Polymorphisms in the Genes for Herpesvirus Entry

To the Editor—We read with interest the article by Struyf et al. [1], which described polymorphisms in herpes simplex virus (HSV) receptor genes that were sought and studied in vitro. We also have searched for polymorphisms in HSV receptors and in a ligand for one of these receptors, and we have examined various patient and control-subject populations to determine whether any of these polymorphisms correlate with HSV-2 infection or symptoms.

Three receptors have been identified for HSV: herpesvirus entry mediator (HVE) A (also referred to as “HVEM”), nectin-1 (also referred to as “HVEC”), and nectin-2 (also referred to as “HVEB”) [2]. We looked for single-stranded conformational polymorphisms in HSV receptor genes from patients who were seronegative for HSV-2 despite having stable partners who were seropositive. In these subjects, we identified a polymorphism in the intracellular domain of nectin-1, located in a region of the carboxy terminus of the protein that contains 8 consecutive glutamic acids (aa 437–444). Sequence analysis of nectin-1 molecules from several individuals showed that polymorphisms were predicted to result in 8–10 consecutive glutamic acids in this region.

To better define the prevalence and clinical significance of these polymorphisms, we developed a polymerase chain reaction (PCR)-based assay, using primers CTGGGTGGAAGCA-GCTATG and TGCCTCGCCCCGCCACCG, to amplify the polyglutamic acid region of nectin-1 from DNA obtained from peripheral blood mononuclear cells. Bands of different lengths (64, 67, and 70 bp) were sequenced and found to contain different numbers of glutamic acid codons. To analyze a large number of samples, PCR products were labeled with T4 polynucleotide kinase and [32P]ATP and were analyzed on 12% polyacrylamide gels, and autoradiography was performed. Bands corresponding to the 3 different sizes were observed. We analyzed 324 samples from subjects with different HSV-1 and HSV-2 serostatus (table 1). The frequency of patients heterozygous for the nectin-1 polymorphism whose HSV-1/HSV-2 serostatus was +/+ +/+−, −/−, or −/+ was 23.1%, 14.8%, 13.6%, or 16.9%, respectively. These differences were not significant (P = .7). Fisher’s exact test. We also analyzed samples from 117 subjects who had symptomatic or asymptomatic recurrences of HSV-2 infection or who were seronegative for HSV-2. The frequency of subjects heterozygous for the nectin-1 polymorphism who were symptomatic for HSV-2 infection, asymptomatic for HSV-2 infection, or uninfected was 22.9%, 26.7%, or 15.4%, respectively. Again, the differences were not significant (P = .4).

In additional studies, we examined the sequences encoding LIGHT (lymphotoxin homolog, exhibits inducible expression and competes, with HSV glycoprotein D, for HVEM, a receptor expressed by lymphocytes), a protein that functions as a ligand for the HSV receptor HVEA [3]. We searched several databases of single-nucleotide polymorphisms and found a polymorphism in the LIGHT gene that changes nt 640 from G to A. This results in a change, near the carboxy terminus of the protein, that substitutes the glutamic acid at aa 214 with lysine. This amino acid is predicted to be located in the extracellular domain of LIGHT, and, on the basis of the corresponding structures of tumor necrosis factor (TNF) and lymphotoxin-α, the amino acid is likely to be part of a β-strand that folds into a “jellyroll” sandwich structure. Of interest, aa 214 of LIGHT, which is changed from an acidic to a basic (lysine) amino acid in the polymorphism, aligns with basic amino acids in TNF, lymphotoxin-β, TRAIL (TNF-related apoptosis-inducing ligand), and Fas ligand.

Using primers TCACAAAAGCTTGCTACTAC and TGCA-CACACGCTCTCCCTTTC, we amplified a 338-bp DNA fragment corresponding to the polymorphism in LIGHT from patient samples and cut the DNA with restriction endonuclease BanRI. Bands of 134, 114, and 90 bp with the wild-type sequence of LIGHT were observed, and bands of 204 and 134 bp with the polymorphism were found. We analyzed samples from 226 subjects, on the basis of HSV serostatus. Only 2 patients were homozygous for the LIGHT polymorphism. The frequency of patients heterozygous for the LIGHT polymorphism whose HSV-1/HSV-2 serostatus was +/+ +/+−, −/−, or −/+ was 9.1%, 12.5%, 9.1%, or 14.3%, respectively. These differences were not statistically significant (P = .5). We also examined DNA from 129 additional subjects with well-defined clinical patterns of infection. The frequency of subjects heterozygous for the LIGHT polymorphism who were symptomatic for HSV-2 infection, asymptomatic for HSV-2 infection, or uninfected was 14.6%, 8.1%, or 9.8%, respectively. Again, the differences were not significant (P = .6).

Although we did not identify any relatively common polymorphisms that correlated with HSV serostatus or symptoms, a prior study identified HLA types that were significantly re-

<table>
<thead>
<tr>
<th>No. (%) of subjects with nectin-1 polymorphisms</th>
<th>Patient HSV status</th>
<th>Heterozygous for mutationa</th>
<th>Homozygous for mutationb</th>
<th>Wild typec</th>
<th>Total</th>
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<tbody>
<tr>
<td>−/−</td>
<td>12 (23.1)</td>
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<td>40 (76.9)</td>
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<tr>
<td>+/−</td>
<td>13 (14.8)</td>
<td>1 (1.1)</td>
<td>74 (84.1)</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>−/+</td>
<td>17 (13.6)</td>
<td>1 (0.8)</td>
<td>107 (85.6)</td>
<td>125</td>
<td></td>
</tr>
</tbody>
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a Heterozygous for glutamic acid (E) repeats, 8 consecutive E (8E) or 9 consecutive E (9E), including 2 patients with 8E/10 consecutive E (10E) and HSV-1/HSV-2 serostatus of +/−.
b Homozygous for polyE repeat 9E9E.
c Wild-type polyE repeat 8E8E.
lated to frequent or infrequent recurrences [4]. We believe that studies of additional polymorphisms in HSV receptors or their respective ligands are warranted, to determine whether any of these polymorphisms might correlate with susceptibility to infection or help to explain why some patients have frequent recurrences and others have infrequent recurrences.

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References

Long-Term Immunological Benefit in Patients with Discordant Responses to Therapy Depends on Both Level of Viremia and Duration of Rebound

To the Editor—The article by Le Moing et al. [1], which reports the results of the large French Antiretroviral Cohort after 24 months of observation, provides important information that may help the clinician who is faced with the dilemma of maintaining or modifying antiretroviral treatment for those patients with satisfactory immunological response to highly active antiretroviral therapy (HAART) in the presence of replicating virus. Nevertheless, Le Moing et al. correctly point out that longer follow-up is needed to confirm the long-term clinical implications of maintaining the current protease inhibitor (PI)–based regimen for patients with low-level viremia. Furthermore, other authors do not recommend such an approach because of the fear of accumulation of drug-specific resistance mutations [2].

We would like to contribute to the debate, presenting the long-term results of the cohort of human immunodeficiency virus (HIV)–infected patients attending the Institute for Infectious and Tropical Diseases of the University of Brescia (Brescia, Italy). Patients started their first PI-based regimen during the period of January 1997 through February 2000, maintained the same therapy for at least 12 months, and were monitored for at least 24 months. Of the 1663 patients whose information is filed in our database and who regularly come to the Institute for Infectious and Tropical Diseases for follow-up visits, 404 patients met the inclusion criteria and were stratified according to their virological response. Complete responders (CRs) had persistent virus suppression to <500 HIV RNA copies/mL, starting from month 8 after HAART initiation (n = 255), and rebounders (REBs) had confirmed detectable viral replication of >500 HIV RNA copies/mL after previously testing negative for virus (n = 149), regardless of treatment adjustments that may have occurred during follow-up. Nonresponders (i.e., patients who never reached virus suppression to <500 HIV RNA copies/mL) are not considered in this analysis.

Baseline median CD4+ cell counts and HIV RNA levels were comparable between CRs (CD4+ cell count, 211 cells/μL; HIV RNA level, 63,600 copies/mL) and REBs (CD4+ cell count, 212 cells/μL; HIV RNA level, 42,200 copies/mL). Median follow-up periods were also comparable between CRs and REBs (33.7 and 34.9 months, respectively). A significant overall difference in the slope of CD4+ cell count gain over time was observed. CRs had better CD4+ cell count gains than did REBs (monthly increase over the follow-up period, 8.4 and 6.4 CD4+ cells/μL, respectively; P = .01). Nevertheless, when the rebound duration (months) and the rebound magnitude (i.e., initial, maximum, and median virus loads) were considered together, a more complex picture was identified.

When the total duration of rebound (viremic period) was <12 months, the long-term (median duration of follow-up, 33.7 [for CRs] and 32.9 [for REBs] months) monthly gain in CD4+ cell count was nearly equal between CRs and REBs (mean, 8.4 and 7.8 cells/μL, respectively, per month of follow-up), regardless of the maximum virus load of the rebound period. When the total duration of rebound was 13–18 months, a significant difference in the long-term (median duration of follow-up, 33.7 [for CRs] and 34.7 [for REB] months) monthly gain in CD4+ cell count was detected between CRs and REBs whose maximum HIV RNA level was >10,000 copies/mL (mean, 8.4 and 4.4 cells/μL, respectively, per month of follow-up; P = .02) but not between CRs and low-replicating (maximum HIV RNA level, <10,000 copies/mL) REBs. When the total duration of viremic period was >18 months, a significant difference in the long-term (33.7 [for CRs] and 41.4 [for REBs] months) monthly gain in CD4+ cell count was detected between CRs and REBs (mean, 8.2 and 4.1 cells/μL, respectively, per month