Major Histocompatibility Complex Genotype Is Associated with Disease Progression and Virus Load Levels in a Cohort of Human Immunodeficiency Virus Type 1–Infected Caucasians and African Americans

Dean L. Mann, Robin P. Garner, Deborah E. Dayhoff, Kai Cao, Marcelo A. Fernández-Viña, Charles Davis, Naomi Aronson, Nancy Ruiz, Deborah L. Birx, and Nelson L. Michael

To assess the influence of HLA on AIDS-free survival, human immunodeficiency virus load, and CD4 cell counts, 91 Caucasian and 48 African-American seroprevalent men were typed for HLA classes I and II and TAP alleles. HLA associations with these markers were assessed by assigning sum integer scores based on 7 class I allele–TAP variants (+1) and 13 class I–class II–TAP combinations (−1) with different AIDS-free survival times found in a prior study. Subjects in both racial groups and combined with positive sum scores were less likely to have CD4 cell decline (P = .0004), to have increased virus burden (P = .014), and to develop AIDS (P = .034) in the follow-up period than were Caucasians and African Americans with scores of 0 or −1. These results confirm the reported associations of specific major histocompatibility complex genes with AIDS-free survival time in Caucasians and specifically extend them to African Americans and to two established markers of disease progression.

The interaction between human immunodeficiency virus type 1 (HIV-1) and the infected host is an integral determinant of the rate of disease progression. After the initial infection, viremia peaks and then declines to set point levels that vary in different persons and remains relatively constant for a period of time [1]. An increase in plasma virus levels from this set point is associated with disease progression [2]. In general, lower virus loads at the set point are found in persons who have longer disease-free intervals.

During acute infection, the host develops antibodies to viral proteins and, in most cases, cytotoxic T cells (CTL) to virus-infected cells [3]. In several studies, vigorous CTL activity was found in the early stages of infection in persons with relatively low virus loads, suggesting a mechanism whereby virus levels may be controlled [4, 5]. The importance of this arm of the immune response in infection control is reinforced by studies that have demonstrated sustained CTL activity during the disease-free interval and the loss of this activity when AIDS develops [6–8].

Genes in the major histocompatibility complex (MHC) encode products that regulate the immune response. These include the class I molecules (HLA-A, -B, -C), which present peptides from self and nonself intracellular proteins that are recognized by CD8-positive CTL. Class II molecules (HLA-DRB1, DRB3, DRB4, DRB5, DQA1, DQB1) present peptides from extracellularly derived proteins to CD4-positive T cells that in turn release cytokines that initiate and augment cellular and humoral immune responses. Other MHC gene products (LMP 2, LMP 7) are part of a cytoplasmic proteolytic structure that cleaves proteins to peptides; others (TAP 1, TAP 2) transport the peptides to the endoplasmic reticulum where they are incorporated into the class I molecule. The extensive polymorphism of the class I and class II genes enables immunologic responses to a wide array of infectious agents, as each allele has preferred combinations of amino acids that constitute the antigenic peptides presented to the T cell. However, every person has a limited set of the total MHC gene pool and thus may be restricted in response to an infectious challenge. In HIV-1 infection, this is reflected in the association of combinations of class I alleles, class II haplotypes, and TAP polymorphisms with different rates of progression to an AIDS-defining illness as observed in 2 cohorts of HIV-1–infected Caucasian men [9]. While those data were statistically robust, HLA associations with any disease outcome are best validated by observing the same asso-
ciations in other cohorts. In HIV-1 infection, the allele combinations might also be expected to be observed with other markers of disease progression, such as changes in CD4 cell numbers and virus load.

In the present study, we examined HLA associations with disease progression as measured by decline in CD4 cells, increasing virus loads, and time to AIDS diagnosis in a cohort of HIV-1-seroprevalent men who were followed 5 years.

Materials and Methods

Study subjects. The study cohort comprised 91 Caucasians and 48 African Americans who volunteered for an immunotherapeutic trial of recombinant gp160. The latter showed no effect on disease progression [10]. All subjects were HIV-1-positive, asymptomatic, ages 18–60 years, and had ≥400 CD4 cells at least three times over 8 weeks before study entry. Lymphocyte subsets and serum virus load were determined on blood samples obtained at 6-month intervals. The enumeration and distribution of lymphocyte subsets was determined by standard flow cytometric techniques. Serum viral RNA was quantified using the Amplicor HIV Monitor Test (Roche Diagnostic Systems, Branchburg, NJ). A diagnosis of AIDS was based on the 1987 CDC definition.

HLA typing and analysis. DNA was prepared from peripheral blood lymphocytes or Epstein-Barr virus-transformed B cell lines that were established from each study subject. To determine HLA-A, -B and -C alleles, intronic primers that flank exons 1, 2, and 3 were used in a polymerase chain reaction to amplify the polymorphic regions of each gene. The amplicons were dotted on nylon membranes. After the membranes dried, the DNA was immobilized by UV irradiation. Oligonucleotide probes were labeled with digoxigenin and hybridized with the dotted membranes. Patterns of reactivity were determined using alkaline phosphatase-labeled antidigoxigenin chemiluminescence and autoradiography. Ambiguous typing results were resolved by molecular cloning and sequencing using dye primer or dye terminator methods. HLA class II alleles and sites of TAP variants were determined by amplifying polymorphic regions with flanking primers and single-stranded conformation polymorphism [11, 12]. We used the same alleles, combinations of alleles, haplotypes, and TAP variants as Kaslow et al. [9] to assign a sum integer value to each subject. Since HLA class I alleles were defined in [9] by serology and in our study by molecular typing, we used the allele’s serologic equivalent in the assignments.

The following alleles and combinations of alleles were assigned a value of +1: HLA-B27, B51, B57, A25, and TAP 2.3; A26 and TAP2.3; A32 and TAP 2.3; B18 and TAP2.3. Alleles and combinations assigned a value of −1 were B37, B49, A28, and TAP2.3; A29 and TAP2.1; B8 and TAP2.1; A23 and not TAP2.3; A24 and TAP2.1 or 2.3; B60 and TAP 2.1 or 2.3; B35 and Cw4. Four class II DRB1-DQA1-DQB1 haplotypes were also assigned −1 when they were present with TAP 1.2: 0401-0300-0301, 1200-0501-0301, 1300-0102-0604, and 1400-0101-0503. All other alleles and haplotypes were scored as 0.

For analysis of progression to AIDS, decline in CD4 cells, and

Figure 1. Kaplan-Meier analysis of AIDS-free survival in a cohort of HIV-1-seroprevalent Caucasian and African-American men separately and combined (P = .034), stratified by sum integer scores derived from combinations of HLA and TAP alleles (HLA profile). A, B, and C designate subject groups by sum integer scores of HLA and TAP alleles (HLA profile: A ≥1, B = 0, C ≤−1).
increase in virus loads, the study subjects were placed in 3 groups on the basis of their integer sum score as follows: ≥ 1 (group A), 0 (group B), and ≤ -1 (group C). Differences between HLA groups with regard to time to an AIDS-defining illness were compared by Kaplan-Meier survival analysis [13]. Differences in declining CD4 cells and increasing virus burden in the 3 groups were evaluated by comparing the normalized areas under the curve (NAUC) for each of the two markers. NAUC is defined as the area under the curve generated by the plot of the marker under consideration and study day, divided by the product of baseline marker value and the amount of time on study. This was calculated individually for each subject. The area under the curve was determined by the trapezoidal method. Values of NAUC >1.0 and <1.0 indicate an increase or decrease over time, respectively, compared with the baseline value. We compared NAUCs among the HLA profile groups by a Kruskal-Wallis nonparametric test for differences between groups, followed by the appropriate multiple comparison at the = .05 level.

CCR5 genotyping was done on genomic DNA extracted from peripheral blood mononuclear samples from the study subjects as described [14].

Results

The distribution of HLA alleles in Caucasians and African Americans is different in the general population and in our patient groups. We therefore examined the effect of HLA type on the time to an AIDS-defining illness in the 2 racial groups separately and combined using Kaplan-Meier analysis (figure 1). The same trends were found when the groups were analyzed separately and combined. The alleles and combinations of alleles that were associated with different rates of disease progress in other studies were confirmed in the Caucasian population studied. Of importance, they were also associated with differing rates of disease progression in African Americans. In the combined population, 34 subjects had HLA profiles in group A, 62 in group B, and 43 in group C. Over the follow-up period, 15% in group A, 29% in group B, and 42% in group C developed an AIDS-defining illness (P = .034).

Two other markers of disease progression, decline in CD4 cell counts and increase in virus burden (NAUC) over the follow-up period were evaluated by HLA group (figure 2). The decline in CD4 cells in group C was significantly greater than in the other 2 groups (P = .05), and the overall difference was highly significant (P = .0004). Virus burden was essentially constant in group A and increased in both other groups but most notably in group C (P = .0142). Thus, the HLA profile of the study subjects appeared to be related to the two markers of disease progression in HIV-1 infection and to the time to an AIDS-defining illness.

Discussion

Host immunoregulatory factors undoubtedly influence disease pathogenesis in HIV-1 infection. In the present study, we examined and confirmed the association of individual alleles and combinations of the MHC that were associated with different rates of disease progression in a prior study of 2 cohorts of infected Caucasian men with closely approximated dates of seroconversion. Our results were obtained in a cohort that differed from the reference study groups in several aspects. The study subjects were seroprevalent at the beginning of a shorter follow-up period (≤ 5 vs. ≥ 8 years) and included Caucasians and African Americans. In addition to an analysis of the time to an AIDS-defining illness, we found these associations were present with two interrelated markers of disease progression, an overall decline in CD4 cells, and an increase in virus burden. To our knowledge, this is the first independent study of Caucasians that has applied this HLA algorithm to these markers of disease progression and the first study that has included African Americans.

The hypothesis that host genetic factors influence HIV-1 disease pathogenesis is best examined in a seroincident population,
because genes that are associated with rapid progression may not be identified. This is particularly true of MHC genes, which have extensive polymorphisms, and was the case in our study with seroprevalent subjects. Three of the four HLA class II haplotypes that were associated with rapid progression in the reference study were not present in Caucasians and were only present once each in the African-American population. As might be expected, frequencies of other alleles differed from those in normal Caucasian and African-American populations (data not shown). Nonetheless, the alleles and allele combinations that stratified the different groups by integer sums were associated with more or less rapid rates of time to AIDS in both African Americans and Caucasians. In the absence of many of the class II haplotypes that are associated with rapid disease progression, the individual class I alleles alone and in combination with variants of the TAP genes contribute largely to the group assignments. This might be expected given their biologic function of presentation of virus-derived antigenic peptides to CTL and the apparent importance of this arm of the immune response in controlling viral infection. In another study, the frequency of potential CTL epitopes in HIV-1 proteins that could be presented by the various alleles strongly correlated with the cognate allele association with disease progression [15]. The finding that the same alleles were associated with different rates of disease progression in the 2 racial groups is substantive confirmation of not only the validity of the observation itself but also the importance of the unique biologic function of each of the alleles.

Another host genetic factor that contributes to the rapidity of disease progression is the 32-bp deletion in the gene encoding the CCR5 chemokine receptor and the coreceptor for binding macrophage-trophic HIV-1 [14]. We found a heterozygous deletion of this gene in 22.5% of our Caucasian study subjects and in 2.1% of the African Americans. In the former, persons with this genotype were distributed about equally in the 3 HLA integer sum groups and thus did not appear to influence the results. However, we cannot entirely exclude the possibility that the CCR5 deletion genotype is a contributing factor in determining the rate of progression to AIDS or to changes in the other markers of progression. Larger cohort studies will be needed to determine the relative contribution of the MHC genes and the chemokine receptor polymorphism to HIV-1 disease-free survival.

References