Association between human leukocyte antigen (HLA) and interferon-α induced thyroid diseases in four patients with HCV-related chronic hepatitis

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Abstract

OBJECTIVES: The interferon-α (IFN-α) therapy for HCV hepatitis may exacerbate or induce underlying thyroid disorders. Besides viral factors, the human leukocyte antigen (HLA) may be an independent risk factor.

METHODS: We evaluated fifteen patients with HCV chronic hepatitis during a period of 40 months. At the enrolment, all the patients were negative for thyroid disorders, excluding one patient with subclinical hypothyroidism. Eleven patients received IFN-α therapy. The HLA system was examined in every patient, evaluating antigens (n=40) of locus A, B and Cw and alleles (n=19) of locus DRB1* and DQB1*. The HLA system was also examined in healthy subjects (n=107) as a control group.

RESULTS: The HCV genotype distribution in patients was: 1b=20%, 2a=60%, 3a=20%. Four IFN-treated patients presented clinical thyroid disorders, including autoimmune hypothyroidism (n=2), transient thyrotoxicosis (n=1) and subacute thyroiditis (n=1). The HLA susceptibility to thyroid disorders (antigen/allele frequency) in the whole group of patients was not different in respect to controls and normal Italian population.

The patients with HCV chronic hepatitis that developed thyroid diseases after IFN-α treatment had a double and specific association with the HLA system (Mantel-Haenszel X²=4.706, p<0.05).

CONCLUSIONS: This case report suggests that HLA system examination is an important and promising diagnostic aspect that may be considered in order to evaluate the appearance of thyroid disorders during the IFN-α treatment for HCV-related chronic hepatitis.
Abbreviations

Abs: autoantibodies  
AIDT: autoimmune thyroid disease  
ANOVA: one-way analysis of variance  
Anti-Tg Abs: anti-thyroglobulin autoantibodies  
Anti-TPO Abs: anti-thyroidperoxidase autoantibodies  
Anti-TSHr Abs: anti-TSH receptor autoantibodies  
FNA: fine needle aspiration  
HCV: hepatitis C virus  
HLA: human leukocyte antigen  
IFN-α: interferon-α  
IRMA: immunoradiometric assay  
McF ratio: male:female ratio  
Ms: months  
Nr: normal range  
Ns: not significant  
PCR: polymerase-chain reaction  
RIA: radioimmunoassay  
SD: standard deviation  
TRAK: radioreceptor antibody assay

Introduction

Chronic hepatitis C virus (HCV) infection may be associated with thyroid autoimmunity and dysfunction, with a mean incidence of 10% and 3% respectively (hypothyroidism: hyperthyroidism ratio approaching 2:1). The interferon-α (IFN-α) therapy for HCV-related chronic hepatitis may exacerbate or induce the underlying latent thyroid disorders, increasing the incidence of thyroid autoimmunity and dysfunction to 20% and 11% respectively [2, 10–12].

Age, female gender and pre-existing positive anti-thyroid Abs are well known risk factors for the development of thyroid diseases induced by IFN-α therapy in HCV patients [2]. The human leukocyte antigen (HLA) may be an independent risk factor either for the liver and the thyroid diseases, suggesting a genetic predisposition to the immune-mediated organ damage [6, 8].

The aim of this case report was to evaluate the association between the HLA system and the development of thyroid diseases in patients with chronic hepatitis C treated with IFN-α.

Materials and methods

Fifteen patients (10 females and 5 males, 38–74 years of age) with chronic hepatitis C were enrolled. The diagnosis of chronic hepatitis C was based on the findings of the serum alanine aminotransferase levels, the liver biopsy, the positive anti-HCV Abs and HCV-RNA (evaluated by the polymerase-chain reaction, PCR, analysis); the genotype of the virus was determined by TRAK assay (nr <10%, Bioline, Belgium).

Eleven patients were treated with recombinant human IFN-α 2a (3–6 x10⁶ IU im three times a week). The treatment was not started in the other four patients because of not compliance to the therapy (patients n.12 and n.14), normal alanine aminotransferase levels (patient n.13) and refusal of the treatment (patient n.15) (Table 1).

The HLA system was examined, evaluating the antigens (n=40) of the locus A, B and Cw (by the complement-dependent lymphocyte cytotoxicity test) and the alleles (n=19) of the locus DRB1* and DQB1* (by the PCR method with the technique of sequence-specific primers, SSP). It is noteworthy that the HLA-DR3 and the HLA-DR5 antigens correspond to the HLA-DRB1.03 and to the HLA-DRB1.11/HLA-DRB1.12 alleles, respectively. The association between the HLA and thyroid disorders during the IFN-α treatment was evaluated, and, in particular:

a) HLA-A2 and thyroid disorders (with clinical features of either thyrotoxicosis/hyperthyroidism or hypothyroidism) during IFN-α therapy for chronic hepatitis C [6];

b) HLA-B35 and the immune-mediated subacute thyroiditis [9];

c) HLA-DR3 and the autoimmune thyrotoxicosis/hyperthyroidism [7];

d) HLA-DR5 and the autoimmune hypothyroidism [3].

The antigen/allele HLA frequency was also evaluated in healthy subjects (n=107) as a control group. All the patients and controls gave their informed consent to the study.

Statistical analysis was performed by the one-way analysis of variance (ANOVA) and by the X²-test, after the correction by the Mantel-Haenszel method.

Results

The patients mean age were 58.73±11.61 y (± standard deviation, SD), the male:female (M:F) ratio was 1:2. The HCV genotype analysis showed 1b=20%, 2a=60%, 3a=20%; no patient presented a mixed HCV genotype. Eleven patients received IFN-α 3–6x10⁶ IU im three times a week; duration of treatment: 3–12 months, ms; follow-up after IFN therapy: 18–42 ms). Four patients (n.12–15) entered the follow-up without receiving the treatment (follow-up: 12–38 ms). All patients were negative for AIDT at the baseline, excluding patient n.7 presenting a clinically inapparent autoimmune hypothyroidism at the first screening; she started substitutive levo-T4 therapy and then she received IFN-α.
The HLA antigens and alleles of the patients are presented in table 1A. The frequency of the HLA antigen/allele associated with susceptibility to the thyroid disorders in the whole group of patients was not significantly (ns) different in respect to controls (HLA-A2 0.266 and 0.238, –B35 0.200 and 0.159, –DRB1.03 0.133 and 0.098, –DRB1.11 0.200 and 0.260, respectively, ns). Six patients presented a double positive result (HLA-A2/B35, HLA-A2/DRB1.03, HLA-A2/DRB1.11, HLA-B35/DRB1.11). Five patients with double positive HLA received IFN-α treatment. Four IFN-treated patients developed AITD, including autoimmune hypothyroidism (n.1 and n.6), transient thyrotoxicosis in autoimmune hypothyroidism before the treatment (n.7) and subacute thyroiditis (n.4).

In particular, patient n.1 developed Abs up to more than 5000 U/l (anti-Tg) and 174 U/l (anti-TPO) after the beginning of IFN-α therapy. Five ms after IFN-α suspension, the thyroid function tests showed a subclinical hypothyroidism (TSH 8.2 µU/ml). In patient n.6, twenty ms after IFN-α therapy, anti-Tg and anti-TPO Abs raised (3400 U/l and 1200 U/l, respectively) and an overt hypothyroidism appeared (TSH 36.3 µU/ml and FT4 0.59 ng/dl). The ultrasonographic scanning showed a highly dishomogeneous structure in both the patients (n.1, n.6). Levo-T4 substitutive therapy successfully normalized the thyroid function. In patient n.4, a typical pain in the anterior neck developed four ms after IFN-α therapy, as it occurs in the De Quervain's thyroiditis. A radioiodine scanning with the almost complete absence of radioiodine uptake and FNA findings with the typical giant cells confirmed the diagnosis. The IFN-α treatment was suspended and the thyroid function returned to normal levels after a transient phase of recovery treated with levo-T4.

Patients were considered with susceptible HLA antigens/alleles if presenting the following associations: HLA-A2/B35, HLA-A2/DRB1.03, HLA-A2/DRB1.11, HLA-B35/DRB1.11.

### Table 1A. Human leukocyte antigen (HLA) in patients with HCV chronic hepatitis (n=15) (1A) and the association with thyroid disorders in the IFN-α treated patients with HCV-related chronic hepatitis (1B).

<table>
<thead>
<tr>
<th>Patients</th>
<th>HLA-A Antigen</th>
<th>HLA-B Antigen</th>
<th>HLA-C Antigen</th>
<th>HLA-DR Allele</th>
<th>HLA-DQ Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.1</td>
<td>A24/A28</td>
<td>B14/B35</td>
<td>Cw4/–</td>
<td>DRB1.11/DRB1.01</td>
<td>DQB1.05/DQB1.06</td>
</tr>
<tr>
<td>n.2</td>
<td>A2/A26</td>
<td>B51/B22</td>
<td>Cw1/Cw3</td>
<td>DRB1.04/DRB1.13</td>
<td>DQB1.03/DQB1.06</td>
</tr>
<tr>
<td>n.3</td>
<td>A2/A31</td>
<td>B35/B50</td>
<td>Cw4/Cw6</td>
<td>DRB1.15/DRB1.07</td>
<td>DQB1.06/DQB1.02</td>
</tr>
<tr>
<td>n.4</td>
<td>A1/A2</td>
<td>B35/B44</td>
<td>Cw4/–</td>
<td>DRB1.01/DRB1.15</td>
<td>DQB1.05/–</td>
</tr>
<tr>
<td>n.5</td>
<td>A23/A11</td>
<td>B35/B17</td>
<td>Cw4/Cw7</td>
<td>DRB1.08/DRB1.14</td>
<td>DQB1.05/DQB1.04</td>
</tr>
<tr>
<td>n.6</td>
<td>A1/A2</td>
<td>B18/B80</td>
<td>Cw7/–</td>
<td>DRB1.03/DRB1.11</td>
<td>DQB1.03/–</td>
</tr>
<tr>
<td>n.7</td>
<td>A30/A2</td>
<td>B7/B53</td>
<td>Cw4/Cw7</td>
<td>DRB1.03/DRB1.13</td>
<td>DQB1.02/DQB1.06</td>
</tr>
<tr>
<td>n.8</td>
<td>A2/A3</td>
<td>B7/B44</td>
<td>Cw7/–</td>
<td>DRB1.15/DRB1.07</td>
<td>DQB1.06/DQB1.02</td>
</tr>
<tr>
<td>n.9</td>
<td>A2/A24</td>
<td>B44/B62</td>
<td>Cw3/Cw5</td>
<td>DRB1.07/DRB1.13</td>
<td>DQB1.02/DQB1.06</td>
</tr>
<tr>
<td>n.10</td>
<td>A32/A33</td>
<td>B14/B18</td>
<td>Cw7/–</td>
<td>DRB1.11/DRB1.01</td>
<td>DQB1.05/DQB1.03</td>
</tr>
<tr>
<td>n.11</td>
<td>A24/–</td>
<td>B45/B50</td>
<td>Cw6/Cw7</td>
<td>DRB1.03/DRB1.11</td>
<td>DQB1.02/DQB1.03</td>
</tr>
<tr>
<td>n.12</td>
<td>A24/A11</td>
<td>B44/B22</td>
<td>Cw1/Cw7</td>
<td>DRB1.11/–</td>
<td>DQB1.03/–</td>
</tr>
<tr>
<td>n.13</td>
<td>A1/A3</td>
<td>B35/B52</td>
<td>Cw4/–</td>
<td>DRB1.15/DRB1.13</td>
<td>DQB1.06/–</td>
</tr>
<tr>
<td>n.14</td>
<td>A1/A24</td>
<td>B35/B44</td>
<td>Cw4/–</td>
<td>DRB1.04/DRB1.07</td>
<td>DQB1.02/DQB1.03</td>
</tr>
<tr>
<td>n.15</td>
<td>A2/A32</td>
<td>B57/B70</td>
<td>Cw6/Cw7</td>
<td>DRB1.03/DRB1.01</td>
<td>DQB1.05/DQB1.03</td>
</tr>
</tbody>
</table>

HLA-DRB1.03 and DRB1.11/DRB1.12 alleles correspond to DR3 and DR5 antigens, respectively.

### Table 1B. HCV patients that developed thyroid disorders HCV patients that did not develop thyroid disorders Total HCV patients

<table>
<thead>
<tr>
<th>With HLA</th>
<th>Without HLA</th>
<th>Total</th>
<th>With HLA</th>
<th>Without HLA</th>
<th>Total</th>
<th>With HLA</th>
<th>Without HLA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFNα-treated patients</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>IFNα-untreated patients</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>9</td>
<td>11</td>
<td>6</td>
<td>9</td>
</tr>
</tbody>
</table>

Corrected $X^2 = 4.076, p<0.05$

Patients were considered with susceptible HLA antigens/alleles if presenting the following associations: HLA-A2/B35, HLA-A2/DRB1.03, HLA-A2/DRB1.11, HLA-B35/DRB1.11.
HCV genotype did not reveal significant association.

**Discussion**

The results of this study showed that the IFN-a treatment induced the development of thyroid disorders in 33% of the patients with pre-treatment negative anti-thyroid Abs. Evidence in the literature suggested that both the environmental (viral agents, IFN-a) and the genetic (susceptibility genes) factors may be involved in the immune-mediated pathogenesis of thyroid diseases [2]. Our patients presenting thyroid diseases during the IFN-a therapy were females, with a mean age of 55.2 y. The HCV infection was due to different genotypes, including a specific association with the thyroid disorder: n.1 B35/DRB1.11, n.4 A2/B35, n.6 A2/DRB1.11, n.7 A2/DRB1.03. The HLA-A2 was not specific for the kind of the disorder, being present in hypothyroidism, in thyrotoxicosis as well as in thyroiditis. Applying the Mantel-Haenszel method, the development of AITD in IFN-a treated patients was significantly associated with the double positive HLA (corrected $X^2$=4.706, p<0.05) (Table 1B). With the same procedure, the analysis of the relationship between the development of AITD and HCV genotype did not reveal significant association.

Our results suggest that HLA system is a susceptibility factor to the development of AITD, in patients presenting two antigens together. The double association seems to increase, exponentially, the risk of thyroid disorders in the IFN-a treated HCV patients rather than to have a mild additional effect. In the paper of Kakizaki et al. [6] and in our results, the HLA-A2 antigen was not specific for the kind of the thyroid disorder; in fact, it was associated with cases of either hypothyroidism, thyrotoxicosis and thyroiditis. A cytotoxic T lymphocyte-mediated mechanism may be involved in the recognition and response against I class HLA-restricted viral as well as cross-reacting self peptides [1]. The HLA-B35, DR3 and DR5 antigens could play a different and more relevant role in the IFN-a induced thyroid alterations, being specifically associated with the kind of thyroid disorders (B35 and subacute thyroiditis, DR3 and hyperthyroidism/ thyrotoxicosis, DR5 and hypothyroidism) [3, 6, 7, 9].

In conclusion, our case report suggests that the HLA system examination may be an important and promising diagnostic aspect that may be considered in order to evaluate the appearance of thyroid disorders during the IFN-a treatment for HCV-related chronic hepatitis. Further studies in larger groups of patients are necessary to confirm the predictive value of the HLA system typing at the basal assessment of the patients on the development of thyroid gland diseases.

**REFERENCES**