

HLA B51 is associated with faster AIDS progression among newly diagnosed HIV-infected individuals in Manitoba, Canada

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Summary

Human leucocyte antigen (HLA) alleles influence the rate of CD4 decline among HIV-infected individuals. We investigated the association between HLA B35 and HLA B51 and the rate of CD4 decline and/or opportunistic infections, among 294 HIV-positive individuals from Manitoba, Canada. All individuals presenting with a CD4 count >200 cells μL^{-1} , who had at least two CD4 counts, and no evidence of co-infection were included. Individuals bearing HLA B35 or HLA B51 were compared to controls. A multivariate model demonstrated that HLA B35 allele was associated with a hazard ratio of 2.05 (95% CI 1.31–3.18) for reaching AIDS and HLA B51 allele with HR of 2.03 (95% CI 1.18–3.49) for reaching the same end-point. High prevalence of HLA B35 was seen in the patient population receiving care in Manitoba. Our observations confirm the association of HLA B35 with rapid disease progression. We report, for the first time, faster CD4 decline among individuals with HLA B51 allele.

Introduction

Human leucocyte antigen (HLA) genes are highly polymorphic genes responsible for expression of cellular surface molecules that present antigens to T lymphocytes. After acquisition of human immunodeficiency virus (HIV), a HIV-specific T-cell response against viral epitopes is generated and this response applies pressure on the virus to mutate to escape recognition by HIV-

specific cells. The balance between the CD8 T-cell response and viral evolution determines the set point of viral load and rate of CD4 loss, and hence disease progression (Carrington *et al.*, 1999; Altfield *et al.*, 2006; Frahm *et al.*, 2006; Duda *et al.*, 2009). A myriad of factors are associated with rate of disease progression: viral factors such as attenuated strains of HIV-1, single nucleotide polymorphisms or large deletions in HIV-1 structure, regulatory, and accessory genes, HIV clade as well as host factors, all play roles in determining the rate of progression. The mechanisms of CD4 demise include caspase-1-mediated pyroptosis, associated with a proinflammatory response to abortive viral replication (Doitsh *et al.*, 2014). Depletion of CD8⁺ cells by administration of an anti-CD8 mAb has been shown to lead to persistence of chimeric simian/human immunodeficiency virus from primary infected rhesus macaques (Matano *et al.*, 1998). Several HLA-B genes have been shown to predict the rate of HIV disease progression, with HLA B53 and HLA B35 being associated with rapid CD4 decline, while HLA B27 and HLA B57 are associated with a slower rate of progression. Consequently, the latter alleles are over-represented among cohorts of individuals who exhibit better control of viral replication (Hendel *et al.*, 1999; Gao *et al.*, 2001; Jin *et al.*, 2002; Huang *et al.*, 2009; Koga *et al.*, 2010). Homozygosity for MHC class I has also been shown to predict faster rates of CD4 decline and disease progression, increasing with the number of homozygous alleles (A, B or C) (Carrington *et al.*, 1999; Tang *et al.*, 1999; Gao *et al.*, 2001). The risk of mother-to-child transmission has also been correlated with maternal homozygosity, and it is speculated that the mechanism responsible for higher transmission rates is higher viral load and cellular immune response that is less efficient in curtailing viral replication (Tang *et al.*, 1999; Mackelprang *et al.*, 2008; Wang *et al.*, 2009). Although the role of these alleles in disease progression has been studied extensively, most of the information is derived from studies of Caucasian or African cohorts and little information is available regarding individuals of Aboriginal descent. It is this population that is over-represented in the Canadian epidemic and contributes to the unique demographic of the Canadian Prairies Provinces. These provinces are

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experiencing the highest rates of new cases, with a trend towards late presentation to care. In Manitoba, 53% of the new HIV cases presenting to care in 2010 had a CD4 of <350 cells μL^{-1} and approximately one-third, presented with CD4 of <200 cells μL^{-1} (MacKenzie *et al.*, 2013). Late presentation is likely the result of multiple factors operating simultaneously, but the role of HLA B alleles contributing to this presentation is supported by some of our recent observations. Specifically, we have noted that among patients that incur a very rapid HIV disease progression, HLA B35, HLA B51 and homozygosity are all over-represented (Keynan *et al.*, 2015).

All persons entering in to care within the Manitoba HIV Program are screened for the HLA-B*57:01 allele. Screening for the HLA-B*57:01 allele has decreased the risk of severe hypersensitivity to the nucleoside reverse transcriptase inhibitor drug abacavir, with a negative predictive value of 100% and a positive predictive value of 47.9%. More than 11 000 HLA-B*57:01 tests have been performed in Canada since 2006, among which, 6.3% are positive (Lalonde *et al.*, 2010). Since 2006, this test has become a standard of care and assists in selection of the appropriate antiretroviral regimen. The aim of the study was to review the HLA typing and to determine the effect of HLA B35 and HLA B51 alleles on the CD4 decline to below 200 cells μL^{-1} .

Materials and methods

The retrospective cohort study was approved by institutional Human Research Ethics Board at the University of Manitoba.

We conducted chart reviews for patients in care at the Health Sciences Centre, Winnipeg, and collected data regarding gender, CD4 at presentation, CD4 rate of decline, HIV viral load and clade, opportunistic infection, comorbidities, ART initiation and antiretroviral regimen.

Genomic DNA samples were sent to the Canadian Blood Services, where HLA typing was performed using LABType (TM) SSO (OneLambda Inc, Canoga Park, CA, USA). If any antigen HLA B57 was identified, further high-resolution MicroSSP™ by OneLambda was used to resolve for allele level typing. The full HLA B typing data were available for analysis.

All 554 individuals enrolled in care at the Health Sciences Centre (one of the clinical sites of the Manitoba HIV Program) at the time of analysis were reviewed. Individuals who presented to care with a CD4 of <200 cells μL^{-1} or evidence of co-infection were excluded. The remaining 294 individuals who met the inclusion criteria and for whom at least two consecutive CD4 counts and clinical information were available are included in this analysis. Controls were defined as individuals who neither carried the HLA B35 nor HLA B51 allele.

Variables

AIDS was defined CD4 ≤ 200 cells μL^{-1} or any category C3 AIDS-indicator conditions according to the CDC classification system for HIV-infected adults and adolescents. All patients with a diagnosis of hepatitis C, tuberculosis, herpes, pneumocystis pneumonia or any other co-infection were considered to have opportunistic infection (thus meeting AIDS end-point). The exposed groups were defined as people who had HLA B35 or HLA B51 and no AIDS at the first time of evaluation. We defined controls as non-HLA B35 nor HLA B51. The endpoint (dependent variable) was defined as AIDS. Data were right-censored when patients did not have AIDS in the last visit to the clinic. Time variable was the time since the first visit to the clinic until the date of AIDS diagnosis or censoring occurred. Antiretroviral therapy is initiated immediately in individuals with opportunistic infections and when CD4 count is between 350 and 500 cells μL^{-1} , in individuals engaged in care (at the time of the study, currently >500 cells μL^{-1}).

Statistics

Data were analysed using spss® version 15.0 (SPSS Inc, Chicago, IL, USA). Results were expressed as percentages, median \pm interquartile range (IQR), hazard ratio (HR) with 95% confidence intervals (CIs) and two-tailed *P*-value. Survival curve for HLA genotype (B35, B51 and controls) was estimated using the Kaplan–Meier method and compared using the log-rank test. To estimate the effect of HLA B35 and HLA B51 on the decline of CD4 to below 200 cells μL^{-1} and/or opportunistic infections, HLA B alleles, age and antiretroviral therapy were incorporated into a step-wise Cox regression. The chi-square was used to test for goodness of fit of the model. The Kaplan–Meier curve and the log–log plot showed that the hazards were proportional across the groups of HLA.

Results

Table 1 describes the demographic features of the 294 patients included in the analysis. The median age was 33. The most common risk factor for HIV acquisition was heterosexual contact (63%), followed by men who have sex with men (MSM) (22%), originating from an HIV endemic area (20%) and approximately 15% are injection drug users. Fifty-six individuals (19%) and 58 (19.7%) were HLA B35 and HLA B51, respectively, and 180 (61.2%) individuals served as controls. There were ten patients that had both HLA B35 and HLA B51. We performed the analysis with and without those patients to verify changes in the HR, and the analyses yielded similar results. The median CD4 T-cell count at the time of initial presentation to care was 424 cells μL^{-1} . (range 321–571). The most common co-infection was Hepatitis C (HCV),

Table 1. Characteristics of HIV-positive persons in care

Variable	Value
Age at HIV diagnosis (yrs), median (P25-P75). <i>n</i> = 289	33 (25–40)
HLA genotype, frequency <i>n</i> (%). <i>n</i> = 294	
B35	56 (19)
B51	58 (19.7)
Control (different to B35 or B51)	180 (61.2)
CD4 count (cells mm ⁻³) at presentation to care, median (P25-P75). <i>n</i> = 292	424 (321–571)
Initial HIV viral load in copies mL ⁻¹ , median (P25-P75). <i>n</i> = 289	13 300 (1200–61 900)
Opportunistic infections at the time of presentation, frequency	
Hepatitis C virus (antibody or PCR positivity)	36
Herpes simplex virus	17
Tuberculosis	12
Varicella-zoster Virus	9
<i>Pneumocystis jirovecii</i> pneumonia	4

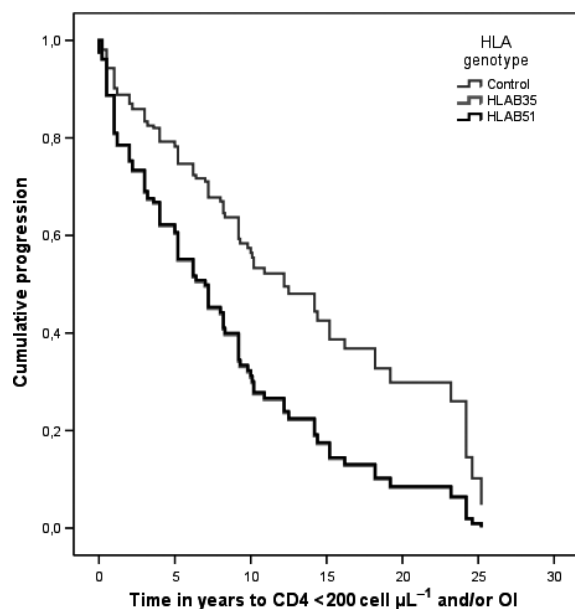


Figure 1. Kaplan–Meier survival curve depicting the CD4 decline to <200 cells μL^{-1} and/or opportunistic infections (OI) (*P*-value by log-rank test = 0.016), adjusted for age and antiretroviral treatment among controls (blue) Human leucocyte antigen HLA B35 (red) and HLA B51 (black). HLA B35 and HLA B51 have higher cumulative progression to CD4 < 200 cells μL and/or OI curves compared to control group.

followed by herpes simplex virus infection, tuberculosis and *Pneumocystis jirovecii* pneumonia.

The multivariate model is depicted in Figure 1.

HLA B35 was found to be associated with a hazard ratio (HR) of 2.05 (95% CI 1.31–3.18) for progression to AIDS. HLA B51 was associated with a HR of 2.03 (95% CI 1.18–3.49) for reaching a CD4 T-cell

Table 2. Multivariate analysis. HLA association with the rate of CD4 decline to below 200 cells μL^{-1} and/or opportunistic infections in 294 HIV-infected patients adjusted for age and antiretroviral treatment

Variable	Wald test	HR	95% CI	<i>P</i> -value
HLA control (ref category)	13.174	1.00		
HLA B35	10.186	2.05	1.31–3.18	0.001
HLA B51	6.622	2.03	1.18–3.49	0.01
Age at diagnosis of HIV	8.560	1.03	1.01–1.05	0.003

HAART was not significant in this model (*P*-value = 0.43). Chi-square test for goodness of fit: 18.293 (*P*-value = 0.001).

count of <200 cells μL^{-1} and/or opportunistic infections (Table 2).

Discussion

Manitoba is experiencing a dramatic increase in the number of newly diagnosed HIV infections and the fastest growing segment of those new infections is among women and those of First Nation's ethnicities. A larger number of new infections are being reported in Saskatchewan, a neighbouring province sharing similar demographic characteristics. Disturbingly a significant proportion of patients present to care with advanced disease and low CD4 counts (MacKenzie *et al.*, 2013). The late presentation is likely the result of multiple factors acting together. Although there is a clear role for socioeconomic parameters in the late presentation, immunogenetic variables may be contributing to the phenomena of late presentation. Since the initial observations of fast disease progression among HIV-infected individuals with HLA B35 over 20 years ago (Cameron *et al.*, 1990), the association of HLA B35 with predisposition for rapid CD4 has been confirmed in diverse cohorts (Gillieatt *et al.*, 1992; Hendel *et al.*, 1999; Gao *et al.*, 2001; Jin *et al.*, 2002; Huang *et al.*, 2009).

We report high rates of HLA B35 in the patient population receiving care in the Manitoba HIV Program. The data demonstrate that individuals with HLA B35 have a HR of 2.05 for reaching a CD4 count of <200 cells μL^{-1} and/or opportunistic infections, in keeping with the observations from other cohorts. The role of HLA B51 is more difficult to ascertain. HLA B51 is common among Asian populations and has been found in approximately 14% of Japanese populations (Mizuki *et al.*, 2001). Carrington & O'Brien, (2003) showed HLA B51 to be associated with low viral loads; however, the mechanism that underlies this improved viral control is still unclear. A recent study of a cohort in Japan has made this association less clear. In this cohort study, the circulating HIV strain was found to have acquired a major escape mutation in one of the most dominant HLA B51-restricted epitopes (Pol 283–289) leading to disappearance of the effect (Kawashima *et al.*, 2009). A recent

study from a Chinese cohort assessed the T-cell responses against three dominant HLA B51-restricted epitopes. Mutations in all three dominant epitopes were commonly associated with HLA B51 in the cohort. A good viral control and higher CD4⁺ counts were associated with at least one detectable T-cell response directed at preserved epitopes. Conversely, patients who developed escape mutations in all three epitopes had significantly higher viral loads and a lower CD4 count (Zhang *et al.*, 2011). In this Manitoba cohort, HIV-infected individuals with HLA B51 allele had a HR of 2.03 for progression to CD4 < 200 cells μL^{-1} and/or opportunistic infections. We have also seen an over-representation of this allele among individuals at the extreme end of the progression spectrum, with declines to CD4 < 200 cells μL^{-1} within a year from HIV seroconversion (Keynan *et al.*, 2015). The ability of the host to control HIV-1 has been shown to be traceable to specific amino acids in key positions of the MHC class I peptide binding groove, involved in the presentation of viral peptides (International HIV Controllers Study *et al.*, 2010). This study, along with data from multiple elite-controller cohorts, may allow for future prediction of the associations of poor viral control and progression in different cohorts.

This study has several limitations: the exclusion of individuals presenting to care with low CD4 counts decreases the total population available for this analysis. However, with the present HIV treatment guidelines, it is impossible to remove this bias. The association of HLA alleles predisposing to rapid progression with Aboriginal ethnicities may introduce bias. The known factors associated with progression were adjusted for, however, the impact of factors that are unique to these individuals, such as access to care, social factors remains unknown and not controlled for. Moreover, the HLA B35 and HLA B51 genes may be in linkage disequilibrium with another factor that may have a functional role in disease progression. A prospective study design will allow for control of some of the potential confounders, and to confirm the association between HLA B51 and AIDS.

This study from our unique cohort confirms the association of HLA B35 with rapid HIV disease progression. In this cohort, individuals carrying the HLA B51 allele have a faster rate of CD4 decline and/or opportunistic infections.

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Conflict of interest

All authors declare no conflict of interest. The manuscript has been seen and approved by all authors; it is not under active consideration for publication, has not

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