Associations of MHC Ancestral Haplotypes with Resistance/Susceptibility to AIDS Disease Development

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Associations of MHC Ancestral Haplotypes with Resistance/Susceptibility to AIDS Disease Development

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We tested the association of MHC ancestral haplotypes with rapid or slow progression to AIDS by comparing their frequencies in the French genetics of resistance/susceptibility to immunodeficiency virus cohort with that reported in a control French population. Seven ancestral haplotypes were identified in the genetics of resistance/susceptibility to immunodeficiency virus cohort with a frequency >1%. The 8.1 (odds ratio (OR) = 3, p = 0.006), 35.1 (OR = 5.7, p = 0.001), and 44.2 (OR = 3.4, p = 0.007) ancestral haplotypes were associated with rapid progression, whereas the 35.2 (OR = 3.6, p = 0.001), 44.1 (OR = 5.4, p < 10\(^{-4}\)), and 57.1 (OR = 5.8, p < 10\(^{-4}\)) ancestral haplotypes were associated with slow progression to AIDS. Although the frequency of each ancestral haplotype is low in the population, the OR were quite higher than those previously obtained for single HLA allele associations, with some p values as low as 10\(^{-4}\). The analysis of the recombinant fragments of these haplotypes allowed the identification of the MHC regions in the 35.1, 35.2, and 44.2 haplotypes associated with rapid progression to AIDS and the MHC regions of the 44.1 and 57.1 haplotypes associated with slow progression to AIDS. Previous studies have identified single HLA alleles associated with disease progression. Our results on recombinant fragments confirm the direct role of HLA-B35 in rapid progression. Associations with HLA-A29 and -B57 might be due to linkage disequilibrium with other causative genes within the MHC region. The Journal of Immunology, 2003, 170: 1925–1929.

Due to the major role of the HLA locus in controlling the immune response, associations between HLA genes and progression to AIDS have been extensively studied (1, 2). Various cohort studies have identified associations between HLA genes (class I and class II) and progression to AIDS. For example, the HLA-B14, -B27, -B57, and -B44 were found associated with slow progression to AIDS (3–6), while the HLA-A29, -DR11, -B22, and -B35 were found associated with rapid progression to AIDS (3, 7–10). However, the biological explanations underlying these associations are not clear because of the complexity introduced by linkage disequilibrium (LD) in the HLA region.

Indeed, the genes located in the HLA region (Fig. 1), a chromosomal segment of ~4000 kb on the short arm of chromosome 6, have the tendency to be inherited in a block due to LD (11). Thus, several combinations of alleles in the HLA-A, -C, -B, -DR, and -DQ loci, the central Bf, C4A, C4B, TNF-α, and TNFβ loci, and genes in between can form what are known as MHC haplotypes (11, 12). These haplotypes have been passed from generation to generation since ancestral times and are therefore referred to as ancestral haplotypes (11). To this day, >30 ancestral haplotypes have been described (13, 14), and they received a denomination in concordance with the HLA-B allele present in the haplotype. For example the ancestral haplotype 8.1 is composed of the alleles A1, Cw7, B8, BfS, C4A0Q, C4B1, DRB1*0301, and DQB1*0201 and is denominated 8.1 after the allele present in this haplotype (11). The complete description of the ancestral haplotypes investigated in the present study is given in Material and Methods.

We hypothesized that the multiplicity of HLA gene variants and loci associations with slow progression or rapid progression to AIDS could be due to LD with a causative gene or causative genes controlling these traits, and that examining the associations of ancestral haplotypes or their recombinant fractions could be useful to map the MHC regions that may contain such gene(s). The fact that ancestral haplotypes have been conserved in blocks during evolution may otherwise suggest that they each could play a role as a whole, possibly regulated at the transcriptional level by “neighborhood effects” (15).

Toward this end, we have used the genetics of resistance/susceptibility to immunodeficiency virus (GRIV) cohort, which is composed of patients with extreme patterns of progression, slow and rapid progression, and is the largest cohort of its kind in the world. HLA genotyping for HLA-A, -B, and -C and HLA-DR and associations with AIDS have already been described in that cohort (3). Further examination of the DQ alleles in this cohort has permitted...
extension of our previous studies and the analysis of ancestral haplotypes. The haplotypes have been imputed by the presence of haplo-
typic allele markers, because it was not possible to strictly prove their
segregation from family studies. The investigation presented in this
study allows the delineation of specific MHC regions likely to be
functionally involved in the different patterns of disease progression.

Materials and Methods

Subjects

The GRIV cohort was established in 1995 in France to generate a large
collection of DNA samples for genetic studies of candidate human poly-
morphisms associated with rapid and slow progression to AIDS (3). To
avoid confounding effects associated with racial/ethnic differences in the
genetic analysis, only Caucasians of European descent were recruited from
hospital-based AIDS units throughout France. Slow progressors (SP) were
defined as asymptomatic individuals who had tested seropositive for HIV-1
8 years with a CD4 cell count of <300/mm³ in a period of time of <3 years after the last sero-
negative testing. We selected for the study individuals with complete in-
termediate to high resolution typings for HLA-A, -C, -B, -DRB1, and
-DQB1 loci. Seventy-four RP and 198 SP to AIDS were included in the
study.

Ancestral haplotypes frequencies for a population of 65,700 French sub-
jects (control French population (CFP)) were previously published by Lon-
jou et al. (16).

Genetic typings and reconstruction of the MHC ancestral haplotypes

The methodology for HLA class I and II, and MHC class I chain-related
gene A (MICA) DNA typings was previously described (3, 17). The an-
cestral haplotypes were reconstructed by imputation of haplo-
typic allele markers as previously described (11, 12, 18) and are putative, because it
was not possible to strictly prove their segregation from family studies. The
most frequent ancestral haplotypes found in the GRIV cohort (frequency
>1%), studied in the present investigation, were the following: 7.1 (HLA-
A*0301, C*07, B*07, DRB1*1301, and DQB1*0603); 8.1 (HLA-A*01,
C*07, B*08, DRB1*0301, and DQB1*0201); 35.1 (C*04, B*35, DRB1*11,
and DQB1*0301); 35.2 (HLA-A*11, C*04, B*35, DRB1*0101, and
DQB1*0501); 44.1 (HLA-A*02, C*05, B*4402, DRB1*04, and DQB1*03);
44.2 (HLA-A*2902, C*16, B*4403, DRB1*07, and DQB1*0201), and 57.1
(HLA-A*01, C*06, B*5701, DRB1*07, and DQB1*0303). Of note, the hap-
loptype 35.1 definition does not include an HLA-A allele.

Statistical analysis

The analysis of statistical significance was done by using Pearson’s χ² and
Fisher’s exact tests, 2 × 2 contingency tables, and the aid of the INSTAT
2.01 computer program (GraphPad, San Diego, CA).

Table 1. Comparative frequencies of ancestral haplotypes

<table>
<thead>
<tr>
<th>Ancestral Haplotypes</th>
<th>Frequency × 100</th>
<th>SP vs CFP</th>
<th>RP vs CFP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP (n=198)</td>
<td>RP (n=74)</td>
<td>p value</td>
</tr>
<tr>
<td>7.1</td>
<td>2.5</td>
<td>0</td>
<td>2.4</td>
</tr>
<tr>
<td>8.1</td>
<td>6.6</td>
<td>10.8</td>
<td>3.9</td>
</tr>
<tr>
<td>35.1</td>
<td>0.5</td>
<td>5.4</td>
<td>1</td>
</tr>
<tr>
<td>35.2</td>
<td>3.5</td>
<td>1.4</td>
<td>&lt;1</td>
</tr>
<tr>
<td>44.1</td>
<td>8.5</td>
<td>4</td>
<td>1.7</td>
</tr>
<tr>
<td>44.2</td>
<td>4</td>
<td>8.1</td>
<td>2.5</td>
</tr>
<tr>
<td>57.1</td>
<td>5.1</td>
<td>2.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

* Information about the frequencies of ancestral haplotypes in the French population was obtained from a previously published database of 65,700 individuals (16). A two-tailed Pearson’s χ² test was used to analyze the data as indicated in Materials and Methods. C.I., Confidence interval.
Results
The 35.2, 44.1, and 57.1, and the 8.1, 35.1, and 44.2 ancestral haplotypes are associated with slow and rapid progression to AIDS, respectively.

To identify associations of ancestral haplotypes with slow or rapid progression to AIDS we compared the frequencies of haplotypes found in the GRIV cohort with the frequencies of haplotypes previously published in a normal French population (16). Among the ancestral haplotypes previously described (13, 14), seven had a frequency >1% in the GRIV cohort: 7.1, 8.1, 35.1, 35.2, 44.1, 44.2, and 57.1 (see Material and Methods). Significant associations with slow progression were obtained for the 35.2 (OR = 3.6, p = 0.001), 44.1 (OR = 5.4, p < 0.0001), and 57.1 (OR = 5.8, p < 0.0001) ancestral haplotypes (Table I). We also found an increased frequency of the 8.1 (OR = 3, p = 0.0057), of the 35.1 (OR = 5.7, p = 0.001), and of the 44.2 ancestral haplotypes (OR = 3.4, p = 0.007) in RP as compared with that in the normal French population (Table I). No association was identified with the 7.1 haplotype (Table I).

Because the 8.1 haplotype has previously been associated with increased TNF-α production due to an LD with the −308 G/A single nucleotide polymorphism (SNP) in the TNF-α promoter (19), we have genotyped the GRIV cohort for the −308 G/A SNP. We retrieve the LD between the rare A allele of that SNP and the 8.1 ancestral haplotype, which could give an explanation of the observed association of this haplotype with rapid progression to AIDS.

Identification of candidate regions associated with slow progression and rapid progression to AIDS in the GRIV cohort.

To identify regions within the HLA locus associated with slow or rapid progression, we used recombinant mapping methodology (20) and compared the frequency of haplotype fragments found in SP with that in RP in the GRIV cohort (Table II).
The 8.1 haplotype was fixed from the HLA-A loci to the HLA-DQB1 loci in the majority of RP to AIDS, making it impossible to map a region specifically associated with that trait by comparison with SP to AIDS (Table II). The haplotype 35.1 exhibited a striking association with rapid progression for all fragments within the C/DQB1 interval. The peak was obtained for the B/DQB1 interval with an OR value of 14.3 ($p = 0.006$). For the haplotype 35.2, a peak association with rapid progression was observed for the interval C/B with an OR of 2.3 and a $p$ value of 0.02. Thus, carrying the whole 35.2 haplotype resulted in the expression of the SP phenotype (Table I), while carrying the HLA-C/B interval of this haplotype resulted in the expression of the RP phenotype (Table II). For the haplotype 44.1, a nearly significant association with slow progression is obtained for the DRB1/DQB1 interval (OR = 0.5, $p = 0.06$). For the haplotype 44.2, an association is observed with rapid progression to AIDS with the intervals A/B and C/B with ORs close to 2.4 and $p$ values close to 0.05 (Table II). The DRB1/DQB1 interval of the 57.1 haplotype was significantly associated with slow progression to AIDS (OR = 0.33, $p = 0.05$); however, all the fragments between C and DQB1 exhibited similar ORs close to 0.4 and low $p$ values such as 0.06 for the C/B interval (Table II).

The associations involving the HLA-B44 allele are of particular interest, because it is the most prevalent allele in the Caucasian population (frequency of $\sim 34\%$). The HLA-B gene is very close to the MICA gene (Fig. 1), and it is known that the HLA-B*4403 allele present in the 44.2 ancestral haplotype is known to be in LD with the MICA*004 allele, while the HLA-B*4402 allele in the 44.1 ancestral haplotype is in LD with the MICA*008 allele (18). The MHC class I chain-related gene B (MICB) allele MICB*01021 is found in both the 44.1 and the 44.2 ancestral haplotypes (18). A preliminary analysis of the GRIV cohort shows that, in fact, most of the HLA-B44 carriers in our sample of RP are MICA*004, while most of the HLA-B44 carriers in the SP are MICA*008 (data not shown).

A recently published study showed an epistatic interaction between the killer Ig-like receptor (KIR) genes and HLA-B genes, with an impact as strong as the CCR5-D32 deletion (21). In that study, an association was observed only when analyzing the KIRs in combination with their HLA ligands. It is known that the KIR2DL2 and 2DL3 receptors on NK cells bind a conserved HLA-C1 group motif (defined by S77 and N80), whereas the KIR2DL1 receptor binds the HLA-C2 group (N77 and K80). We tried to compare the RP and SP to AIDS by sorting them in the HLA-C1 or HLA-C2 group motifs, but we could not see any difference. As described by Martin et al. (21), it might be necessary to analyze the combination of HLA and KIR gene polymorphisms to observe an association.

Discussion

Although the frequencies of ancestral haplotypes in all populations are extremely low (11, 12, 16), we are reporting for the first time an association of the 35.2, 44.1, and 57.1 ancestral haplotypes, and of the 35.1 and 44.2 ancestral haplotypes with slow progression and rapid progression to AIDS, respectively. Previous reports have also found that the 8.1 ancestral haplotype is associated with rapid progression to AIDS (22, 23). Of note, these associations between ancestral haplotypes and AIDS progression withstand Bonferroni corrections. The OR in this study, comprised between 2.9 and 5.9, are globally higher than those obtained for single HLA alleles (3–10). This can be explained by two reasons: a statistical explanation based on the small number of subjects carrying ancestral haplotypes and a biological explanation based on the importance of the contextual arrangement of genetic elements for successful immunologic control of HIV-1 infection. For instance, the single allele HLA-B8 alone is not associated with rapid progression to AIDS (3); the HLA-B8 allele is associated with rapid progression only in the full context of the ancestral haplotype 8.1. Our results do not exclude that ancestral haplotypes are associated with susceptibility to infection, because they are generally higher in both the SP and RP populations than in the CFP population. It is unlikely that there is a bias in the CFP population, because the haplotyping involved 65,700 individuals (16) and the frequencies are very similar to the ones from other large studies (24).

Until now, the most widely viewed explanation for HLA influence is that class I and class II genes act directly by restricting the immune response—Ag peptidic presentation—inducing selection of HIV-1 viral escape mutants: in fact, the direct interaction between HLA and the generation of escape mutants can be analyzed directly (25). Our approach based on the analysis of recombinant ancestral haplotypes (fragments of these haplotypes) should permit further mapping and identification of new disease susceptibility genes within the HLA locus acting in concert with the classical MHC class I and II genes (20). Our results suggest that the HLA-C/B regions of the 44.2 and 57.2 ancestral haplotypes are associated with rapid progression to AIDS, and that the DRB1/DQB1 regions of the 44.1 and 57.1 ancestral haplotypes are associated with slow progression to AIDS. For the haplotype 35.1, all intervals were associated with rapid progression to AIDS with a peak for B/DQB1. It is important to emphasize that these data do not integrate Bonferroni corrections, and only the associations of the B/DQB1 and DRB1/DQB1 fragments of the 35.1 haplotype with rapid progression to AIDS would remain significant if such corrections were applied. It was not possible to determine the effect of specific recombination events within the 8.1 ancestral haplotype because the haplotype was totally conserved among RP subjects; however, we found it in total LD with the previously described 308 A allele of TNF-α promoter (19).

Interestingly, among the seven ancestral haplotypes studied, four of them involve HLA gene alleles that were previously associated with disease progression (3): HLA-B35 for haplotypes B35.1 and B35.2, HLA-DRB11 for the haplotype 35.1, HLA-A29 for the haplotype B44.2, and HLAB57 for the haplotype B57.1. Unlike HLA-B35, the ancestral haplotype B35.2 exhibited an association with slow progression to AIDS (Table I). However, the C/B fragment of this ancestral haplotype seemed to be associated with rapid progression to AIDS (Table II) and tends to confirm the direct role of HLA-B35 allele in AIDS progression. A corrective effect may occur elsewhere in the haplotype, whether between the A and C gene, suggesting a possible role for HLA-E, or in the CFP regions of the 35.1 haplotype with promoter (19).

The analysis of the recombinant fragments of 35.1 suggests a strong association with rapid progression to AIDS for all the recombinant fragments, highly significant for the B/DQB1 fragment (Table II): the association of the B35.1 haplotype appears bimodal with a peak in the OR at 14.3 linked with the combined association of the alleles B35 and DR11, and two smaller ORs but very significant ($p = 0.02$ and $p = 0.004$) at the level of the C/B and DRB1/DQB1 fragments, respectively, which correspond to the associations of B35 and DR11 with rapid progression to AIDS we previously described (3). This could suggest the tracking effect of an association for a gene located within the B/DRB1 fragment, or a synergistic effect for the combination of the individual alleles B35 and DR11. A recent work has shown that the HLA-B35 allele effect was associated with the subtypes 02, 03, and 04 of B35 (9). It is not known whether the ancestral haplotypes 35.1 and 35.2 are associated with a given subtype of B35 and that analysis will need to be performed. Of interest, the association with rapid progression to AIDS found on
the C/B region for both the 35.1 and 35.2 haplotypes in the recombinant analysis had an OR of 2.3, similar to the one published for the B35 subtypes 02, 03, and 04 (9). The association of the 44.2 haplotype with rapid progression to AIDS is similar in amplitude to the one we previously described for A29 (3); however, the recombinant fragment C/B of the 44.2 haplotype appears also sufficient to track that association. Finally, for the HLA-B57 association, the recombinant analysis of the 57.1 haplotype points to the whole C/DQB1 region; unlike the 35.1 haplotype, the ORs are similar for all fragments, and all the genes in the C/DQB1 region could thus be good candidates to explain the association. In this study of recombinant fragments, no discrepancy is observed between B and C genes, on the one hand, and DRB1 and DQB1, on the other hand, reflecting their close proximity on chromosome 6.

In the DR/DQ region, besides the HLA DR/DQ genes themselves, the neighboring genes known as transporter associated with Ag processing and large multifunctional protease are interesting candidate genes that are functionally involved in the Ag processing by class I molecules (26). Around the HLA B/C genes, promising candidates are the genes of the MICA and MICB, but the HLA-E locus located in between the HLA-A and HLA-C is also polymorphic (27, 28). MICA and MICB proteins are ligands of the NK cell-activating receptor NKG2D (29), while HLA-E is the ligand of the inhibitory NKG2A, -B, and -C receptors (30–32).

In conclusion, our study confirms that the molecular mechanisms underlying the associations between HLA genes and AIDS disease progression are not always direct effects of HLA restriction but can also be indirect effects due to LD, or both. Our analysis through the ancestral haplotypes has brought some insight into the possible mechanisms by pointing out class I, II, or III MHC regions linked to various HLA alleles susceptible to being associated with disease progression. The combined analysis of the TNF-α, MICA/B, HLA-E, transporter associated with Ag processing, large multifunctional protease, and KIR killer cell-activating receptor gene polymorphisms as well as of the viral gene sequences (25) and their interactions (21) should certainly bring a definitive answer to these questions.

Acknowledgments

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References