Gender Susceptibility to Chronic Hepatitis C Virus Infection Associated with Interleukin 10 Promoter Polymorphism

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Elevated levels of interleukin 10 (IL-10) were previously described for chronically hepatitis C virus (HCV)-infected patients. We determined by a sequence-specific oligonucleotide probing technique the IL-10 promoter genotypes in 286 Argentinean HCV patients grouped according to disease outcome. The GG genotype (position −1082) is known to be associated with high IL-10 production, GA is considered an intermediate producer, and AA is associated with low IL-10 production. We found an increase in frequency of the GG genotype in female patients who do not eliminate the virus (RNA+). In these patients, the GG frequency was 0.19, versus 0.10 in controls (P = 0.03). This association became more significant in those RNA+ female patients with elevated hepatic transaminases (GG frequency of 0.25; P = 0.0013). Additionally, this genotype frequency was higher in noncirrhotic female patients than in controls (GG frequency for noncirrhotic female patients was 0.31; P = 0.009). In RNA− patients, the GA frequency was elevated compared with that in controls (GA frequency of 0.76 in RNA− patients versus 0.48 in controls; P = 0.01), that in all HCV patients (GA frequency of 0.43; P = 0.001), and that in RNA+ patients (GA frequency of 0.40; P = 0.0005). We conclude that a gender effect is observed with women carrying the GG high IL-10 producer genotype. The higher levels of IL-10 present in those individuals are associated with a higher risk of an inefficient clearance of the HCV and the development of a chronic HCV infection together with a lower risk of progression to cirrhosis in female patients.

Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease worldwide. About 60 to 80% of patients develop chronic infection, which may progress to cirrhosis and hepatocellular carcinoma (2, 26). The mechanisms whereby HCV causes acute liver injury and initiates the cascade of events leading to the establishment of persistent infection and development of chronic liver disease are not clearly established. Many factors, including age, gender, alcohol consumption, body mass index, steatosis, and human immunodeficiency virus (HIV) or hepatitis B virus (HBV) coinfection, affect disease outcome but are insufficient to explain it. Immunologic and genetic factors may play an important role (2).

A strong natural killer (NK) cell- and T helper 1 (Th1) cell-mediated immune response seems to be a key factor in the protection against HCV infection (9, 11). In addition, viral persistence and a deficient response to antiviral therapy have been associated with the production of inappropriate levels of cytokines (2). Interleukin 10 (IL-10) is a Th2 cytokine which down-regulates the Th1 effector mechanisms. Elevated serum levels of this cytokine have been observed to occur in patients with untreated chronic HCV infection (3). Moreover, in vitro production of IL-10 by peripheral blood mononuclear cells of chronically infected patients is higher than that observed with individuals showing a self-limited HCV infection (12, 31). This increase in IL-10 levels was also observed in a prospective study of patients with acute infection who developed a chronic disease (29).

Functional polymorphism was described for the IL-10 gene promoter. The single-nucleotide polymorphisms (SNP) at positions −1082 (G/A), −819 (C/T), and −592 (C/A) from the transcriptional start site are in linkage disequilibrium, and they are responsible for three different haplotypes: GCC, ACC, and ATA. There is a correlation between IL-10 genotype and cytokine production, i.e., ACC/ACC, ACC/ATA, and ATA/ATA (designated the AA genotype) are associated with low IL-10 production, GCC/ACC and GCC/ATA (GA genotype) are considered intermediate producers, and GCC/GCC (GG genotype) is considered a high producer (S). The −1082G allele, by having a lower binding affinity to the transcription factor PU.1, shows an increased transcriptional activity of the IL-10 promoter (27).

The present study was designed to retrospectively analyze the frequencies of IL-10 haplotypes and genotypes in anti-HCV-positive patients, taking into account the different outcomes of HCV infection.

MATERIALS AND METHODS

Patients. This retrospective study included 209 healthy controls (HC) and 286 anti-HCV-positive individuals (HCV patients) derived from the hepatology units of the Infectious Diseases Hospital F. J. Muñiz, Gastroenterology Hospital Dr. C. Bonorino Udaondo, and Buenos Aires Italian Hospital, Buenos Aires, Argentina. The ethnicity of this population is known as Latin American Caucasoid.

The clinical features of HCV patients included in this study are described in Table 1. All individuals showed the presence of anti-HCV antibodies detected by a third-generation enzyme-linked immunosorbent assay (version 4.0; Abbott-
null
SSPE 2× for 10 min at room temperature. After 5 min of washing in buffer 1 (0.1 M Tris, 0.15 M NaCl, pH 7.5), the membranes were blocked to reduce nonspecific binding in buffer 1 containing 1% nonfat milk (buffer 2) and incubated with 1/10,000 alkaline phosphatase-conjugated antidigoxigenin antibody (Roche Diagnostics, Indianapolis, Ind.) in buffer 2 for 30 min at room temperature. The membranes were washed twice in buffer 1 (10 min each wash) and once in buffer 3 (0.1 M Tris, 0.05 M MgCl2, 0.1 M NaCl, pH 9.5; 5 min), incubated with 25 mM chemiluminescent substrate CSPD (Roche Diagnostics, Indianapolis, Ind.) in buffer 3 for 20 s at room temperature, and stored for 15 min at 37°C. X-ray films were exposed for 30 min before being developed. Allele-specific probes were used to determine each SNP genotype, and the IL-10 promoter haplotypes were inferred after compound analysis of the three SNP results.

**Statistical analysis.** We compared haplotype and genotype frequencies between the groups of patients and controls with the chi-square test for independence for 3 by 2 contingency tables (GG versus GA versus AA) or the two-sided Fisher exact test for 2 by 2 tables (GG versus non-GG and GA versus non-GA). P values of <0.05 were considered statistically significant, P values of <0.01 very significant, and P values of <0.001 extremely significant. Bonferroni’s correction (p) was applied to P values obtained by the Fisher test (three groups). The odds ratio (OR) with a 95% confidence interval (CI) was calculated to evaluate the relative risk in each patient group.

**RESULTS**

Haplotype and genotype frequencies of IL-10 promoter in different populations. Ethnic differences in the haplotype and genotype frequencies within the IL-10 promoter have been reported previously (8, 18, 25). The genotype frequencies of GG, GA, and AA observed in the Argentinean population were quite similar to the frequencies found in the Brazilian Caucasian population (23). However, as depicted in Table 2, these frequencies differed from those previously reported for other populations. For example, in England and Australia the GCC haplotype is present in approximately 50% of white individuals (5, 13). With Norwegian, North American, and Italian individuals, 16 to 26% of the population is homozygous for this haplotype (14, 15, 19, 30). On the other hand, in Japan, the GCC haplotype is less frequent (4 to 6%) and the GG homozygous genotype was not found in any control individual (7, 8, 17).

**TABLE 4. Frequencies of IL-10 promoter genotypes according to outcome of HCV infection**

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of controls or patients</th>
<th>Genotype frequency</th>
<th>Groups compared</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
</tr>
<tr>
<td>HC</td>
<td>209</td>
<td>0.10</td>
<td>0.48</td>
<td>0.42</td>
</tr>
<tr>
<td>HCV patients</td>
<td>286</td>
<td>0.15</td>
<td>0.43</td>
<td>0.42</td>
</tr>
<tr>
<td>HCV RNA⁺</td>
<td>25</td>
<td>0.04</td>
<td>0.76</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>261</td>
<td>0.16</td>
<td>0.40</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>0.19</td>
<td>0.31</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>154</td>
<td>0.14</td>
<td>0.46</td>
<td>0.40</td>
</tr>
<tr>
<td>HCV RNA⁺ normalALT</td>
<td>61</td>
<td>0.13</td>
<td>0.32</td>
<td>0.55</td>
</tr>
<tr>
<td>HCV RNA⁺ elevatedALT</td>
<td>200</td>
<td>0.19</td>
<td>0.42</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>0.25</td>
<td>0.30</td>
<td>0.45</td>
</tr>
<tr>
<td></td>
<td>126</td>
<td>0.16</td>
<td>0.49</td>
<td>0.36</td>
</tr>
</tbody>
</table>

a GG, GCC/GCC frequency; GA, GCC/ACC plus GCC/ATA frequencies; AA, ACC/ACC plus ACC/ATA plus ATA/ATA frequencies.

b GG versus GA versus AA genotypes were compared by the chi-square test.
decrease of GA frequencies in the same group of female patients \((P = 0.03, \rho_c = 0.09, OR = 0.9, CI = 0.4 \text{ to } 0.95)\) and a tendency to an increase of the GG frequency \((0.17 \text{ in female HCV patients versus } 0.10 \text{ in controls})\).

We found no differences in the IL-10 promoter haplotype and genotype frequencies according to viral genotype or age of HCV patients (data not shown).

In our control population, the IL-10 promoter haplotype and genotype frequencies showed no differences between males and females (Table 3). Therefore, all subsequent comparisons involving female or male patients were made with the total number of control individuals (males plus females).

**Gender effect on IL-10 promoter frequency for those HCV patients who do not eliminate the virus \((\text{RNA}^+)\).** The genotype frequencies \((\text{GG/GA/AA})\) showed significant differences when we compared \(\text{RNA}^+\) female patients either with controls \((P = 0.004)\) or with male \(\text{RNA}^+\) patients \((P = 0.04)\) (Table 4). These frequencies showed an even stronger significance in those \(\text{RNA}^+\) female patients with elevated levels of ALT in comparison with controls \((P = 0.0006)\) or with males who also had elevated levels of ALT \((P = 0.028)\) (Table 4).

The analysis of GG versus non-GG genotypes showed a significant increase of GG frequency in \(\text{RNA}^+\) female patients compared with that in controls \((P = 0.03, \rho_c = 0.09, OR = 2.2, CI = 1.1 \text{ to } 4.2)\). This comparison became even more significant in \(\text{RNA}^+\) female patients with elevated ALT versus controls \((P = 0.0013, \rho_c = 0.039, OR = 3.3, CI = 1.6 \text{ to } 6.5)\) (Fig. 1). This increase in GG frequency of \(\text{RNA}^+\) female patients was also accompanied by a decrease in the GA frequency compared with that for controls \((P = 0.004, \rho_c = 0.012, OR = 0.5, CI = 0.3 \text{ to } 0.8)\) or with that for \(\text{RNA}^-\) male patients \((P = 0.015, \rho_c = 0.045, OR = 0.5, CI = 0.3 \text{ to } 0.9)\) (Fig. 1).

**IL-10 promoter frequencies in self-limited HCV infection \((\text{RNA}^+)\).** In patients with self-limited HCV infection, the genotype distributions were significantly different when patients who had cleared the virus were compared with controls \((P = 0.03)\), with all HCV patients \((P = 0.005)\), or with \(\text{RNA}^+\) HCV patients \((P = 0.002)\) (Table 4). As depicted in Fig. 2, this difference was caused by an increase in the frequency of \(\text{GA}^-\) versus \(\text{HC}\) \((P = 0.01, \rho_c = 0.03, OR = 3.1, CI = 0.1 \text{ to } 76)\); \(\text{RNA}^-\) versus total HCV patients, \(P = 0.001, \rho_c = 0.003, OR = 2.3, CI = 0.09 \text{ to } 0.26\); \(\text{RNA}^+\) versus RNA \(^+\), \(P = 0.0005, \rho_c = 0.0015, OR = 0.2, CI = 0.08 \text{ to } 0.5\).

In females, the high IL-10 producer genotype \((\text{GG})\) seems to be associated with an antircirrhotic effect. We next addressed the association of IL-10 promoter genotypes and the development of cirrhosis. As depicted in Table 5, the genotype frequencies \((\text{GG/GA/AA})\) in cirrhotic patients are quite similar to those in controls. Again, a significant difference was present only when we evaluated these frequencies in noncirrhotic female patients in comparison with frequencies either in controls \((P = 0.003)\) or in male noncirrhotic patients \((P = 0.009)\).

Further analysis of genotype combinations confirmed the increase of the GG frequency in female noncirrhotic patients \((P = 0.009, \rho_c = 0.027, OR = 0.3, CI = 0.15 \text{ to } 0.7)\), indicative of the antifibrotic role of the high IL-10 producer genotype (Fig. 3). In this group of patients, we found that the increase in GG frequency was also accompanied by a decrease in GA frequency, in comparison with results for controls \((P = 0.009, \rho_c = 0.027, OR = 2.4, CI = 1.3 \text{ to } 4.7)\) and for male noncirrhotic patients \((P = 0.003, \rho_c = 0.009, OR = 3, CI = 1.4 \text{ to } 6.2)\) (Fig. 3).

It is known that the age at which a patient became infected influences the outcome of HCV infection. Thus, we analyzed the IL-10 promoter genotypes according to age/gender and development of fibrosis (Table 6). Differences in the genotype frequencies were observed for noncirrhotic female patients whose age was above 40 years old either versus controls \((P = 0.015, \rho_c = 0.045, OR = 0.5, CI = 0.3 \text{ to } 0.9)\) (Fig. 1).
0.0026) or versus males with the same features (P = 0.02). These differences were caused by an increase of the GG frequency (for noncirrhotic females of >40 years versus HC, P = 0.007, p<sub>c</sub> = 0.021, OR = 0.29, and CI = 0.13 to 0.66; for noncirrhotic females of >40 years versus noncirrhotic males of >40 years, P = 0.056) and a decrease of GA frequency in those female patients (for noncirrhotic females of >40 years versus HC, P = 0.015, p<sub>c</sub> = 0.045, OR = 2.5, and CI = 1.2 to 5.4; for noncirrhotic females of >40 years versus noncirrhotic males of >40 years, P = 0.02, p<sub>c</sub> = 0.06, OR = 2.9, and CI = 1.2 to 6.9).

Frequencies found in female patients less than 40 years old did not prove to be significantly different from those of the controls, but the number of patients included in the former group was smaller.

We also analyzed the IL-10 promoter polymorphism in 55 patients with chronic pulmonary disease (Table 3) and 102 patients with celiac disease (data not shown). The IL-10 promoter haplotype and genotype frequencies in these samples resulted in no significant differences from frequencies in HC samples.

### DISCUSSION

The present study demonstrated an increase in the GG frequency as well as a decrease of the GA frequency in female HCV patients, in particular, in those RNA<sup>+</sup> patients with elevated levels of ALT. Additionally, we found an increased frequency of the GA genotype in self-limited HCV infection.

In spite of ethnic differences, our results are in line with similar findings reported by the Mayo Clinic (30) and by an extensive study performed in the United Kingdom (10). These studies have also found that the GG frequency increased in patients with chronic HCV infection. Also in accordance with our report, the ATA haplotype (15) and ATA/ATA and −1082A/G genotypes (10) were found to be associated with the self-limited infection.

This study addressed for the first time the gender effect in the association between IL-10 promoter polymorphism and the HCV infection. Our understanding is that the gender effect described in the present study might explain many contradictory reports regarding the effect of the IL-10 promoter associ-
ated with HCV infection. As examples of these contradictory results, Edwards-Smith et al. (5) found no significant differences in genotype frequencies including a gender effect, but the small number of patients analyzed might preclude detection of this effect. Along the same lines, additional reports were unable to confirm differences in the haplotype or genotype frequencies between patients and controls (15, 16, 24). However, those studies failed to analyze a gender effect or also were based on a small number of cases. On the other hand, Lio et al., whose study was based on data from only 60 HCV patients (18 RNA + and 42 RNA − patients), reported that the GG genotype was associated with a self-limited HCV infection (14).

A vigorous CD4 + and CD8 + T-cell response with a predominant Th1 cytokine profile seems to be responsible for recovery from an HCV infection (4, 11). Conversely, patients who develop a chronic infection show a predominant Th2 response that down-regulates the Th1 response and therefore favors persistent HCV infection (6, 26). In this context, it is tempting to speculate that those individuals carrying the high IL-10 producer genotype (GG) will be prone to down-regulate the Th1 response, resulting in a failure of the HCV clearance.

Experimental and clinical data suggest a protective role of IL-10 in hepatic fibrogenesis (20, 28). Accordingly, we found that the increased frequency of GG was observed only with noncirrhotic female patients, mainly in those with stages 1 and 2 of liver fibrosis (not shown), but not in patients with stage 4 (cirrhosis). A study of Japanese chronically HCV-infected patients reported the GCC haplotype to be associated with less hepatic fibrosis. In line with our findings, this Japanese study suggests that high production of IL-10 may cause inhibition of liver fibrosis progression (7).

Chromically HCV-infected patients who received a short treatment with recombinant IL-10 showed a decreased hepatic inflammation and reduced liver fibrosis (20). On the other hand, and in concordance with our findings, a 12-month IL-10 therapy in patients with advanced fibrosis led to increased levels of serum HCV RNA and a reduction in fibrosis score (21), suggesting that high levels of IL-10 not only decrease fibrogenesis but also lead to an increased HCV viral burden. This could be achieved by decreasing the number of HCV-specific CD4 + and CD8 + gamma interferon-secreting T cells and polarizing the immune response towards a Th2-dominant profile.

Similarly, it has been published that the antibody-induced blockage of the IL-10 receptor generates a favorable balance of CD4 + T-cell response to HCV. Also, this anti-IL-10 receptor reverses the inhibitory effect of IL-10 on HCV-specific T-cell proliferation, demonstrating the major role of IL-10 in suppressing antiviral T-cell responses (28). Moreover, clinical evidence suggests that individuals with cellular immune dysfunction, such as that due to HIV infection, show a much faster disease progression (11).

In conclusion, the present study confirms that the host’s genetic background plays a significant role in the outcome of HCV infection. In particular, we demonstrate a gender effect associated with the susceptibility to develop a persistent HCV infection and a chronic liver disease together with an inhibition of fibrogenic process in women carrying the GG IL-10 promoter genotype.


