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CASE REPORT

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 X_c^2=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
AITD:	autoimmune thyroid disease
ANOVA:	one-way analysis of variance
Anti-Tg Abs:	anti-thyroglobulin autoantibodies
Anti-TPO Abs:	anti-thyroperoxidase 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
M:F 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 antithyroid 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. The thyroid function tests (serum TSH, FT3 and FT4 concentrations), the anti-thyroperoxidase (anti-TPO), anti-thyroglobulin (anti-Tg) and anti-TSH receptor (anti-TSHr) antibodies (Abs) were monitored at the baseline of the study (before IFN-α therapy) and, subsequently, every three months. The thyroid ultrasonographic scan were obtained at the baseline (before IFN-α therapy) and at the end of the study. Serum FT3 and FT4 concentrations were measured by RIA (normal ranges, nr, 1.8–5.6 pg/ml and 0.7–1.8 ng/dl, respectively, Ares-Serono, Italy). TSH levels was determined by IRMA (nr 0.25–4.0 μU/ml, Ares-Serono). Anti-Tg and anti-TPO Abs were detected by RIA (nr <150 U/l and <100 U/l, respectively) and anti-TSHr Abs were determined by TRAK assay (nr <10%, Bioline, Belgium).

Eleven patients were treated with recombinant human IFN-α 2a (3–6 x10^6 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) (Table1).

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 X^2-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 (3–6×10^6 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 AITD 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.

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).**

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

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		
	With HLA	Without HLA	Total	With HLA	Without HLA	Total	With HLA	Without HLA	Total
Groups	A	B	N1	C	D	N2	M1	M2	T
IFNα-treated patients	4	0	4	1	6	7	5	6	11
IFNα-untreated patients	0	0	0	1	3	4	1	3	4
Total	4	0	4	2	9	11	6	9	15

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/DRB1.115, HLA-B35/DRB1.11.

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/B-35, –A2/DRB1.03, –A2/DRB1.11 or –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-a 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-a treatment was suspended and the thyroid function returned to normal levels after a transient phase of recovery treated with levo-T4. In patient n.7, before IFN-a therapy, the thyroid function test showed a pattern of mild primary hypothyroidism (TSH 4.5 μU/ml and FT4 0.7 ng/dl), not clinically evidenced before. The thyroid volume was at the lower limit by the ultrasonographic scanning, without any modification of the glandular pattern. The anti-TPO Abs were only moderately increased (361 U/l). The levo-T4 therapy was started aiming to normalize the thyroid function, and five ms later she received IFN-a. After three ms, the patient showed clinical and laboratory signs of thyrotoxicosis (anti-TPO Abs 1426

U/l, anti-Tg Abs 96 U/l, anti-TSHr Abs 6%, TSH 0.01 μU/ml, FT3 8.1 pg/ml and FT4 25.4 ng/dl). The IFN-a and the l-T4 therapy was suspended. Abs levels began to reduce after two ms; the thyroid function test and Abs normalized after twelve ms.

The mean age (± SD) of the patients with AITD was 55.2±8.88 y (not significantly different in respect to the patients without AITD), all were females. These patients presented a double positive HLA susceptibility, 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.

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 (HCV genotype distribution 1b:2a:3a=1:2:1). Previous studies in IFN-a treated HCV patients showed an association with the female gender, the older age, pre-existing positive anti-thyroid Abs and the mixed genotype infection [2, 10, 11].

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

1 Brazillet MP, Batteux F, Abehsira-Amar O, Nicoletti F, Charreire J. Induction of experimental autoimmune thyroiditis by heat-denatured porcine thyroglobulin: a CTL-mediated disease. Eur J Immunol 1999; **29**:1342–1352.

2 Broussolle C, Steineur MP, Bailly F, Zoulim F, Trepo C. Hepatitis C virus infection and thyroid diseases. Rev Med Interne 1999; **20**:766–773.

3 Farid NR, Sampson L, Moens H, Barnard JM. The association of goitrous autoimmune thyroiditis with HLA-DR5. Tissue Antig 1981; **17**:265–268.

4 Fernandez-Soto L, Gonzalez A, Escobar-Jimenez F, Vazquez R, Ocete E, Olea N et al. Increased risk of autoimmune thyroid disease in hepatitis C vs hepatitis B before, during, and after discontinuing interferon therapy. Arch Intern Med 1998; **158**: 1445–1448.

5 Ganne-Carrie N, Medini A, Coderc E, Seror O, Christidis C, Grimbert S et al. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. J Autoimmun 2000; **14**:189–193.

6 Kakizaki S, Takagi H, Murakami M, Takayama H, Mori M. HLA antigens in patients with interferon-a-induced autoimmune thyroid disorders in chronic hepatitis C. J Hepatol 1999; **30**: 794–800.

7 Kinney JS, Hurwitz ES, Fishbein DB, Woolf PD, Pinsky PF, Lawrence DN. Community outbreak of thyrotoxicosis: epidemiology, immunogenetic characteristics, and long term outcome. Am J Med 1988; **84**:10–18.

8 Martocchia A, Labbadia G, Paoletti V, Gargano S, Grossi A, Trabace S et al. Hashimoto's disease during interferon-alpha therapy in a patient with pre-treatment negative anti-thyroid autoantibodies and with the specific genetic susceptibility to the thyroid disease. Neuroendocrinol Lett 2001; **22**:49–52.

9 Ohsako N, Tamai H, Sudo T, Mukuta T, Tanaka H, Kuma K et al. Clinical characteristics of subacute thyroiditis classified according to human leukocyte antigen typing. J Clin Endocrinol Metab 1995; **80**:3653–3656.

10 Oppenheim Y, Ban Y, Tomer Y. Interferon induced Autoimmune Thyroid Disease (AITD): a model for human autoimmunity. Autoimmun Rev 2004; **3**:388–393.

11 Prummel MF, Laurberg P. Interferon-alpha and autoimmune thyroid disease. Thyroid 2003; **13**:547–551.

12 Rocco A, Gargano S, Provenzano A, Nardone M, De Sanctis GM, Altavilla N et al. Incidence of autoimmune thyroiditis in interferon-alpha treated and untreated patients with chronic hepatitis C virus infection. Neuroendocrinol Lett 2001; **22**:39–44.