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The Major Histocompatibility Complex Conserved Extended Haplotype 8.1 in AIDS-related Non-Hodgkin's Lymphoma

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Abstract

Two single nucleotide polymorphisms (SNPs) in adjacent genes, lymphotoxin alpha (LTA +252G, rs909253 A>G) and tumor necrosis factor (TNF −308A, rs1800629 G>A), form the G-A haplotype repeatedly associated with increased risk of non-Hodgkin's lymphoma (NHL) in individuals uninfected with HIV-1. This association has been observed alone or in combination with HLA-B* 08 or HLA-DRB1*03 in the major histocompatibility complex (MHC). Which gene variant on this highly conserved extended haplotype (CEH 8.1) in Caucasians most likely represents a true etiologic factor remains uncertain. We aimed to determine whether the reported association of the G-A haplotype of *LTA-TNF* with non-AIDS NHL also occurs with AIDS-related NHL. SNPs in *LTA* and *TNF* and in six other genes nearby were typed in 140 non-Hispanic European American pairs of AIDS-NHL cases and matched controls selected from HIV-infected men in the Multicenter AIDS Cohort Study. The G-A haplotype and a 4-SNP haplotype in the neighboring gene cluster (rs537160 (A) rs1270942 (G), rs2072633 (A) and rs6467 (C)) were associated with AIDS-NHL (OR=2.7, 95% CI: 1.5–4.8, p=0.0009 and OR=3.2, 95% CI: 1.6–6.6 p=0.0008; respectively). These two haplotypes occur in strong linkage disequilibrium with each other on CEH 8.1. The CEH 8.1-specific haplotype association of MHC class III variants with AIDS-NHL closely resembles that observed for non-AIDS NHL. Corroboration of an MHC

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determinant of AIDS and non-AIDS NHL alike would imply an important pathogenetic mechanism common to both.

Keywords

Human Leukocyte Antigen; HIV; CD4; Multicenter AIDS Cohort NHL Study

INTRODUCTION

In individuals not infected with human immunodeficiency virus (HIV), single nucleotide polymorphisms (SNP) in the gene encoding lymphotoxin alpha (*LTA*) and those in the gene for tumor necrosis factor (*TNF*) have been associated with non-Hodgkin's lymphoma (NHL)[1–8]. Specifically, the 'G' allele at nucleotide position +252 (rs909253 A>G) of *LTA* alone or in combination with the 'A' allele at position −308 (rs1800629 G>A) of *TNF* have been repeatedly associated with NHL in some [2–5, 7] but not other [1, 6, 8] studies. Associations with alleles at specific human leukocyte antigen (HLA) loci in the vicinity of *TNF* and *LTA* have also occasionally been reported [9–11]. However, for several reasons it remains uncertain whether variants in LTA (+252G) and TNF (−308A) or markers in adjacent loci represent true etiologic factors. First, NHL often occurs in the context of autoimmunity, immunosuppression, infection [12] and atopy 13; but neither high levels of the immunostimulatory TNF and LTA molecules nor other indications of their direct involvement in NHL have been well documented. Second, while the −308A variant has been associated with high levels of TNF production, the numerous attempts to relate sequence variation in those genes to functional variation as measured by production, levels, or biologic activity of the molecules have been controversial [14–19]. Finally, the SNPs in those two genes repeatedly associated with NHL most often occur on one of the most conserved extended haplotypes yet found in the human genome—the Caucasian HLA-B*08-containing ancestral haplotype CEH 8.1 [20, 21] spanning more than 2 Mb in the MHC region.

No study to date has examined the MHC-related genetic determinants of NHL in the context of HIV/AIDS; and no class III genetic markers other than those in the LTA-TNF have been examined, leaving unexplored a large (~ 1 Mb) chromosomal region. We have sought to confirm the relationships seen in non-AIDS NHL through a case-control study of AIDS-NHL within a large cohort study by analyzing haplotypes formed by SNPs in the central MHC class III region. For this reason, we designed the present study to incorporate two sets of MHC class III SNPs in weak or no LD with each other (based on publicly available population data) to evaluate the effects associated with extended haplotypes in central MHC. In addition to the target *TNF-LTA* gene cluster, we selected SNPs from four genes in the region that lies about 330 Kb centromeric to *TNF*. We chose this region because: 1) it is one of the most polymorphic regions of the human genome; 2) it hosts a complement gene cluster including *CFB* (complement factor B) involved in the proliferation of B-lymphocytes, a hallmark of NHL pathogenesis; and 3) it lies between *TNF* and *HLA-DR*, two loci previously associated with NHL.

Taken together, our observed association of AIDS-NHL with the same LTA (+252G)-TNF (−308A) haplotype repeatedly associated with non-AIDS NHL, along with a comparable association of a nearby segment of CEH 8.1 bearing the *C2* and *CFB* genes, corroborates the overall haplotypic effect of CEH 8.1 on NHL pathogenesis within and outside of the context of HIV/AIDS.

MATERIAL AND METHODS

Cohort characteristics and study design

We studied participants in the Multicenter AIDS Cohort Study (MACS), a prospective investigation of the natural history of HIV infection and AIDS 22 among 4954 homosexual men enrolled in 1984–1985 plus 668 men enrolled in 1987–1991 at four study centers in the US (Baltimore, Chicago, Los Angeles and Pittsburgh). Clinical information and blood samples were obtained at six month intervals. The MACS cohort study was approved by the Institutional Review Board at each center, where individual participants gave informed consent; testing for genetic variants potentially related to HIV/AIDS outcomes was included. Within the cohort, a case-control study was designed to compare serologic features in NHL cases and HIV-infected controls 23. Cases were participants who were diagnosed with AIDS-NHL as of April 2002, had at least one serum sample from a time point preceding the diagnosis, and could be a matched with HIV-infected control as described below (n=180). Longitudinal serum samples were available at all or at any of the three designed time points prior to NHL diagnosis: > 3 years pre-NHL (closest to 4 years), 1–3 years pre-NHL (closest to 2 years) and/or 0–1 year pre-NHL (closest to 0.5 year).

Controls were HIV+ participants who had not been diagnosed with NHL as of April 2002. Matching criteria were: 1) duration of HIV infection based on the known date of seroconversion (n=21) or date of entry to the study as HIV seroprevalent (n=159), and 2) expected sample availability at equivalent time points (± 1 year). One-to-one matching of a case with a control minimized the case-control differences in the duration of HIV infection (6.7 and 6.9 years, respectively).

To facilitate control for confounding by HIV duration, up to 39 serial measurements of CD4+ count for each subject permitted estimation of a slope of the CD4+ count over time and backward imputation of the count in the control at the time of diagnosis in the index case (see Statistical analyses). Eleven matched pairs had no CD4+ count measurement in the cases (n=7) or in the controls (n=2) or in both cases and controls (n=2) within two years preceding the NHL diagnosis. Exclusion of these subjects led to a total of 169 matched case-control pairs.

Subphenotypic classification based on 112 (67%) of the NHL cases identified diffuse large B cells (28.0%); large B cell diffuse, immunoblastic (22%); Burkitt lymphoma (BL) and BL-like (17%); lymphoma/NHL, not otherwise specified (22%); and other (11%). An overlapping subset of NHL cases (n=95) were further classified as either systemic (56%) or central nervous system (CNS) (44%). Finally, 46 controls (29%) and 73 cases (44%) were also diagnosed with Kaposi's sarcoma (KS) during the course of their HIV infection.

The analysis reported here was restricted to subjects of non-Hispanic European ancestry (n=140 pairs) because of the strong differentiation of HLA genes in ethnically diverse populations and because of insufficient numbers of subjects in other ethnic groups. Only crude screening analysis of NHL subphenotypes could be conducted in a study of this size and degree of histologic heterogeneity.

Laboratory methods

DNA polymorphisms at candidate MHC class III genes were genotyped using a commercially available genotyping platform (BeadArray®, Illumina Inc., San Diego, CA). SNP selection and genotyping.

A total of 63 SNPs were selected in candidate genes clustering in two regions of central MHC (Fig.1). A first telomeric set of 27 SNPs was selected from across *LTA* and *TNF* and

extending up to *NCR3* (natural cytotoxicity triggering receptor 3 gene) and a second centromeric set of 36 SNPs in the region containing genes from *C2* (complement component 2) to *CYP21A2* (cytochrome P450, family 21, subfamily A, polypeptide 2 gene). Within each candidate gene, a systematic search for informative SNPs in populations of Western European ancestry was conducted in public databases (HapMap I and SNP500 cancer database) as well as in private databases (Illumina Technologies, San Diego, CA and Applied Biosystems Inc., Foster City, CA). Primary criteria for SNP selection included a) aggressive ($r^2 > 0.80$) haplotype tagging potential (htSNP) across gene loci 24, b) minor allele frequency (MAF) > 5%, c) predicted functionality (identified in PupaSuite) 25 or documented association with NHL or location in genes relevant to B-cell activation and lymphomagenesis, and d) two-hit SNPs (or Illumina-validated) with a "designability" score of 1 (anticipated success rate > 80%). Quantification of DNA was performed with Molecular Probes (Invitrogen, Carlsbad, CA) prior to genotyping on the Illumina platform.

Portions of the subjects had previously been typed at HLA class I and class II loci for several different studies of HIV-related outcomes. HLA typing methods and the relationship between the effects at the HLA loci and the effects of the MHC class III loci are detailed in Supplementary Material.

Quality Control

Reliability of SNP typing was assessed through comparisons of duplicate data available for an average of 31 SNPs typed in a parallel study including a subset of NHL cases (n=65) and controls (n=44). Whenever departure from Hardy-Weinberg equilibrium (HWE) was observed for a SNP, the genotypic call score was checked prior to including a given SNP for analyses.

STATISTICAL ANAYSES

Statistical tests were performed in SAS 9.1 (SAS Institute Inc., Cary, NC), unless otherwise indicated. The effects of the genetic variants were evaluated in a case-control design after adjustment for other covariates as shown below.

Covariates

The distributions of ages between cases and controls were comparable in a previous study of these subjects [23]. To control for potential confounding by differential decline in $CD4^+$ count during HIV disease progression, a well known effect of variants in HLA class I genes [26], we assumed that the raw $CD4^+$ count declined according to a linear function and used regression methods to impute the $CD4^+$ count in each control at the time of NHL diagnosis in his matched case. In a minority of subjects (n=13) where data were insufficient to estimate the slopes reliably, the actual $CD4^+$ count at the study visit closest to but preceding (within 2-years) NHL diagnosis was used. For the seroprevalent subjects we included all available $CD4^+$ counts in the estimation of the slopes; for the seroconverters we included counts during the interval between one year post-seroconversion and the NHL diagnosis. Following log-transformation, standardization of the raw $CD4^+$ counts and removal of the outliers (> 3 standard errors from zero), backward imputations of the $CD4^+$ counts in the matched controls at the date of the index diagnoses were performed. The resulting covariate was included in the multivariate models for estimating the risk associated with selected DNA polymorphisms.

Population structure

Because cases and controls were selected from the same large cohorts of heterogeneous non-Hispanic European Americans recruited into the study before any NHL occurred,

inadvertent bias due to systematic population stratification should have been minimal. Although we did not include standard genomic control markers [27] in this analysis, the contribution of population structure among those of non-Hispanic European descent was nevertheless evaluated by testing the allelic diversity at the highly polymorphic and population-specific HLA class I and II loci among cases and controls (See Supplementary Data). We estimated the degree of gene differentiation among cases and controls using Weir and Cockerham's variance-based method [28] to estimate θ*s*, an approximation of the Wright's F-statistic [29] and to assess the correlation of pairs of alleles between cases and controls. Confidence intervals (95%) around the estimates of θ*s* were assessed with 10,000 bootstrap replicates and differences in overall allelic distributions among cases and controls were assessed using a G-like exact test as implemented in Genepop 4.0 [30].

Single locus analysis

SNP markers were examined separately in case and control groups for adherence to HWE using Pearson's chi-squared test. Pairwise LD between SNPs were measured using the coefficient of determination r^2. Graphical representations of LD patterns across the central MHC were obtained separately for cases and controls using Haploview [31]. Estimates of the risk associated with each marker genotype were determined by the prevalence odds ratio (OR) and corresponding 95% confidence interval (95% CI) using logistic regression with or without adjustment for the imputed $CD4^+$ counts. In the OR calculations the most frequent homozygous genotype in the controls served as the referent genotype. If a high level of heterozygosity or a low level of homozygosity was observed, dominant and additive (p for trend) genetic models were considered.

Multiple locus analysis

Haplotypes were assigned from the unphased genotypes at SNPs showing significant association ($p < 0.05$) in the single-locus tests. Haplotype frequencies were estimated using the expectation-maximization (EM) algorithm 32. To account for the long-range LD in the MHC, maximum likelihood estimates of haplotype frequencies were obtained iteratively starting with initial frequencies of 1/h, where h denotes the number of possible haplotypes in the sample. The 95% confidence intervals for haplotype frequencies were calculated using a binomial method 33. Frequency estimates were obtained for the common haplotypes (>3%) 34. MHC haplotypes were constructed by expanding the associated TNF-LTA haplotypes to include haplotypes formed by the associated SNPs in the complement region.

Overall differences in the distribution of haplotypes between cases and controls were assessed using the haplotype trend regression (HTR) approach [35]. This approach, assuming an additive model, estimates posterior probabilities for each subject for all EM-inferred haplotypes. These posterior probabilities were treated as independent variables in the HTR model with the weights in the design matrix reflecting various alternative inferences about haplotypes. A logistic regression model containing weighted haplotypes was applied to accommodate our case-control design 36 and to allow control for confounding by differential CD4 effects. In the multivariate logistic model, the adjusted odds ratios represent the risk increase per haplotype copy. Haplotypes with a frequency < 3% were aggregated as a single term in the model. Haplotype associations were tested using the most prevalent haplotype in the controls as reference.

Multiple testing

No formal correction for comparison of multiple class III SNPs was applied because the primary objective of the study was to test the prior hypothesis that the two linked SNPs previously associated with non-AIDS NHL (LTA +252 and TNF −308), along with others in CEH 8.1, were also associated with AIDS NHL.

RESULTS

Marker statistics

Of the 63 SNPs assayed in the central MHC; 47 could be amplified and were polymorphic. For those SNPs, we compared 137 cases and 140 controls. The estimated average frequency of typing error (2–4%) was unlikely to affect the results materially.

Allele frequency and HWE

The panel of 47 SNPs analyzed is shown in Table 1. The SNPs (m30–m76) are shown ordered in telomere-to-centromere orientation from the *LTA/TNF* region (top) toward the complement gene region located 333 kilobases (Kb) away (bottom). Minor allele frequency (MAF) and deviation from HWE are shown separately for the cases and controls. Several markers (~ 38%) that had MAFs < 5% in cases and controls were excluded from further analyses. We considered the SNPs whose proportions departed from HWE only in cases or controls but not in both as potentially indicative of marker-disease associations (cases) or signs for selective constraints (controls) and analyzed them carefully. No significant ($p < 0.05$) departure from HWE was found in both cases and controls at any SNP loci, with exception to TNF-863 SNP ($p=0.078$ and $p=0.004$ in the cases and controls, respectively).

Single locus analysis

Globally, 9 SNPs were found to be associated with increased risk for AIDS-NHL (Table 2). Of these, four located in the *LTA/TNF* gene cluster (m33, m34, m37, m39) included the commonly studied LTA +252G (m34) and TNF−308A (m39) polymorphisms, and five other polymorphisms (m51, m67, m70, m71 and m75) occurred in genes in and around the complement gene cluster. Three LTA markers (m33, m34 and m37) were in complete LD, and LTA +252G (m34) was subsequently used to tag all three in analyses. In univariate logistic models with matched data and adjustment for the $CD4^+$ counts, 4 of these SNPs (m39, m67, m70 and m71) were found to be associated with increased risk for NHL (ORs=2.1 to 3.2). With the exception of SNP m70, the elevations of the ORs were of modest significance ($0.01 < p < 0.06$) and occurred with the heterozygous genotype at every marker. The low frequencies of the variant homozygotes and the excess of heterozygotes observed at several SNPs supported analyses under dominance and additive genetic models. Similar increases of ORs were observed under dominant and additive genetic models (p for trend: 0.009–0.08). We excluded possible distortion of estimates due to a combination of matching by duration of HIV infection and controlling for the rate $CD4^+$ change by comparing risk estimates from a model tested without adjustment for the $CD4^+$ count with those obtained from a logistic model using unmatched data and adjustment for the effect of $CD4^+$ counts. The two models yielded comparable risk estimates and more consistent trends in the genotypic risks (Table 2). We have concentrated on the unconditional model with adjustment for the $CD4^+$ counts because it was based on a larger sample size (n=277 vs. n=238). Markers that significantly (p for trend < 0.05) modified the risk for NHL (LTA m34, TNF m39, CFB m67 and m70, RDBP m71 and CYP21A2 m75) were selected for multilocus-based tests of association.

Linkage disequilibrium

To examine the relationships among the tested SNPs, long-range LD patterns were examined separately for cases and controls (supplemental Figs 1 & 2). The LD patterns and haploblock [37] structures indicated that LTA/TNF markers are not in strong LD with markers in the complement gene cluster, with the exception of the TNF m39 and CFB m70 markers ($r^2=0.54$ and $r^2=0.58$ in cases and controls, respectively).

Haplotype analysis

With no overall correlation found between markers of the *LTA/TNF* and the complement gene clusters, we evaluated the risk associated with the MHC haplotypes separately across the two gene clusters. We first inferred haplotypes across the well-studied markers LTA +252G (m34) and TNF−308A (m39) and tested whether specific haplotypes containing these two SNPs were associated with AIDS-NHL. Sixty-one cases (44.5%) and 32 controls (22.8%) carried the LTA+252(G)-TNF−308(A) haplotype (henceforth haplotype G-A or hap1) (Table 3A). Consistent with previous studies of non-AIDS NHL [1], the haplotype G-A conferred a 2.7-fold increase of risk (p=0.0009).

For haplotypes across the complement gene region we found a unique haplotype [CFB m67(A)-CFB m70(G)-RDBP m71(A)-CYP21A2 m75(C)] (hap2) associated with a 3-fold increase in risk (OR=3.2; 95% CI:1.6–6.6; p=0.0008). To determine whether the risk haplotypes hap1 and hap2 are independent of each other, we evaluated the association with the combined 6-locus haplotypes (m34-m39-m67-m70-m71-m75). A haplotype formed by the juxtaposition of hap1 and hap2 [(m34(G)-m39(A)-m67(A)-m70(G)-m71(A)-m75(C)] (hap3) was the only haplotype that modified the risk for AIDS-NHL (OR=4.2; 95% CI: 2.0–8.9; p=0.0002).

The apparent discrepancy between the estimated ORs for Hap3 and Hap2 in all of the three sets A, B and C is due to the varying frequencies of the referent haplotype; adjustment had only a minor effect. Referent haplotype G-A-G-A used to estimate the OR for hap2 in the set A occurred in 28.7% of cases and 38.7% of controls. Referent haplotype A-G-G-A-G-A occurred in 19.3% of cases and 32.5% of controls.

Examination of the haplo-specific alleles at the 6 SNP sites on the CEH 8.1 contig NT_113891 (c6_COX cell line-derived genomic contig 38) revealed that this haplotype is part of the conserved CEH 8.1. Interestingly, approximately one-third of the cases with G-A carried this haplotype in combination with haplotypes other than hap2 suggesting that the G-A haplotype may occur on other common haplotypes across the class I and III regions of the MHC.

With the partial existing HLA data we examined the connection between the early reported associations of NHL with HLA-DR3 and HLA-B8 and the more recently reported MHC class III associations. To this end, we expanded hap3 to include the closest telomeric (HLA-B) and centromeric (HLA-DRB1) HLA genes and found that hap4 (a CEH 8.1-specific haplotype formed by the combination of HLA-B*0801, hap3 and HLA-DRB1*0301) is the only 8-locus haplotype that was significantly associated (OR=7.8; 95% CI: 2.5–24.0, p=0.0004) with the NHL (supplementary Table S1). Further support for a CEH 8.1 effect comes from analyses that included partial data from other HLA class I (namely *HLA-Cw*) and class II (*HLA-DQA1* and *HLA-DQB1*) genes (supplementary Table S1).

We evaluated the potential confounding or additional risk associated with comorbidity due to Kaposi's sarcoma (KS) in a subset of cases (n=62) and controls (n=44) who also developed that condition during the course of their HIV infection. In the KS-free subset, the associations with haplotypes hap1–3 remained significant (Table 3B). The subset of subjects who developed KS was too small to permit meaningful estimates of risk (data not shown).

The availability of subphenotypic data for the anatomic location of AIDS-NHL [systemic vs. central nervous system (CNS)] permitted limited analysis of stratified data. For the subset of systemic cases, all three haplotypes (hap1–3) were positively associated with AIDS-NHL (Table 3C), whereas for the much smaller subset of CNS cases (n=45), the ORs could not be calculated with confidence (Supplementary Table S2).

DISCUSSION

In our case-control study of homosexual men in the MACS, carriage of LTA (+252G) and TNF (−308A), which closely tag the conserved extended MHC haplotype CEH 8.1, were associated with an approximately two-fold higher risk of AIDS-NHL. These *LTA* and *TNF* variants have repeatedly been reported in association with NHL unrelated to HIV infection [2–5, 7]. We detected associations of similar magnitude with SNPs tagging a nearby segment of the CEH 8.1 that contains a complement gene cluster. Alleles of HLA genes present on CEH 8.1 and less frequently studied in association with non-AIDS NHL [9–11] also showed comparable relationships among the subset of MACS subjects with available HLA typing. This first study of MHC effects in AIDS-NHL thus succeeded in its purpose of replicating the established association with non-AIDS NHL.

The multiple previous positive studies of the associated SNPs may have focused attention on *TNF* because its encoded protein is involved in a range of neoplastic processes [39, 40] and because its promoter variant −308A has been implicated, albeit not invariably, in relatively high TNF production [15, 16]. However, that *TNF* marker and its companion in *LTA* are well documented elements of the most extensively conserved haplotype in the genome yet observed. CEH 8.1 stretches for at least 2 Mb between HLA class I and class II loci [20, 21]. Although this inordinately strong conservation of the haplotypic relationships of those two SNPs to CEH 8.1 is not in doubt, we nevertheless verified that our observed associations did not mainly reflect unusual recombinant events in that central MHC region. We demonstrated that more cases than controls who displayed the LTA-TNF G-A combination carried other alleles in the neighboring loci from *LTA* to *CYP21A2* that are also recognized CEH 8.1 variants [38]. While these associations increase the likelihood that one or more causal loci in this extended haplotype will be implicated in NHL, they also diminish the probability that any population association with a given marker in that haplotype will actually signify a causal relationship. In order to emphasize the CEH 8.1-wide effect, we have reported associations with haplotypes rather than with SNPs.

Our study tested a specific hypothesis, and it is most unlikely that our detection of the identical associations seen multiple times before in non-AIDS NHL represents a chance finding. On the other hand, the small size of our study sample may have led to unstable and potentially somewhat inflated risk estimates; replication in another HIV cohort would not only confirm but also better quantify the risk.

We analyzed different groups of cases and controls and applied alternative statistical models to ensure that our results were not method-dependent. We have emphasized the unmatched case-control design with adjustment for the $CD4^+$ count because it provided greater statistical power and probably yielded a less conservative statistical test. Further, the CEH 8.1 was earlier thought to be associated with a rapid decline of $CD4^+$ cells in HIV-1 infection [26]. It was therefore important that an analysis of cases and controls matched by duration of HIV infection effectively eliminated the possibility of confounding by differential decline of $CD4^+$ cells among pairs with CEH 8.1-positive NHL cases.

The extraordinary conservation in CEH 8.1 will make identification of the precise causal determinant on this haplotype by typical genetic approaches more difficult. By the same token, we documented that one-third of the G and A alleles were carried on non-HLA-B*08 lineages. Smaller numbers of subjects appear to carry both G and A alleles either on the same or the opposite chromosome but in the absence of the full CEH 8.1. If these cases represent recombinant chromosomes carrying CEH 8.1 with reduced conservation, they could be highly informative for fine mapping of the candidate region of the central MHC. However, since the EM-inferred haplotypes may not be entirely accurate for LTA +252 and

TNF −308 double heterozygotes, direct experimental assessment of the phase in such subjects is warranted for future studies.

NHL is a neoplasm for which the strongest risk factors identified to date reflect dysregulation of the immune system. However, regardless of whether immune deficiency is congenital, iatrogenic in the setting of post-transplant immunosuppression, or acquired as a consequence of HIV infection, its presence could complicate the evaluation of true causal factor(s) whose function is altered in NHL. In the context of HIV/AIDS, factors other than the decline of $CD4^+$ count with disease progression are suggested by 1) a disproportionally higher risk of NHL (10–100-fold higher than the population risk) in HIV-infected individuals even in the setting of moderate immune deficiency [41, 42] and 2) a less dramatic decline of systemic NHL incidence as compared with that of KS and primary CNS lymphoma in the era of highly active antiretroviral therapy[43–45]. Prolonged immune deficiency and low $CD4^+$ count one year prior to the time of NHL diagnosis have also been reported as independent predictors of NHL outcome[46]. As noted above, we controlled for possible confounding of the observed genetic relationships by the underlying decline in immunity.

Inclusion of a substantial proportion of subjects who also developed KS did not distort the association with CEH 8.1 because the HLA alleles of that haplotype have not been associated with HIV-related KS in the MACS [47]. Clinical information on the histological type was available for only a subset of our cases; that limitation precluded statistically meaningful tests by tumor type. Likewise, the study showed a clear association with systemic AIDS-NHL but that with central nervous system AIDS-NHL could not be accurately assessed because of the small sample size; however the trend was similar to that observed with the unstratified sample (Supplementary Table S2).

Recent reports [1, 6, 8] have described a protective association with TNF −857T, a SNP that was not included in our genotyping scheme. Although no earlier study detected significant associations with both the TNF −308A - LTA +252G haplotype and the TNF −857 SNP, we cannot exclude a protective role for the latter in our AIDS-NHL. The documented occurrence of the two TNF SNPs on distinct MHC lineages [8] suggests that they are differentially distributed in different Caucasian populations. That may explain the consistency in the findings for the haplotype containing −308A in our study and the others including relatively heterogeneous European populations, and the contrasting –857T association in subjects of predominantly British ancestry. Moreover, the –857T association has appeared stronger with follicular lymphoma [1, 8], while the −308A association has appeared stronger with diffuse large B cell disease [4, 6], the form more frequently represented in our population as well.

The pathogenetic mechanisms underlying the increased susceptibility of CEH 8.1 carriers to NHL and to a number of other conditions of autoimmunity and immune dysfunction 21, are poorly understood. Adaptive and demographic considerations have been invoked to account for the apparent contrast between increased disease susceptibility and high population frequency (selective advantages) of CEH 8.1 [48]. Our data show that, except for TNF −308A and CFB m70, the remaining four SNPs associated with AIDS-NHL have elevated minor allele frequencies in both the controls (MAF ≥ 0.27) and the cases (MAF≥ 0.38), emphasizing the necessity to focus future mapping efforts on those MHC markers in strong LD with the presumably less extended TNF −308(A)-CFB m70(G) subhaplotype.

To summarize, the present study extends previously reported association of LTA +252G and TNF −308A from non-AIDS to AIDS-NHL and, as with the recent observations in non-AIDS NHL [10, 11], shows that the positive association with these two polymorphisms extends

across CEH 8.1. In light of the extraordinary allelic invariance across several megabases of central and extended MHC of CEH 8.1-bearing chromosomes [20, 38, 49–51], our results as well as those reported for non-AIDS NHL strongly support an association with one or more genetic variants somewhere in the G-A-bearing haplotypes rather than suggesting an *LTA-TNF*-specific causal relationship. Fortunately, few large HIV-infected populations now go untreated for long enough to permit NHL to develop in numbers sufficient for an investigation similar to ours. It could therefore be difficult to use another population study to confirm our observation of an MHC haplotype determinant common to the pathogenesis of AIDS- and non-AIDS NHL alike.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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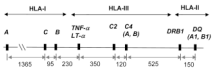

Figure 1.

Table 1

Allele frequency and HWE at markers in the major histocompatibility complex [a]

Obs[b]	SNP	intermarker distance (bp)	MAF[c] ctrl (n=140)	MAF[c] case (n=137)	HWE[d] ctrl	HWE[d] case
LTA						
M30	rs2009658		0.15	0.16	0.573	0.809
M31	rs2516312	1191	0.02	0.01	0.829	0.931
M32	rs2844482	332	0.15	0.16	0.573	0.736
M33	rs1800683 [f]	304	0.27	0.40	0.272	0.909
M34	rs909253 [f] (LTA +252)	242	0.27	0.40	0.272	0.909
M35	rs2857713	243	0.28	0.23	0.084	0.821
M36	rs3093542	137	0.04	0.02	0.596	0.793
M37	rs1041981 [f] (LTA +723)	91	0.27	0.40	0.272	0.774
TNF						
M38	rs1800630 (TNF −863)	1692	0.16	0.17	0.004	0.078
M39	rs1800629 (TNF −308)	555	0.12	0.24	0.531	0.311
M40	rs3093661	727	0.06	0.02	0.473	0.759
M41	rs3093662	431	0.09	0.05	0.358	0.528
M42	rs3093665	1202	0.01	0.02	0.863	0.828
M43	rs3093668	1104	0.06	0.02	0.478	0.759
LST1						
M44	rs986475	10214	0.09	0.10	0.904	0.688
NCR3						
M45	rs11575840	931	0.01	0.02	0.864	0.829
M46	rs11575839	151	0.02	0.02	0.796	0.793
M47	rs2736191	3119	0.02	0.04	0.762	0.624
C2						
M48	rs2734335 [e,f]	333034	0.48	0.49	0.512	0.103
M49	rs3020644 [e,f]	682	0.39	0.39	0.089	0.858

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Obs[b]	SNP	intermarker distance (bp)	MAF[c] ctrl (n=140)	MAF[c] case (n=137)	HWE[d] ctrl	HWE[d] case
M50	rs9332704	993	0.02	0.01	0.830	0.862
M51	rs7746553	354	0.18	0.10	0.758	0.186
M52	rs9332735	5800	0.02	0.02	0.830	0.759
M53	rs635348	516	0.05	0.06	0.533	0.468
M54	rs621701	832	0.05	0.06	0.533	0.468
M55	rs9332739	683	0.04	0.04	0.596	0.592
M56	rs1042663	1326	0.08	0.08	0.237	0.976
M57	rs2507953	198	0.08	0.08	0.237	0.969
M58	rs550605	1819	0.08	0.08	0.237	0.969
M59	rs638383	1077	0.03	0.03	0.694	0.690
M60	rs497239	537	0.08	0.08	0.237	0.969
M61	rs609061	1401	0.08	0.08	0.237	0.969
M62	rs2242572	767	0.07	0.08	0.802	0.969
CFB						
M63	rs12614	3250	0.11	0.07	0.270	0.383
M64	rs4151670	1353	0.02	0.03	0.830	0.725
M65	rs4151651	82	0.02	0.03	0.762	0.725
M66	rs512559	448	0.03	0.03	0.694	0.691
M67	rs537160 *f*	338	0.28	0.38	0.439	0.260
M68	rs2072634	891	0.02	0.02	0.830	0.793
M69	rs4151657 *e,f*	249	0.35	0.36	0.854	0.680
M70	rs1270942	1320	0.08	0.17	0.874	0.255
RDBP						
M71	rs2072633 *f*	718	0.38	0.48	0.103	0.652
M72	rs4151672	252	0.04	0.04	0.628	0.624
M73	rs522162	87	0.08	0.08	0.874	0.969
M74	rs550513	770	0.08	0.08	0.874	0.969
CYP21A2						

Obs[b]	SNP	intermarker distance (bp)	MAF[c] ctrl (n=140)	MAF[c] case (n=137)	HWE[d] ctrl	HWE[d] case
M75	rs6467 *e, f*	86171	0.36	0.48	0.485	0.982
M76	rs1040312	450	0.04	0.02	0.595	0.759

(a) Subjects are all HIV-infected non-Hispanic European American men with NHL cases diagnosed either as the initial AIDS-defining condition or subsequent to another AIDS-defining illness.

(b) Single nucleotide polymorphisms (SNPs) in eight genes in the major histocompatibility complex (MHC) are shown in a centromere-to-telomere orientation along their respective gene. SNPs were selected in the genes for lymphotoxin-α (*LTA*): M30-A37; tumor necrosis factor-alpha (*TNF*): M38–M43; leukocyte specific transcript 1 (*LST1)* M44; natural cytotoxicity triggering receptor 3 (*NCR3*): M45–M47; complement component 2(*C2*): M48–M62; complement factor B (*CFB*): M63–M70; RD RNA binding protein (*RDBP*): M71–M74; cytochrome P450, family 21 or steroid 21 hydroxylase (*CYP21A2*): M75–M76.

(c) Minor allele frequency (MAF) in percent.

(d) Estimates of Hardy-Weinberg equilibrium are shown separately for cases and controls with the single significant ($p < 0.05$) departure underlined.

Heterozygous genotypes are the most frequent genotypes in the controls (e) or in the cases (f).

Table 2

Association of selected SNPs in the MHC with AIDS-NHL in a case-control study of HIV-1-infected European American men

				Conditional logistic (n=115–119 pairs)						unconditional logistic		
		n (%)		unadjusted*			adjusted*			adjusted*		
SNP	genotype	controls	cases	OR	(95% CI)	p	OR	(95% CI)	p	OR	(95% CI)	p
rs1800683	GG	71 (50.7)	50 (36.5)	referent		na	referent		na	referent		na
m33	AG	61 (43.6)	65 (47.4)	1.5	(0.8–2.5)	0.17	1.4	(0.6–3.1)	0.47	1.6	(0.9–3.1)	0.12
	AA	8 (5.7)	22 (16.1)	2.8	(1.2–6.6)	0.01	1.9	(0.6–5.9)	0.28	3.9	(1.3–12.0)	0.01
	AG or AA	69 (49.3)	87 (63.5)	1.8	(1.1–3.0)	0.02	1.6	(0.7–3.4)	0.22	2.1	(1.1–3.9)	0.02
	Trend	140 (100)	137 (100)	1.7	(1.2–2.5)	0.006	1.5	(0.8–2.5)	0.16	1.9	(1.2–3.1)	0.005
rs909253	AA	71 (50.7)	50 (36.5)	referent		na	referent		na	referent		na
(LTA +252)	AG	61 (43.6)	65 (47.4)	1.5	(0.8–2.5)	0.17	1.4	(0.6–3.1)	0.47	1.6	(0.9–3.1)	0.12
m34	GG	8 (5.7)	22 (16.1)	2.8	(1.2–6.6)	0.01	1.9	(0.6–5.9)	0.28	3.9	(1.3–12.0)	0.01
	AG or AA	69 (49.3)	87 (63.5)	1.8	(1.1–3.0)	0.02	1.6	(0.7–3.4)	0.22	2.1	(1.1–3.9)	0.02
	Trend	140 (100)	137 (100)	1.7	(1.2–2.5)	0.006	1.5	(0.8–2.5)	0.16	1.9	(1.2–3.1)	0.005
rs1041981	CC	71 (50.7)	50 (37.0)	referent		na	referent		na	referent		na
(LTA +723)	AC	61 (43.6)	63 (46.7)	1.3	(0.8–2.3)	0.28	1.1	(0.5–2.6)	0.77	1.5	(0.8–2.9)	0.17
M37	AA	8 (5.7)	22 (16.3)	2.8	(1.2–6.4)	0.02	1.8	(0.6–5.6)	0.33	3.8	(1.3–11.5)	0.02
	AC or AA	69 (49.3)	114 (63.0)	1.8	(1.1–3.0)	0.02	1.5	(0.7–3.1)	0.32	2.1	(1.1–3.8)	0.02
	Trend	140 (100)	135 (100)	1.7	(1.1–2.4)	0.007	1.4	(0.8–2.4)	0.22	1.9	(1.2–3.1)	0.006
rs1800629	GG	108 (77.1)	76 (55.5)	referent		na	referent		na	referent		na
(TNF −308)	AG	29 (20.7)	55 (40.1)	2.7	(1.4–5.0)	0.002	3.0	(1.2–7.6)	0.02	3.3	(1.7–6.7)	0.0007
m39	AA	3 (2.1)	6 (4.4)	1.9	(0.4–8.1)	0.39	0.7	(0.1–3.9)	0.68	2.6	(0.5–13.3)	0.25
	AG or AA	32 (22.8)	61 (44.5)	2.7	(1.5–4.9)	0.0009	2.4	(1.1–5.6)	0.03	3.5	(1.8–6.8)	0.0003
	Trend	140 (100)	137 (100)	2.4	(1.4–4.0)	0.001	1.9	(0.9–3.7)	0.08	2.7	(1.5–4.8)	0.0009
rs2734335	GG	95 (67.9)	111 (81.0)	referent		na	referent		na	referent		na
m51	CG	40 (28.5)	23 (16.8)	0.5	(0.3–1.0)	0.04	0.7	(0.3–1.8)	0.46	0.6	(0.3–1.2)	0.17
	CC	5 (3.6)	3 (2.2)	0.5	(0.1–2.7)	0.42	0.7	(0.1–7.5)	0.74	0.8	(0.1–5.1)	0.80
	CG or CC	45 (32.1)	26 (19.0)	0.5	(0.3–0.9)	0.03	0.7	(0.3–1.7)	0.41	0.6	(0.3–1.3)	0.22
	Trend	140 (100)	137 (100)	0.6	(0.3–0.9)	0.03	0.7	(0.3–1.5)	0.42	0.7	(0.4–1.3)	0.28

				Conditional logistic (n=115–119 pairs)						unconditional logistic		
		n (%)		unadjusted*			adjusted*			adjusted*		
SNP	genotype	controls	cases	OR	(95% CI)	p	OR	(95% CI)	p	OR	(95% CI)	p
rs537160	GG	74 (52.9)	49 (35.8)	referent		na	referent		na	referent		na
m67	AG	53 (37.9)	71 (51.8)	1.9	(1.1–3.3)	0.02	3.2	(1.2–8.0)	0.01	2.3	(1.3–4.3)	0.01
	AA	13 (9.2)	17 (12.4)	2.2	(0.8–6.0)	0.12	2.3	(0.3–10.8)	0.29	1.5	(0.5–4.1)	0.42
	AG or AA	66 (47.1)	88 (63.2)	2.0	(1.2–3.4)	0.007	3.4	(1.3–8.6)	0.01	2.3	(1.2–4.3)	0.008
	Trend	140 (100)	137 (100)	1.8	(1.2–2.8)	0.008	2.4	(1.1–5.2)	0.02	1.6	(1.0–2.6)	0.04
rs1270942	AA	119 (85.0)	93 (67.9)	referent		na	referent		na	referent		na
m70	AG	20 (14.3)	42 (30.7)	3.4	(1.6–7.2)	0.001	4.2	(1.4–12.4)	0.009	3.2	(1.5–6.7)	0.003
	GG	1 (0.7)	2 (1.5)	1.0	(0.1–16.0)	1.0	nd		nd	4.2	(0.3–65.2)	0.30
	AG or GG	21 (15.0)	44 (32.2)	3.5	(1.7–7.4)	0.0008	4.2	(1.4–12.3)	0.009	3.4	(1.6–7.2)	0.001
	Trend	140 (100)	137 (100)	3.5	(1.7–7.4)	0.0008	4.2	(1.4–12.3)	0.009	3.0	(1.5–6.1)	0.001
rs2072633	GG	58 (41.4)	36 (26.3)	referent		na	referent		na	referent		na
m71	AG	57 (40.7)	71 (51.8)	1.9	(1.1–3.4)	0.02	2.1	(0.8–5.3)	0.06	2.1	(1.1–4.1)	0.03
	AA	25 (17.9)	30 (21.9)	1.8	(0.8–3.9)	0.12	2.9	(0.9–8.9)	0.11	1.9	(0.8–4.5)	0.12
	AG or AA	82 (58.6)	101(73.7)	2.0	(1.1–3.5)	0.01	2.6	(1.1–6.2)	0.03	2.3	(1.2–4.4)	0.01
	Trend	140 (100)	137 (100)	1.5	(1.0–2.2)	0.03	1.9	(1.1–3.4)	0.03	1.6	(1.0–2.4)	0.03
rs6467	AA	54 (39.4)	37 (27.2)	referent		na	referent		na	referent		na
m75	AC	67 (48.9)	68 (50.0)	1.8	(1.0–3.3)	0.05	1.3	(0.5–3.2)	0.55	1.5	(0.8–2.9)	0.23
	CC	16 (11.7)	31 (22.8)	3.2	(1.5–7.0)	0.003	2.5	(0.8–8.2)	0.13	4.3	(1.6–11.0)	0.003
	AC or CC	83 (60.6)	99 (72.8)	2.2	(1.2–3.39)	0.009	1.6	(0.7–4.0)	0.28	2.0	(1.0–3.8)	0.03
	Trend	137 (100)	136 (100)	1.9	(1.3–2.8)	0.002	1.7	(0.9–3.1)	0.09	2.0	(1.3–3.1)	0.003

(*) Adjusted for the rate of change in CD4 count.

Table 3

Risk of AIDS-NHL associated with MHC haplotypes in HIV-1 infected European American men

	Hap #	LTA (+252)	TNF (−308)	CFB		RDBP	CYP21A2	Frequency total, n (% hap)		adjusted* odds ratio		
		m34	m39	m67	m70	m71	m75	controls	cases	OR¶	95% CI	p
A												
	1	G	A					140, 32 (12.5)	137, 61 (24.4)	2.7	1.5–4.8	0.0009
	2			A	G	A	C	137, 20 (7.7)	136, 43 (16.4)	3.2	1.6–6.6	0.0008
	3	G	A	A	G	A	C	137, 20 (7.7)	136, 41 (15.7)	4.2	2.0–8.9	0.0002
B												
	1	G	A					96, 20 (11.4)	75, 39 (28.0)	3.2	1.6–6.5	0.001
	2			A	G	A	C	95, 16 (8.9)	74, 29 (20.2)	2.8	1.3–6.2	0.01
	3	G	A	A	G	A	C	95, 16 (8.9)	74, 26 (18.9)	3.5	1.5–8.0	0.003
C												
	1	G	A					77, 21 (15.6)	92, 45 (26.6)	2.1	1.1–4.0	0.02
	2			A	G	A	C	75, 14 (10.0)	92, 26 (18.8)	2.5	1.1–5.5	0.02
	3	G	A	A	G	A	C	75, 13 (7.5)	92, 25 (13.8)	2.9	1.2–6.3	0.01

Subjects are all HIV-infected non-Hispanic European American men who later in the course of their HIV infection have either developed (cases) or not (controls) Non-Hodgkin's lymphoma (NHL).

(¶)The odds ratios (OR) were estimated by unconditional logistic regression and by modeling posterior haplotype probabilities as independent variables (see Methods) in the entire case-control set (A), in the subset of Kaposi-free AIDS-NHL cases (B) and of systemic AIDS-NHL cases (C).

(n) Number of subjects carrying the associated haplotypes.

(% hap) Expectation-Maximization (EM) algorithm-based estimates of haplotype frequencies (%).

(*)Adjusted for the rate of change in CD4 count.

Note that the set of cases sampled for the estimation of ORs associated with hap2 (n=43) and hap3 (n=41) is the same except for two subjects who are LTA +252(G)-TNF −308(A) negative.