A Polymorphism That Reduces RANTES Expression Is Associated with Protection from Death in HIV-Seropositive Ugandans with Advanced Disease

Graham S. Cooke,1 Kerrie Tosh,1 Patricia A. Ramaley,1 Pontiano Kaleebu,2 Joanna Zhuang,3 Jessica S. Nakkyingi,1 Christine Watera,4 Charles F. Gilks,5 Neil French,5,6 James A. G. Whitworth,7 and Adrian V. S. Hill8

1Wellcome Trust Centre for Human Genetics, Churchill Hospital, University of Oxford, Oxford, 2Imperial College, London, and 3Liverpool School of Tropical Medicine, Liverpool, United Kingdom, 4Medical Research Council Programme on AIDS, Uganda Virus Research Unit, Entebbe, Uganda

We investigated the effect of RANTES polymorphisms on human immunodeficiency virus type 1 (HIV-1) disease progression in an urban population of Uganda. HIV-positive individuals homozygous for the INT1.1C polymorphism, which had been associated previously with low RANTES expression, were less likely to die than were those with other genotypes (hazard ratio, 0.53 [95% confidence interval, 0.33–0.83]; P = .007). This report of a non–human leukocyte antigen genetic association with HIV-1 and/or acquired immunodeficiency syndrome disease progression in an African population reveals a genetic effect different from that reported elsewhere for African Americans and may impact therapeutic strategies targeting the RANTES pathway in HIV infection.

Among the many molecules of the human immune system involved in the pathogenesis of HIV infection, RANTES has emerged as one of the most important. RANTES is an endogenous ligand for CCR5, the key coreceptor for M-tropic HIV-1 strains commonly seen in early infection. Since the in vitro observation was made that the anti-HIV CD8+ T cell response is reduced substantially by blocking the activity of RANTES [1], there has been interest in the therapeutic potential of manipulating this β-chemokine, and several new CCR5 antagonists are in the late phases of clinical development. However, there is evidence that RANTES might promote the replication of some HIV strains, particularly at high concentrations [2, 3], and this has raised the troubling question of whether its activity is beneficial or harmful in vivo (reviewed in [4]).

In the absence of convenient in vivo assays to address this question, the study of host genetic polymorphism has provided important evidence that control of RANTES expression might influence the risk and outcome of infection, with studies implicating promoter polymorphisms at nucleotide positions −403 and −28 [5, 6]. The most comprehensive study to date has come from An et al. [7], who, after extensive sequencing, identified an intronic C→T polymorphism (INT1.1) within the RANTES gene. In vitro functional data, acquired using luciferase reporter assays in transfected Jurkat cells, demonstrated that the INT1.1C allele was associated with reduced RANTES expression. Investigation of the polymorphism’s role in US cohorts of HIV-positive patients found evidence that the INT1.1C allele was associated with more rapid progression to AIDS in HIV-positive individuals and possibly with increased susceptibility to HIV infection. The effect was most clearly seen in African Americans, which led the authors to propose that this might be an important allele determining HIV disease progression in African populations.

We investigated the impact of RANTES polymorphisms on HIV-1 infection and disease progression in an urban Ugandan population. The study was approved by the Uganda Virus Research Institute Ethics Committee.

Methods. Participants were enrolled as part of a pneumococcal vaccine study conducted in Entebbe, Uganda, between 1995 and 1998, before antiretroviral therapy was available [8]. For the purposes of this study, patients were followed up until 2002. Patients were recruited from 2 community-based HIV clinics and were eligible if they were ≥15 years of age, lived within 15 km of the study clinics, and had World Health Organization (WHO) clinical disease stage 1, 2, or 3 [9]. Patients were excluded if they were pregnant, were older than 55 years, were receiving rifampicin-based antituberculous medication, had an acute febrile illness, or had WHO clinical disease stage 4. All patients provided written, informed consent. Baseline characteristics of the cohort were as follows: mean age, 31.8 years; sex, 69.8% female and 30.2% male; mean CD4+ cell count, 299 cells/mm3; ethnicity, 96.1% Bantu; and viral clade, 50.9% clade A, 47.6% clade D, and 1.5% other clade. Viral load measurements were...
not performed. The largest single ethnic group, Bagandans, accounted for 80.1% of the population. Clinical evaluation was done 1 month after enrollment and every 6 months thereafter. Those who did not attend the clinic were visited by a field worker. Patients who moved away from the area, refused follow-up, or did not have a clinical visit were (right) censored at the date of move from the area, the date of refusal, or the date of the last clinical appointment.

An HIV-negative cohort was identified from individuals who attended the study centers for voluntary HIV tests but were found to be uninfected. The case-control study had 195% power to detect an odds ratio 1.2 for a genotype with a frequency of 80%.

Both contingency table analysis of genotype versus infection status and Cox regression survival analysis were conducted using SPSS (version 12.0; SPSS) [10]. In the analysis of predictors of survival, Cox proportional hazards multiple regression analysis was used, with the covariates sex, ethnicity, viral clade, and CD4+ cell count at the time of study recruitment. Results are given as relative risks (i.e., hazard ratios [HRs]). All measures of significance were 2-tailed.

Results. Four polymorphisms were investigated—at nucleotide positions −403 and −28, INT1.1, and INT1.2 [7]. In a randomly selected sample of 94 cases and 94 controls, 3 polymorphisms had minor-allele frequencies >1% in this population (−403G/A, INT1.1T/C, and INT1.2C/T), and these 3 were studied in all available samples. The allele frequencies were 53% G and 47% A for −403G/A, 80% T and 20% C for INT1.1T/C, and 93% C and 7% T for INT1.2C/T.

A total of 791 HIV-infected individuals were studied. After 7 years, 240 individuals (30.1% of the cohort) were alive. Survival rates among those with the INT1.1TT and INT1.1CT genotypes were not significantly different, with 356 deaths (68.6%) among 519 individuals and 178 deaths (75.1%) among 237 individuals, respectively (Cox regression analysis HR, 1.02 [95% confidence interval {CI}, 0.82–1.23]; P = .81) (figure 1). However, those with the INT1.1CC genotype were significantly protected from death, with 20 deaths (52.6%) among 38 individuals (Cox regression analysis HR, 0.53 [95% CI, 0.33–0.83]; P = .007). There were no significant differences in ethnic composition between each INT1.1 genotype group. Bagandans accounted for 76.6%, 80.2%, and 81.1% of groups with genotypes TT, CT, and CC, respectively. A total of 213 patients were right censored: 104 patients between days 0 and 1200 and 109 patients between days 1200 and 1400.

A total of 285 HIV-negative controls were studied. The distribution of ethnic groups in the controls did not differ significantly from that in HIV-positive individuals, with Bagandans forming the largest single group among both case patients (86.3%) and controls (88.2%). None of the polymorphisms studied showed a significantly different genotype frequency between case and control groups. For the INT1.1C/T variant, a nonsignificant excess of INT1.1CC was observed among uninfected controls. Genotype distributions for INT1.1 were as follows: cases, 519 (65.4%) TT, 237 (29.8%) CT, and 38 (4.8%) CC; and controls, 178 (62.5%) TT, 86 (30.2%) CT, and 21 (7.4%) CC. There was no overall significant difference (P = .24). Nei-

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of patients included in analysis at each time point (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TT</td>
<td>517</td>
</tr>
<tr>
<td>CT</td>
<td>237</td>
</tr>
<tr>
<td>CC</td>
<td>37</td>
</tr>
</tbody>
</table>
ther the −403A/G nor the INT1.2C/T polymorphism was associated with infection or progression of disease.

Discussion. The clear finding here is that homozygosity for the INT1.1C allele was associated with protection from death in HIV-positive individuals in this population. The confirmation that RANTES polymorphism is associated with disease progression is significant, because this is the first report of any such effect in an African population, and it follows the previous observation that chemokine receptor polymorphisms are not associated with disease in the same cohort [11].

Importantly, however, our results are in contrast to previous work in an African American population in which the INT1.1C allele was found to have a dominant effect, increasing susceptibility to both infection and disease. There are several reasons why such a difference could be seen between these 2 populations. One explanation is that another, as-yet-unidentified genetic variant exists, with patterns of linkage disequilibrium differing between African Americans and this population from urban Uganda. If this is the case, it reinforces the difficulties in extrapolating data from one population to another, even if the populations might appear to be closely related. However, the extensive sequencing of RANTES by An et al. [7] and the similarity between the allele frequencies of the polymorphisms studied here in Ugandans and those in African Americans suggest that another genetic variant might not be the explanation.

Perhaps more importantly, there are significant differences in design and recruitment between this study and previous genetic studies. Considerable insight has been gained into the pathogenesis of HIV disease from large, predominantly US-based cohorts [12]. These cohorts were recruited prospectively at a time before antiretroviral medications (ARVs) were available. However, there are no such cohorts for African populations, and, with the availability of ARVs, the recruitment of such a cohort would not be ethical. The most likely explanation for the differences is that the patients recruited here are likely to represent the later stages of HIV infection, with a worse prognosis. The 30% 6-year survival rate of the Entebbe cohort is far lower than that of the cohorts used for the largest study of RANTES polymorphism to date [7]. The exclusion of patients with WHO stage 4 disease is unlikely to have contributed significantly to the overall conclusions, since this group represented only 2% of the original cohort. Other differences in opportunistic infection patterns, contrasts in routes of viral transmission (largely heterosexual contact in Uganda in contrast to intravenous drug use in the US study), and different infecting viral clades [13] are possible but less likely explanations of the differences seen.

In the absence of prospective data from seroconversion, the CD4+ cell count at baseline was used as a surrogate marker to determine disease stage. It is possible that the protection from death observed is an artefact of early deaths in rapid progressors who were not ascertained, in particular those with the INT1.1CC genotype. However, no significant difference was observed in the frequency of the INT1.1CC genotype between infected individuals and healthy controls.

Despite the methodological differences mentioned above, these data raise the possibility of a biological difference in the role played by RANTES in these HIV-positive populations, suggesting that, in this Ugandan cohort, down-regulation of RANTES expression protects HIV-positive individuals from progression to death. Any such difference in the role played by RANTES would, if confirmed, have important implications for strategies targeting the molecule for therapeutic intervention. In particular, these data raise the possibility that different manipulations of RANTES might benefit an individual patient at different stages of disease, with an up-regulation of RANTES activity potentially being beneficial in early infection and harmful in later disease. It would be interesting to know whether similar findings for late-stage infection can be replicated in other populations. The contrasting findings between Ugandans in this cohort and African Americans in US cohorts highlight the difficulties in extrapolating research from one population to another and emphasize the importance of performing studies in populations with the greatest burden of disease.

Acknowledgments

We thank all those who consented to participate in this study, Victor Appay for his helpful comments on the manuscript, and Alison Rodger for statistical advice.

References