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*J Immunol* 1999; 162:6942-6946; ;
http://www.jimmunol.org/content/162/11/6942

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New Class I and II HLA Alleles Strongly Associated with Opposite Patterns of Progression to AIDS

Houria Hendel,* Sophie Caillat-Zucman,† Hélène Lebuanec,* Mary Carrington,‡ Steve O’Brien,§ Jean-Marie Andrieu,* François Schächter,* Daniel Zagury,* Jay Rappaport,‖ Cheryl Winkler,‡ George W. Nelson,‡ and Jean-François Zagury‡*

The genetics of resistance to infection by HIV-1 cohort consists of 200 slow and 75 rapid progressors to AIDS corresponding to the extremes of HIV disease outcome of 20,000 Caucasians of European descent. A comprehensive analysis of HLA class I and class II genes in this highly informative cohort has identified HLA alleles associated with fast or slow progression, including several not described previously. A quantitative analysis shows an overall HLA influence independent of and equal in magnitude (for the protective effect) to the effect of the CCR5-Δ32 mutation. Among HLA class I genes, A29 (p = 0.001) and B22 (p < 0.0001) are significantly associated with rapid progression, whereas B14 (p = 0.001) and C8 (p = 0.004) are significantly associated with nonprogression. The class I alleles B27, B57, C14 (protective), and C16, as well as B35 (susceptible), are also influential, but their effects are less robust. Influence of class II alleles was only observed for DR11. These results confirm the influence of the immune system on disease progression and may have implications on peptide-based vaccine development.

of HIV infection, which we show to be comparable in magnitude to the protective influence of CCR5-Δ32 (16).

Materials and Methods

Subjects

The GRIIV cohort was established in 1995 in France to generate a large collection of DNAs for genetic studies of the candidate human polymorphisms associated with rapid and slow progression to AIDS (12). To avoid the confounding effects associated with racial/ethnic differences in genetic analyses, only Caucasians of European descent were recruited from hospital AIDS units throughout France. SPs were defined as asymptomatic individuals seropositive for ≥8 years with a CD4 cell count of ≥500/mm³ in the absence of antiretroviral therapy. A seropositive test older than 8 years was necessary for inclusion in the study. RPs were defined by a CD4 count of <300/mm³ at ≥3 years after the last seronegative testing. Upon enrollment, each patient signed an informed consent form and donated 40 ml of blood. Blood was shipped overnight from the collection centers and immediately processed in the laboratory. PBMCs were collected, and EBV-transformed B cell lines were generated as a renewable source of genetic material. Some serum (one tube) was spared, allowing for some analyses, only Caucasians of European descent were recruited from hospital AIDS units throughout France.

HLA genotyping

HLA class I and II DNA typing was performed by hybridization with sequence-specific oligonucleotide probes following amplification of the corresponding genes in the PCR according to the 12th International Histocompatibility Workshop and Conference protocols (14). We first used a sequence-specific oligonucleotide probe typing system that detects alleles at the HLA-A, -B, and -C loci (Life Codes, Stamford, CT) and DRB1, DRB3, DRB4, and DRB5 loci (BioMérieux, Lyon, France). In a second step, subtyping was performed for selected generic class I or II alleles (A29, B17, B27, and DR11) using sequence-specific primer amplification (Dynal, Oslo, Norway).

Statistical analysis

The odds ratio (OR) as an estimate of risk and the Fisher’s exact test were used to determine the strength of the allele-specific associations in the SP vs RP groups. The OR is used to estimate risk in case-control studies in which the relative risk computation is not appropriate. An OR of <1 indicates protection, whereas an OR of >1 indicates increased risk. Bonferroni corrections were conducted by multiplying the Fisher’s exact test p values by the number of allelic comparisons. p values of <0.05 were considered significant.

Results

Associations with disease progression

Table I presents the alleles exhibiting allelic/genotypic frequency differences between the SP and RP categories. A number of alleles were associated with nonprogression, such as B14, B27, B57, C8, and C14, whereas A29, B22, B35, C16, and DR11 favored rapid progression. The results obtained were essentially identical whether computing the allelic or genotypic frequencies in the two categories of progression. In addition, several alleles exhibited a trend toward allelic/genotypic frequency differences between the SP and RP groups, with p values ranging between 0.06 and 0.1: C2 (SPs at 7.5% vs RPs at 3.3%, p = 0.09), C4 (SPs at 10.75% vs RPs at 16.45%, p = 0.078), C6 (SPs at 8.75% vs RPs at 3.95%, p = 0.057), and DR14 (SPs at 3.23% vs RPs at 7.24%, p = 0.057). Except for B14 and B35, the frequencies found in the French control population were in between the frequencies of the SP and RP groups, providing additional support that the alleles are involved in the dichotomous SP and RP phenotypes.

After performing Bonferroni corrections for each HLA gene, only A29, B14, B22, and C8 remained significant. The DR11 effect remained significant, but only among women in the RP group (Table II). We did not detect any frequency differences between the two groups for any allele of the HLA-DQ locus.

The association of the alleles HLA-A22 (A54/A55/A56), A29, B17 (B57/B58), B27, and DR11 with progression was not due to differences in subtypes. Sequence-specific primer genotyping did not reveal differences in subtype frequencies for these broad serological alleles between SP and RP groups (data not shown).

To determine whether homozygosity had an effect on progression, we compared patients who were heterozygous at all four loci with patients who were homozygous at one or more loci. The
frequency of homozygosity was similar between the SP and RP groups. However, the frequency of homozygotes at two or more loci was significantly increased within the RP group (p < 0.025).

Of interest, we computed whether some HLA associations would specially arise when combined with sex or specific routes of infection (homosexual, heterosexual, transfusion, and i.v. drug use); no association could be found, with the exception of DR11 and women.

**Known HLA linkage disequilibrium**

Some HLA alleles are known to be in linkage disequilibrium and commonly occur on the same haplotype. We found the following disequilibria to be equally represented in both the SP and RP groups: A29-C16, B8-C7, B14-C8, B27-C52, B35-C4, B51-C14, B57-C6, B57-DR7, and A1-B8-DR3. This may explain the similar association observed for some of the A, B, and C alleles, which are in positive linkage disequilibrium (Table I). Among C alleles, only C14 had a stronger individual effect (p = 0.03) than its counterpart B51 (p = 0.57). Unlike the findings reported in other studies (11, 18), we did not observe a significant frequency difference between the two groups for the A1-B8-C7-DR3 haplotype.

**DR11 allele**

Because of the unusual effect of DR11 with gender on progression, we studied more carefully the patients carrying this allele. Unexpectedly, there was a complete reversal of the DR11-negative effect in the presence of DR4: the 12 subjects in the cohort who are DR4-negative patients carrying HLA alleles associated with rapid progression did not show an increase in the protective effect of DR11.

**Discussion**

This study affirms several previously reported associations with progression to AIDS: B27 and B57 (19) have been reported to be associated with slow progression, and DR11 (20) and B35 (21) have been found to accelerate progression to AIDS (reviewed in Refs. 11–13). This study has identified several additional HLA alleles, not previously reported, that have a profound effect on progression to AIDS. The HLA alleles B14, C14, and C8 were also found in a French longevity study (22) examining the genetic determinants of aging. This suggests that there may be a hormonal component to immune control of HIV that is not observable in the usual male cohorts.

**Table III. Comparison with CCR5 effect**

<table>
<thead>
<tr>
<th></th>
<th>SP (n = 200)</th>
<th>RP (n = 76)</th>
<th>p Value (SP vs RP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protect&quot;/suscept&quot;</td>
<td>61 (30.5%)</td>
<td>2 (2.7%)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Suscept&quot;/protection&quot;</td>
<td>40 (20%)</td>
<td>35 (46%)</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Suscept&quot;/protection&quot;</td>
<td>16 (8%)</td>
<td>5 (6.5%)</td>
<td>p = 0.8</td>
</tr>
<tr>
<td>CCR5-Δ32</td>
<td>55 (27.5%)</td>
<td>2 (2.7%)</td>
<td>p &lt; 0.0001</td>
</tr>
</tbody>
</table>

*a The HLA alleles chosen were the most significant ones (p ≤ 0.01 in Table I): Protect" = A25, B14, B57, or C8, whereas Suscept" = A29, B22, or DR11; Protect' = subjects with at least one of the Protect alleles; Suscept' = subjects with at least one of the Suscept alleles; Protect" = none of the alleles Protect; Suscept" = none of the alleles Suscept".
Our work shows that HLA alleles are influential on slow or rapid progression, and that the strength of the protective HLA alleles is comparable with and independent of the protective effect afforded by CCR5-Δ32, as shown in Table III. The fact that CCR5-Δ32 and some HLA alleles have independent protective effects reflects the duality of their action on viral expansion: the first by limiting viral colonization by decreased coreceptor availability and the second by mounting an efficient immune response against HIV. Because the CCR2 and SDF-1 protective effects have been observed to be as strong as CCR5 in other cohorts (of all-stages patient) but occurring later in infection (23, 24), the weakness of these effects in the GRIV cohort (16) suggests that this cohort emphasizes early effects. Indeed, the B14 allele, unlike the other HLA protective alleles, has an increased prevalence among SPs, but no decrease among RP patients (Table I); it seems to prevent the initiation of disease progression. Moreover, this allele was not detected in the other cohorts, which confirms that the B14 effect must occur before the start of the disease process. This early influence of HLA is in line with the results of Pantaleo et al. (25). We also believe that the inclusion of 75 extremely rapid progressors defined by the stringent criteria of a CD4 T cell count within 3 years of the last seronegative HIV test increases the power to detect deleterious HLA alleles that may be missed by other studies with less sensitivity. This may be because many cohort studies have a frailty bias that tends to exclude the most rapid progressors (26).

The efficiency of the CTL response against HIV may be severely compromised by viral mutations that abrogate either peptide binding to HLA or CTL recognition of the HLA-peptide complex. The likelihood of such escape mutations occurring in an HLA peptide epitope is determined by two factors: whether mutations can occur without eliminating viral viability, and whether the HLA binding and the recognition of the epitope is eliminated by a given mutation. Conversely, presentation of an HIV epitope by a particular HLA allele will tend to be resistant to escape mutation if the epitope is in a region of the HIV genome for which detailed structure is essential for viral function, and if the peptide binding groove of the allele is tolerant of limited mutations in the peptide. In line with these ideas, the protective alleles we identified, namely B27, B14, and B57, have been shown to tolerate mutations in their epitopes, as shown B27 (27), B14 (28), B57 (29, 30). Reciprocally, the recognition by susceptibility alleles A29 and B35 has been shown to be sensitive to mutations (31, 32). The case of B35 is notable for the large number of epitopes recognized (32). It is possible that this is a consequence of the instability of its presentation, with repeated immune escape followed by a response to new epitopes. These concepts offer important support to the existing theory that protective CTL responses are those that resist escape mutation. Following this theory, a plausible vaccine approach would involve the selection of those HIV peptides that, presented as epitopes, would have the maximum resistance to escape mutations. Those already identified as persistent epitopes associated with long-term survival, presented by HLA alleles associated with nonprogression, are obvious candidates. The case of the B14-associated epitopes is of interest, because B14 seems to favor the prevention of entry in disease progression, while not having an effect on more advanced stage patients (not detected by other all-stages patient cohorts, not decreased among RPs). However, such epitopes would not be sufficient, because a vaccine designed around them might not offer protection to individuals lacking these protective HLA alleles. For alleles that elicit a less protective response, a possible strategy would be to seek out HIV epitopes, among all those potentially presented by the allele, in which the mutations that would abrogate class I binding are most strongly constrained by viral function. To do this effectively may require a more precise ability to predict peptide binding to HLA receptors than currently exists, but advances both in empirical studies of peptide HLA binding (6, 7) and in numerical modeling of peptide binding may offer this knowledge in the near future.

Because the constraints on HIV are not sufficient to control viral infection even in individuals carrying protective alleles, it is clear that other processes are involved in the escape of HIV from immune control. The progressive loss of CD4+ T cells undoubtedly weakens the immune response, and may account for the failure of the CTL response against new escape mutant strains that arise late in infection. A number of HIV immunosuppressive factors have been identified; in particular, our group has shown that Tat protein can act as a potent immunosuppressive toxin (33), and disease progression correlates with the loss of anti-Tat Abs (17). Such an effect could explain the ultimate ineffectiveness of even the protective HLA alleles.

To conclude, the quality of the highly selected GRIV cohort has allowed us to identify HLA alleles with effects as influential as the CCR5-Δ32 mutation on HIV disease progression and to identify, tentatively, a pattern determining the protective or susceptible effects of a genotype. It must be emphasized that no HLA alleles are truly protective in the very long term, and that HIV immune escape and pathogenesis involve other immune evasive and destructive factors, such as Tat, which are also potential targets for vaccine approaches (34).

Acknowledgments
We thank Patricia Przednowed for kind and excellent technical assistance.

References


