, DQ-3, DRB4-53; DRB1-11, DQ-3, DRB3-52B, DRB1-12, DQ-3, DRB3-52B were associated with illness, but not unique to any category. Pairs of DRB1-8, with DQ 3,4,5,6 were not associated with illness. Prospective acquisition of illness was seen in 5 members of the control group, with initial known exposure resulting in illness (3 *Borrelia*, 2 fungal toxin), correlated with genotype. Family studies showed conservation of allele linkages in offspring, with unique illness susceptibility also conferred to offspring by genotype.

Statistics: HLA antigen frequencies were compared between ill groups and control using the chi-square test, and the odds ratio (OR) was calculated by the cross-product ratio. The Fisher exact test was used as appropriate. All comparisons used inferred genotype rather than allele frequency, following long-established practice. Prior analysis showed no significant difference in associations when allele rather than genotype frequencies were used (data not shown). Bonferroni corrections were carried out. Correction for multiple comparisons was used where patient and control groups were divided into subgroups and compared with each other. Haplotypes were derived by eye from the genotypes, confirmed by computer analysis. Linkage disequilibrium between alleles was assessed using the standard delta formula, normalized to take account of allele frequencies, compared to that expected by Hardy-Weinberg equilibrium.

Discussion: This large study suggests there is a susceptibility to biotoxin illness that affects individuals exposed to toxigenic dinoflagellates, fungi and spirochetes based on unique genotypes in HLA DR. Traditional population studies demonstrate the association when few genotypes confer susceptibility, but are inadequate to assess susceptibility when multiple genotypes confer susceptibility. Knowledge of individual genotypes in evaluation of an at-risk group, such as a group of individuals exposed to a dinoflagellate bloom, workers in a fungal contaminated building or a cohort of patients with illness, possibly caused by persistence of *Borrelia*, can guide diagnosis and therapy. Certain genotypes are non-specific in their association with chronic, fatiguing illness following exposure to biotoxins; others are non-specific in their lack of association with illness. Prospective studies of known well patients are underway to validate risk of acquisition of illness by genotype. Presence of biotoxin-associated illness should prompt measurement of HLA DR in offspring to assess possible future risk of disease acquisition.