

# Slower Progression of HIV-1 Infection in Persons with GB Virus C Co-Infection Correlates with an Intact T-Helper 1 Cytokine Profile

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**Background:** Progression to AIDS is slower in persons infected with both HIV-1 and GB virus C (GBV-C), also known as hepatitis G virus.

**Objective:** To compare clinical, virologic, and immunologic variables in HIV-1-seropositive patients with and without GBV-C co-infection.

**Design:** Subanalysis of a prospective cohort study.

**Setting:** Institute of Infectious Diseases, University of Catania, Catania, Italy.

**Patients:** 80 asymptomatic HIV-1-seropositive patients.

**Measurements:** GBV-C RNA level; plasma HIV-1 viral load; CD4<sup>+</sup> cell counts; and serum levels of interleukin (IL)-2, IL-4, IL-10, and IL-12.

**Results:** At the start of the study, plasma GBV-C RNA was detected in 17 patients (21%). During follow-up, IL-2 and IL-12 levels decreased significantly ( $P = 0.005$  and  $P = 0.01$ , respectively) and IL-4 and IL-10 levels increased significantly ( $P = 0.01$  and  $P = 0.004$ , respectively) in the GBV-C-negative group but did not change substantially in the GBV-C-positive group. Each measured variable differed significantly between GBV-C-positive and GBV-C-negative groups during follow-up ( $P < 0.001$  for IL-12, IL-4, and IL-10;  $P = 0.002$  for IL-2).

**Conclusion:** GB virus C may immunologically interfere with progression of HIV-1 infection to AIDS by maintaining an intact T-helper 1 cytokine profile.

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Hepatitis G virus, also called GB virus C (GBV-C), is a recently identified RNA virus belonging to the Flaviviridae family (1, 2). It is usually transmitted parenterally (2, 3). The prevalence of GBV-C viremia ranges from 20% to 24% among persons who use intravenous drugs (4, 5). Higher rates have been seen in patients with HIV-1 infection, regardless of intravenous drug use (4, 6).

In recent surveys, HIV-1-infected persons with GBV-C co-infection had better AIDS-free survival rates and higher CD4<sup>+</sup> cell counts than HIV-1-infected patients who were GBV-C negative (7–10). It has also been shown that GBV-C inhibits HIV-1 replication in vitro (11). Our objective was to evaluate AIDS-free survival rates, plasma HIV-1 viral load, and selected immunologic variables in 80 HIV-1-seropositive patients with and without GBV-C co-infection. We sought to determine possible immunologic mechanisms involved in these co-infection scenarios.

## METHODS

The study was initiated between January and March 1989 at the Institute of Infectious Diseases, University of Catania, Catania, Italy. Institutional review boards at the Institute of Infectious Diseases, University of Catania, approved the follow-up protocol. Signed informed consent was obtained from each patient. Among 319 HIV-1-seropositive patients, 240 used intravenous drugs, 60 were homosexual men, and 19 had received many blood transfusions. Eighty of these 319 patients (25%), all of whom used intravenous drugs, were asymptomatic and were enrolled in a prospective follow-up study to evaluate the pro-

gression of HIV-1-related disease. All 80 patients underwent physical examination and routine blood biochemistry examinations every 6 months. Blood samples were obtained annually from each patient, and serum was stored at  $-80^{\circ}\text{C}$  until use.

The analyses for the current study were begun in January 1997. Quantitative plasma HIV-1 RNA levels and circulating levels of specific interleukins (ILs)—IL-2, IL-4, IL-10, and IL-12—were retrospectively determined in all serum samples. We determined GBV-C RNA levels in all serum specimens collected at the beginning of the study and at the end of follow-up. Analyses were repeated in January 2001.

Anti-E2 antibodies were detected by using the Enzygnon-Test Anti-HGenv (Boehringer Mannheim Corp., Indianapolis, Indiana). Plasma HIV-1 RNA copy numbers were determined by using the nucleic acid sequence-based amplification method (NASBA, Organon Teknika, Boxtel, the Netherlands). The sensitivity limit of the assay was 400 copies/mL. Interleukin-2 was analyzed by using a quantitative enzyme immunoassay (Predicta, Genzyme Diagnostics, Cambridge, Massachusetts) with a sensitivity limit of 4 pg/mL. Interleukin-4 was tested by using a quantitative enzyme immunoassay (InterTest, Genzyme Diagnostics) with a sensitivity limit of 0.045 ng/mL. Interleukin-10 was measured by using a competitive enzyme immunoassay (Cytokit Red 10, Genzyme Diagnostics), which had a range of detection between 0.195 and 200 ng/mL. Interleukin-12 levels were determined by using an enzyme immunoassay provided by R&D Systems (Oxon, United Kingdom) that had a lower sensitivity limit of 5 pg/mL. We measured GBV-C RNA level by using

reverse transcriptase polymerase chain reaction, as described elsewhere (12).

### Statistical Analysis

Plasma HIV-1 RNA levels were logarithmically transformed to normalize their distribution. Categorical variables were analyzed by using the Fisher exact test. The group means were compared by using the Student *t*-test. Variations of all interim values of plasma HIV-1 RNA level, CD4<sup>+</sup> cell count, and IL levels were analyzed within each group by using two-way analysis of variance. We compared GBV-C RNA–positive and GBV-C RNA–negative groups by using the Mann–Whitney U test to examine percentage variations from baseline values to values at the end of follow-up. Curves reflecting variations by time in immunologic and virologic variables were compared by using univariate repeated-measures analysis that followed an analysis-of-variance structure (13).

Progression to AIDS was defined as the development of an opportunistic infection or malignant condition. We used the Kaplan–Meier method to evaluate the effect of GBV-C infection on AIDS-free survival by assuming that GBV-C and HIV-1 infection status was fixed at the beginning of follow-up. A *P* value less than 0.05 was considered statistically significant. Statistical analyses were performed by using SAS software, version 6.12 (SAS Institute, Inc., Cary, North Carolina).

### Role of the Funding Sources

The funding sources had no direct control over the analysis of the study.

## RESULTS

The mean age of the study patients ( $\pm$ SD) was  $24.6 \pm 2.2$  years. Fifty-two patients were men, and 28 were women. Mean duration of intravenous drug use ( $\pm$ SD) was  $24.2 \pm 1.8$  months, and mean duration of known HIV-1 seropositivity ( $\pm$ SD) was  $9.2 \pm 1.6$  months. The mean baseline CD4<sup>+</sup> cell count ( $\pm$ SD) was  $471 \pm 55 \times 10^9$  cells/L. Fifty-eight patients declined to take any antiretroviral drug, and 6 were treated with zidovudine alone throughout the follow-up period. In 16 patients, didanosine was added to zidovudine, starting in 1993. In January 1997, follow-up was interrupted and all 80 patients began to receive different highly active antiretroviral therapy (HAART). At this time, 6 patients were asymptomatic, 24 had stage B disease, and 50 had stage C disease, according to stages defined by the U.S. Centers for Disease Control and Prevention. At the end of follow-up, the mean CD4<sup>+</sup> cell count ( $\pm$ SD) was  $77 \pm 33 \times 10^9$  cells/L.

Seventeen of the 80 serum specimens collected at the beginning of follow-up (21%) were positive for GBV-C RNA. All of these 17 patients maintained GBV-C viremia to the end of the follow-up period, and none of the 63 patients who were GBV-C RNA negative acquired the in-

### Context

Patients infected with HIV-1 progress to AIDS more slowly if they are co-infected with hepatitis G virus, also called GB virus C (GBV-C), than if they are not. The mechanism of this effect of GBV-C infection is not known.

### Contribution

Among 80 asymptomatic HIV-1–infected patients, T-helper 1 cytokine profiles changed unfavorably in those without GBV-C infection and remained stable in those with GBV-C infection.

### Implications

Co-infection with GBV-C may slow progression of HIV-1 infection through a mechanism related to T-helper 1 cytokines.

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fection. Patients who were GBV-C negative and those who were GBV-C positive did not significantly differ in age, sex, duration of intravenous drug use and HIV-1 seropositivity, or rate of hepatitis C virus and hepatitis B virus infection (Table).

### Clinical Outcomes

At the end of the 8-year follow-up, 3 of 17 GBV-C–positive patients (18%) remained asymptomatic (stage A), 7 (41%) had stage B disease, and 7 (41%) had stage C disease. In the 7 patients with stage C disease, the following AIDS-defining diseases were observed: AIDS dementia complex ( $n = 7$  [100%]), *Pneumocystis carinii* pneumonia ( $n = 4$  [57%]), esophageal candidiasis ( $n = 3$  [43%]), and *Toxoplasma encephalitis* infection ( $n = 1$  [14%]). Three of 63 GBV-C RNA–negative patients (5%) had stage A disease, 17 (27%) had stage B disease, and 43 (68%) had stage C disease. Among those with stage C disease, the following diseases were observed: *P. carinii* pneumonia ( $n = 18$  [42%]), esophageal candidiasis ( $n = 11$  [26%]), *Toxoplasma encephalitis* infection ( $n = 5$  [12%]), cryptococcal meningitis ( $n = 4$  [9%]), intestinal cryptosporidiosis ( $n = 3$  [7%]), Kaposi sarcoma ( $n = 2$  [5%]), AIDS dementia complex ( $n = 2$  [5%]), and disseminated cytomegalovirus disease ( $n = 1$  [2%]).

According to Kaplan–Meier curves showing progression to AIDS in HIV-1–infected persons, cumulative AIDS-free survival rates at 24 and 48 months, respectively, were 0.5 (95% CI, 0.3 to 0.6) and 0.4 (CI, 0.3 to 0.5) in the GBV-C–negative group and 0.9 (CI, 0.7 to 1.0) and 0.7 (CI, 0.5 to 0.9) in the GBV-C–positive group ( $P = 0.02$  for between-group comparisons). Mean AIDS-free survival time was 73 months (CI, 59 to 87 months) among GBV-C–positive persons and 45 months (CI, 35 to 54 months) among GBV-C–negative persons.

Table. Epidemiologic and Virologic Variables Measured at Baseline and at the End of Follow-up\*

Characteristic	Values in the GBV-C-Positive Group (n = 17)	Values in the GBV-C-Negative Group (n = 63)	P Value
Age at baseline, y	24.2 ± 1.6	24.8 ± 2.3	
Men/women at baseline, n/n	11/6	41/22	
Duration of HIV-1 seropositivity at baseline, mo	9.1 ± 1.5	9.3 ± 1.7	
Duration of intravenous drug use at baseline, mo	24.1 ± 1.7	24.3 ± 1.9	
Hepatitis C seropositivity at baseline, n (%)	8 (47)	30 (48)	
Hepatitis B seropositivity at baseline, n (%)	1 (6)	4 (6)	
ALT level at baseline, U/L	47.4 ± 32.6	53.0 ± 33.5	
Receiving therapy at baseline, n (%)	5 (29)	17 (27)	
Zidovudine, n	2	4	
Zidovudine + didanosine, n	3	13	
IL-2 level			
At baseline, pg/mL	157.3 ± 38.3	161.4 ± 30.6	
Change at end of follow-up ± SE, %	-8 ± 5	-85 ± 18	0.003
IL-12 level			
At baseline, pg/mL	100.2 ± 14.5	101.1 ± 21	
Change at end of follow-up ± SE, %	38 ± 7	-85 ± 2	0.001
IL-4 level			
At baseline, ng/mL	0.15 ± 0.05	0.14 ± 0.04	
Change at end of follow-up ± SE, %	33 ± 10	674 ± 29	0.002
IL-10 level			
At baseline, ng/mL	29.1 ± 13	28.2 ± 15	
Change at end of follow-up ± SE, %	16 ± 16	419 ± 28	0.003
CD4 <sup>+</sup> cell count			
At baseline, × 10 <sup>9</sup> cells/L	508 ± 44	462 ± 63	
Change at end of follow-up ± SE, %	-68 ± 2.5	-88 ± 1	0.041
Plasma HIV-1 RNA level			
At baseline, log <sub>10</sub> copies/mL	3.9 ± 0.2	4.1 ± 0.2	
Change at end of follow-up ± SE, %	26 ± 1.4	42 ± 0.8	0.033

\* Percentage variations from baseline to the end of follow-up were compared between the two groups by using the Mann-Whitney U test. Values presented with plus/minus signs are means ± SD unless otherwise indicated. ALT = alanine aminotransferase; GBV-C = GB virus C; IL = interleukin.

### Immunologic and Virologic Evaluations

The **Figure** shows the cross-sectional averages of IL levels, CD4<sup>+</sup> cell counts, and HIV RNA levels during the follow-up period. Annual plasma HIV-1 RNA levels significantly increased during follow-up in both the GBV-C-negative and GBV-C-positive groups, whereas CD4<sup>+</sup> cell counts significantly decreased ( $P < 0.01$  for all comparisons). In the GBV-C-negative group, IL-4 and IL-10 levels increased significantly from baseline ( $P = 0.01$  and  $P = 0.004$ , respectively) but IL-2 and IL-12 concentrations decreased significantly throughout the entire follow-up period ( $P = 0.005$  and  $P = 0.01$ , respectively). In contrast, in the GBV-C-positive group, none of the measured cytokine levels changed significantly during follow-up. The **Table** shows the percentage variation from baseline to the end of follow-up in plasma HIV-1 RNA levels, CD4<sup>+</sup> cell counts, and cytokine concentrations between the two groups.

### Response to HAART

In January 1997, all 80 patients began taking HAART. Among the 17 GBV-C-positive patients, 10 received zidovudine, lamivudine, and saquinavir and 7 received zidovudine, lamivudine, and indinavir. Among the 63 GBV-C-negative patients, 20 were treated with zidovudine, lamivudine, and saquinavir; 20 were treated with zidovudine, lamivudine, and indinavir; 13 were treated with zidovudine, didanosine, and indinavir; and 10 were

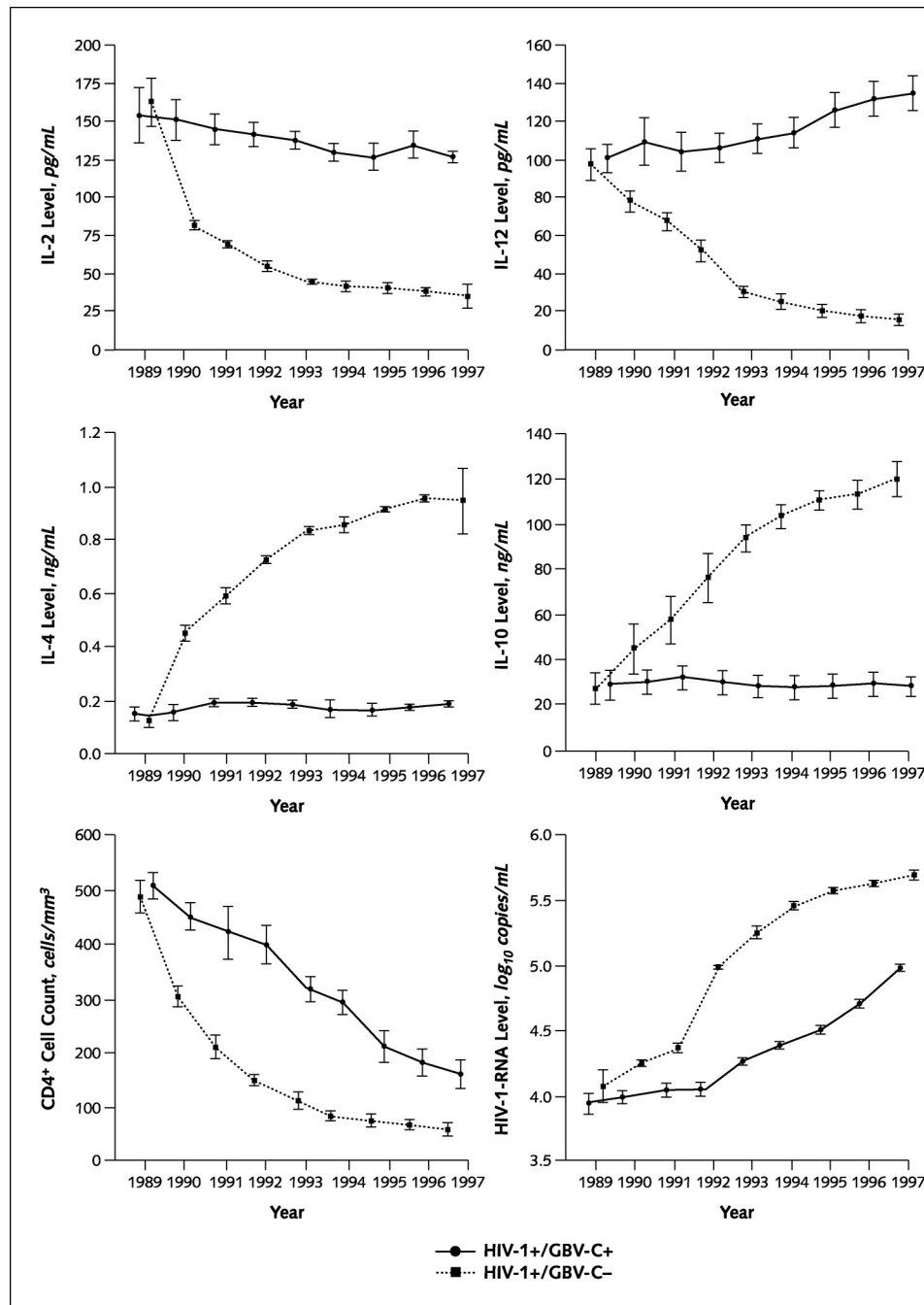
treated with zidovudine, lamivudine, and ritonavir. Fourteen patients, 2 in the GBV-C-positive group and 12 in the GBV-C-negative group, stopped taking HAART between 1997 and 2001 because of personal preference, lack of adherence, or severe side effects. Antiretroviral treatment was not restarted in any of these patients. The remaining 66 patients continued to receive HAART. During the remaining 4 years of follow-up, none of the GBV-C-negative patients became positive for GBV-C RNA or anti-E2 antibodies.

After 4 years of uninterrupted HAART, a significantly greater proportion of GBV-C-positive patients had a plasma HIV-1 viral load less than 400 copies/mL (11 of 15 [73%] vs. 20 of 51 [39%];  $P = 0.03$ ) and CD4<sup>+</sup> cell counts greater than  $350 \times 10^9$  cells/L (13 of 15 [87%] vs. 22 of 51 [43%];  $P = 0.01$ ) compared with GBV-C-negative patients. Moreover, significantly fewer treatment failures were observed among GBV-C-positive patients than among GBV-C-negative patients (1 of 15 [7%] vs. 23 of 51 [45%];  $P = 0.01$ ).

### DISCUSSION

In this 8-year longitudinal follow-up study, HIV-1-seropositive patients co-infected with GBV-C maintained an intact T-helper 1 cytokine profile, had lower plasma HIV-1 RNA levels, and had a better AIDS-free survival

Figure. Cross-sectional averages of interleukin (IL) levels, CD4<sup>+</sup> cell counts, and HIV-1 RNA levels in GB virus C (GBV-C)-positive and GBV-C-negative patients during 8 years of follow-up.



Comparison of GBV-C-positive and GBV-C-negative patients was based on each immunologic and virologic variable examined during follow-up (analysis of variance for repeated measures).  $P < 0.001$  for all variables except IL-2, for which  $P = 0.002$ .

rate than those not co-infected with GBV-C. Moreover, GBV-C viremia was associated with better survival even after HAART (10). We also found that in HIV-1-infected persons, co-infection with GBV-C may improve the efficacy of HAART and extend the rate of response to anti-retroviral therapy.

Our study points to a new pathogenic hypothesis that can be added to Xiang and colleagues' findings regarding

the anti-HIV-1 replicative activity of GBV-C (11). We found that patients infected with both HIV-1 and GBV-C had stable serum levels of T-helper 1 cytokines during follow-up but that in GBV-C-negative patients, T-helper 1 cytokine levels significantly decreased. In addition, serum levels of T-helper 2 cytokines (IL-4 and IL-10) progressively increased in the GBV-C-negative group while the GBV-C-positive group maintained an intact cytokine pro-

file. The importance of the T-helper response against HIV-1 is supported by numerous studies demonstrating that progression to AIDS is correlated with the inability of mononuclear cells to produce IL-2, IL-12, and interferon- $\gamma$  and with increased production of IL-4 and IL-10 (14).

It is unclear whether the inhibited expansion of T-helper 2 response and the predominant production of T-helper 1 cytokines in co-infected patients represent a cause or a consequence of delayed progression to AIDS. The recent evidence that CD4<sup>+</sup> cells may support GBV-C replication (15, 16) strengthens the hypothesis that GBV-C may interfere immunologically with HIV-1 infection. Nonetheless, whether GBV-C affects the polarization of T-helper cells is unknown. The relatively small number of patients in our study should be considered a possible limitation. Further studies are necessary to understand which molecular mechanism or mechanisms are involved in the interference observed during co-infection with HIV-1 and GBV-C.

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