CONCISE COMMUNICATION

Protective Effect of Interleukin-4 –589T Polymorphism on Human Immunodeficiency Virus Type 1 Disease Progression: Relationship with Virus Load

Emi E. Nakayama, Laurence Meyer, Aikichi Iwamoto, Anne Persoz, Yoshiyuki Nagai, Christine Rouzioux, Jean-François Delfraissy, Patrice Debre, Dorian McIlroy, Ioannis Theodorou, Aikichi Iwamoto, Anne Persoz, Yoshiyuki Nagai, Emi E. Nakayama, Laurence Meyer, Tatsuo Shioda, and the SEROCO Study Group

The interleukin (IL)–4 –589T allele bears a single nucleotide polymorphism at position –589 upstream from the open-reading frame of the IL-4 gene. To determine the influence of this allele on human immunodeficiency virus (HIV) type 1 disease, disease progression and serum virus load were assessed by IL-4 genotype in 427 white patients with known seroconversion dates who were followed in the French SEROCO cohort between 1988 and 1996. Serum virus load was 0.20 log lower during the 6–24-month plateau phase after seroconversion in patients with IL-4 –589T than in those without this allele (P = .02). Kaplan-Meier analysis survival curves showed a slower progression to clinical AIDS in carriers of IL-4 –589T (P = .04). Adjustment for early serum virus load greatly diminished the strength of this association. These results suggest that IL-4 –589T protects against HIV-1 disease progression by reducing virus load.

Interleukin (IL)–4 has multiple immune response–modulating functions, including induction of IgE production in B lymphocytes and the differentiation of precursor T helper (Th) cells toward the Th2 subset that mediates humoral immunity [1]. With respect to human immunodeficiency virus (HIV) type 1 infection, IL-4 differentially regulates 2 major HIV-1 coreceptors, CCR5 and CXCR4 [2–4]. CCR5 is a coreceptor used by non-syncytia-inducing/macrophage-tropic/R5 viruses, which are more likely to be transmitted through sexual contact [5] and are present early after infection in nearly all HIV-1–infected persons. In contrast, CXCR4 is a coreceptor used by syncytia-inducing T cell line–tropic/X4 variants, which are usually present in the latter stages of infection [6]. IL-4 down-regulates CCR5 expression and thus inhibits replication of R5 HIV-1 in human T cells and macrophages [2, 3], while it up-regulates the expression of CXCR4 and enhances the replication of X4 variant [4].

Recently, polymorphisms in the HIV-1 coreceptor, their natural ligand, and cytokine genes were shown to modify HIV-1 transmission and disease progression [7, 8]. In the IL-4 gene, Rosenwasser et al. [9] reported a polymorphism with a C to T exchange at position –589 upstream of the open-reading frame of the IL-4 gene, IL-4 –589T, that is associated with increased promoter activity for IL-4 transcription and elevated levels of serum IgE in asthmatic families [9]. Previously, we reported that IL-4 –589T is associated with increased rates of the acquisition of X4 variants and elevated serum IgE levels in HIV-1–infected Japanese persons [10]. We also showed a lower frequency of IL-4 –589T in persons infected with HIV-1 who reported heterosexual contact as a risk factor than in uninfected persons. These results suggested that IL-4 –589T has a protective effect against replication of R5 virus via CCR5 down-modulation. To evaluate the impact of the IL-4 –589T allele on HIV-1 disease progression, we analyzed IL-4 genotypes of 427 HIV-1–infected subjects with a documented date of seroconversion who were enrolled in the French SEROCO cohort.

Patients and Methods

Patients. Since January 1988, the multicenter French SEROCO cohort has included 1516 HIV-1–infected adults without hemophilia. The 6 monthly follow-up visits included a full physical examination and collection of blood samples that were stored at –180°C. A detailed description of the SEROCO cohort is given elsewhere [11]. In all, 454 patients had a documented date of infection within an interval between their last negative and first positive
HIV antibody test of <24 months or incomplete/complete Western blot results. The date of infection is defined as the date of an incomplete Western blot less 1 month, the date of primary symptomatic infection less 15 days, or the midpoint between the 2 tests. These patients were enrolled in the cohort <2 years after infection (median, 8.0 months). Stored peripheral blood mononuclear cell samples were available for 428 patients (median follow-up from seroconversion until September 1996, 116 months). DNA of 1 patient was not amplified for this study.

IL-4 genotyping. Polymerase chain reaction (PCR) amplification of the IL-4 promoter was done by using genomic DNA extracted from cryopreserved lymphocytes. The IL-4 –589T mutation was detected by PCR restriction fragment–length polymorphism analysis as described elsewhere [10].

HIV-1 RNA quantification. Serum HIV-1 RNA levels were measured on samples taken at baseline by using a PCR-based assay with a lower limit of detection of 200 HIV RNA copies/mL (AmpliCor HIV Monitor assay; Roche) according to the manufacturer’s instructions.

Statistical analysis. We used the χ² test to compare qualitative variables and Student’s t or Wilcoxon rank sum tests to compare continuous variables. The Kaplan-Meier analysis method was used to construct survival curves, which were compared by the log-rank test. Crude and adjusted relative risks (RRs) and their 95% confidence intervals (CIs) were calculated by Cox proportional hazards models. The cutoff date was 15 September 1996.

Results

The frequency of the IL-4 –589T allele was 0.15 in 427 patients (14 T homozygotes, 98 C/T heterozygotes, and 315 C homozygotes), and this proportion was not different from that of a healthy French population (0.15; 2 T homozygotes, 21 C/T heterozygotes, and 63 C homozygotes). Since there were few homozygous patients and the disease progression to AIDS or death did not differ between heterozygotes and homozygotes (data not shown), these 2 categories were considered together in the analysis.

As shown in table 1, the frequency of patients bearing IL-4 –589T did not differ significantly by sex. The risk factor for HIV infection, age at infection, or year of infection did not differ significantly between patients with and without IL-4 –589T. The median period between infection and enrollment in the cohort (8 months) was similar in the patients with and without IL-4 –589T. The frequency of symptomatic primary infection (isolated minor signs such as isolated fever or pharyngitis or rash were excluded from the definition) did not differ significantly between patients with IL-4 –589T and homozygous wild-type patients (table 1). Virus load could be quantified in frozen serum samples of 400 patients. Early virus load (first sample HIV RNA assay in the 6–24 months after infection) was significantly lower in the patients carrying IL-4 –589T than the wild-type homozygotes (median, 3.85 and 4.05 log₁₀ HIV RNA copies/mL, respectively; P = .02, Wilcoxon test). Only 17 patients had undetectable early virus load. However, baseline CD4 and CD8 cell counts were similar in persons with IL-4 –589T and wild-type homozygotes.

In total, 148 AIDS cases were diagnosed during follow-up; of these, 30 were in persons carrying IL-4 –589T. The AIDS-free survival curves showed that the mutation was associated with a significantly delayed progression to clinical AIDS (figure 1A; P = .04, log-rank test). Thus, the risk of progression to clinical AIDS for persons carrying IL-4 –589T was lower than in wild-type homozygotes (figure 1A; RR, 0.66, Cox model; 95% CI, 0.43–0.99). On the other hand, the protective effect of the CCR5Δ32 depletion and the M280 mutation in the CX3CR1 gene [12] (adjusted RR, 0.65; 95% CI, 0.43–1.02) or further adjustment for the CCR5Δ32 deletion and the M280 mutation in the CX3CR1 gene (adj) was not modified by adjustment for IL-4 –589T in this study population. These results suggested that there was no confounding by other known genetic factors. However, after adjustment for early serum virus load (in the 6–24 months after infection), the protective effect of IL-4 –589T on progression to AIDS was no longer significant (adjusted RR, 0.82; 95% CI, 0.54–1.23; P = .33). In other words, the protective effect of the IL-4 –589T allele was mediated through a lower early virus load.

The same protective effect associated with IL-4 –589T was observed for progression to death (log-rank test, P = .03; figure

### Table 1. Baseline and clinical characteristics of 427 human immunodeficiency virus (HIV) type 1–infected patients according to the mutated interleukin (IL-4) –589T allele (SEROCO cohort).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients without IL-4 –589T allele (n = 315)</th>
<th>Patients with IL-4 –589T allele (n = 112)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, % (no.)</td>
<td>76.8 (242)</td>
<td>80.4 (90)</td>
<td>NS</td>
</tr>
<tr>
<td>Exposure group, % (no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Homosexual contact</td>
<td>64.1 (202)</td>
<td>65.2 (73)</td>
<td>NS</td>
</tr>
<tr>
<td>Heterosexual contact</td>
<td>26.0 (82)</td>
<td>22.3 (25)</td>
<td></td>
</tr>
<tr>
<td>Injection drug user</td>
<td>7.0 (22)</td>
<td>7.1 (8)</td>
<td></td>
</tr>
<tr>
<td>Other or unknown</td>
<td>2.9 (9)</td>
<td>5.4 (6)</td>
<td></td>
</tr>
<tr>
<td>Age at infection, mean years (SD)</td>
<td>30.4 (8.4)</td>
<td>29.2 (7.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Year of infection, % (no.)</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Before 1988</td>
<td>27.0 (85)</td>
<td>25.9 (29)</td>
<td></td>
</tr>
<tr>
<td>1988</td>
<td>33.0 (104)</td>
<td>30.4 (34)</td>
<td></td>
</tr>
<tr>
<td>After 1988</td>
<td>40.0 (126)</td>
<td>43.8 (49)</td>
<td></td>
</tr>
<tr>
<td>Symptomatic primary infection, % (no.)</td>
<td>30.5 (96)</td>
<td>26.8 (30)</td>
<td>NS</td>
</tr>
<tr>
<td>CD4 cell count, median cells/mL</td>
<td>528.5</td>
<td>529</td>
<td>NS</td>
</tr>
<tr>
<td>CD8 cell count, median cells/mL</td>
<td>875</td>
<td>935</td>
<td>NS</td>
</tr>
<tr>
<td>Serum virus load, median log₁₀ HIV RNA copies/mL</td>
<td>4.05</td>
<td>3.85</td>
<td>.02c</td>
</tr>
<tr>
<td>AIDS cases during follow-up, % (no.)</td>
<td>37.5 (118)</td>
<td>26.8 (30)</td>
<td>.04d</td>
</tr>
</tbody>
</table>

NOTE. NS, not significant.

a First measurement in 6–24 months after infection.

b No. available for 293 and 107 patients, respectively.

c Wilcoxon test.
d Log-rank test.
and for progression to <200 CD4 cells/mL, although the difference failed to reach statistical significance \( (P = 0.06, \text{log-rank test; figure 1B}) \). The risk of progression to death was also lower in persons with IL-4 \( <589T \) than in wild-type homozygotes (RR, 0.58 by Cox model; 95% CI, 0.35–0.96; \( P = 0.03 \); figure 1C). It was not influenced by adjustment for age at seroconversion, but, after further adjustment for early virus load, a significant protective effect was no longer found (adjusted RR, 0.77; 95% CI, 0.46–1.29; \( P = 0.31 \)).

Discussion

Our previous cross-sectional study in Japanese seroprevalent subjects showed a protective effect of IL-4 \( <589T \) against HIV-1 transmission in heterosexual contact and a clear effect of X4 variant acquisition [10]. It was speculated that IL-4 \( <589T \) had a protective effect against HIV-1 disease progression via CCR5 down-modulation in the absence of X4 variants, but, once an X4 variant emerges, IL-4 \( <589T \) no longer exerts a protective effect and would accelerate HIV-1 disease progression due to CXCR4 up-regulation. Further analysis in a well-organized cohort of seroconverters was required since the Japanese cross-sectional study was not adequate to evaluate these dual effects that transit during disease progression.

In the present study, we analyzed a large cohort of 427 HIV-1–infected white subjects with known dates of seroconversion. We found that the IL-4 \( <589T \) was indeed associated with delayed progression to AIDS and death. Virus load was lower in patients with IL-4 \( <589T \), and adjustment for early virus load greatly diminished the strength of the association with disease progression. These results suggest that the protective effect is due in part to a reduction of HIV replication and HIV dynamics in vivo and is in good agreement with previous reports of in vitro experiments that showed that IL-4 down-regulates CCR5 expression and inhibits replication of R5 HIV-1 strains [2].

In this cohort, the allelic frequency of IL-4 \( <589T \) was 0.15 lower than in Japanese seroprevalent subjects (0.64) [10]. Contrary to our previous observation, the T allele frequency was not significantly lower in the heterosexual group in the SEROCO cohort (0.12) than in the homosexual group in this cohort (0.15) or in healthy French persons (0.15). At present, the discrepancy between the Japanese cross-sectional and the cohort studies in white subjects cannot be clearly explained, but it may be due to lower frequency of the T allele in whites or to study design differences.

At first, we expected that the slower clinical progression in persons carrying the IL-4 \( <589T \) allele compared with wild-type homozygotes would be evident in the early period of infection and not in the late period because of the emergence of X4 variants, since IL-4 \( <589T \) enhances X4 variant acquisition. Contrary to our expectation, the protective effect of IL-4 \( <589T \) was shown also for progression to death. It is possible that the impact of X4 variant acquisition on disease progression is not so strong in overall follow-up. Also, of note, the emergence of X4 variant in the Japanese study was associated only with IL-4 \( <589T \) homozygotes, which are lower in the French population.

Figure 1. Effect of interleukin (IL)–4 promoter polymorphism (IL-4 \( <589T \)) on human immunodeficiency virus type 1 disease progression in infected white subjects (SEROCO cohort) for 315 patients without and 112 patients with IL-4 \( <589T \). Time from seroconversion to clinical AIDS (1993 European definition) (A), to a decline in CD4 cells to <200 cells/mL (B), or to death (C) were examined. \( P \) values were determined by log-rank test. Relative risk (RR) and 95% confidence intervals (CIs) were determined by Cox proportional hazards models.
Of interest, all 3 patients with AIDS who were homozygous for the IL-4 –589T allele died ≤3 years after developing AIDS, while only 53.6% (15 of 28) of patients heterozygous for the mutation and 63.1% (77 of 122) of patients with the wild-type allele died. These effects of the IL-4 –589T allele on disease progression and X4 virus acquisition appear to be similar to those of the CCR2 64I mutation described in the Amsterdam cohort. In CCR2 64I–bearing patients, the delay in disease progression was more pronounced when only R5 viruses were present and was not observed after conversion to X4 variants, although a higher rate of emergence of X4 variants was observed in patients with CCR2 64I than in wild-type homozygotes [13]. Since the phenotype of HIV-1 strains was not routinely analyzed in the SEROCO cohort, these results should be confirmed in relation to the presence or absence of X4 variants.

SEROCO Study Group Members

C. Rouzioux, M. Bary, and M. Burgard, ACCTES (Paris); P. Dellamonica and J. Durant, Hôpital L’Archet (Nice); H. Gallais and A. M. Quinson, Hôpital de la Conception (Marseille); J. F. Delfraissy, P. Lebras, C. Goujard, Y. Quertainmont, and M. T. Ranou, Hôpital du Kremlin-Bicêtre (Le Kremlin-Bicêtre); M. T. Ranou, Hôpital du Kremlin-Bicêtre (Le Kremlin-Bicêtre); J. J. Lefrère, J. Lerable, J. Salpetrier, and M. C. Meyohas, Hôpital Saint-Antoine, (Paris); J. A. Gastaut, G. Fabre-Coste-lier, M. Bonmarchand, C. Katlama, M. Richard, and C. Rivièere, Hôpital Pitié-Salpêtrière (Nice); B. Dupont, V. Feuillie, and M. P. Treilhou, Hôpital de l’Institut Pasteur (Paris); D. Vittecoq and L. Escaut, Hôpital Paul Brousse (Villejuif); S. Herson, A. Coutelhou, Hôpital de l’Institut Pasteur (Paris); S. Herson, A. Coutelhou, Hôpital de l’Institut Pasteur (Paris); J. F. Delfraissy, P. Lebras, C. Goujard, Y. Quertainmont, and M. C. Meyohas, Hôpital Saint-Antoine, (Paris); J. P. Cassuto and M. Quaranta, Hôpital Cimiez (Nice); J. F. Delfraissy, P. Lebras, C. Goujard, Y. Quertainmont, and M. C. Meyohas, Hôpital Saint-Antoine, (Paris); J. A. Gastaut, G. Fabre-Coste-lier, M. Bonmarchand, C. Katlama, M. Richard, and C. Rivièere, Hôpital Pitié-Salpêtrière (Paris); J. A. Gastaut, G. Fabre-Coste-lier, M. Bonmarchand, C. Katlama, M. Richard, and C. Rivièere, Hôpital Pitié-Salpêtrière (Paris); D. Vittecoq and L. Escaut, Hôpital Paul Brousse (Villejuif); S. Herson, A. Coutelhou, Hôpital de l’Institut Pasteur (Paris); D. Vittecoq and L. Escaut, Hôpital Paul Brousse (Villejuif); S. Herson, A. Coutelhou, Hôpital de l’Institut Pasteur (Paris); J. L. Vilde, C. Leport, U. Colassante, and W. Nouiouia, Hôpital Bichat (Paris); A. Sobel and M. Lechevalier, Hôpital Henri Mondor (Créteil); M. Kazatchkine, M. Buissson, and J. Vrtousnik, Hôpital Broussais (Paris); L. Guillevin, B. Jarrousse, P. Cohen, and P. Deny, Hôpital Avicenne (Bobigny).

Acknowledgment

We thank Noriko Teramoto for manuscript preparation.

References