Research Article

Hypothyroidism in Noninterferon Treated-HCV Infected Individuals Is Associated with Abnormalities in the Regulation of Th17 Cells

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Received 11 August 2009; Revised 18 December 2009; Accepted 15 February 2010

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HCV-Ag-specific TH17 cells secrete IL17, a cytokine involved in autoimmune diseases and regulated by IL10 and TGF-b. 5–12% of patients with chronic HCV infection have hypothyroidism. We evaluated the role of these cytokines in this patients by determining serum concentration of TSH, T3, free T4, IL2, IL10, IL12, IL17, TGF-b, anti-TG, TPO, CCP, GBM, and cardiolipin antibodies in 87 chronically noninterferon treated HCV-infected patients. 20 patients (group A) had elevated TSH values (>5 μUI/ml) whereas the remaining 67 (group B) had normal values. The percentage of anti-TPO, TG, GBM, and cardiolipin antibodies in group A patients (33%, 41%, 5% and 5%, resp.) as well as IL17, IL2 and TGF-b concentrations (25 ± 23 pg/ml, 643 ± 572 pg/ml, and 618 ± 221 pg/ml, resp.) were significantly higher than group B. Abnormal Th17 regulation mediated by IL-2 and low TGF-b concentrations is associated with hypothyroidism in chronically-infected HCV patients.

1. Introduction

There is ample evidence showing that 7–11% of HCV-infected children and adult individuals have thyroid dysfunction, mainly subclinical hypothyroidism, prior to the initiation of treatment [1, 2]. The association between autoimmune thyroiditis in HCV-infected patients, in particular in subjects with HCV-related mixed cryoglobulinemia, has also been confirmed [3]. Once combined pegIFNα2b + ribavirin therapy is initiated, the percentage of patients with thyroid dysfunction goes up to 15–20% [4]. Immunological mechanisms at the origin of this thyroid dysfunction are presumed but the precise mechanism still is unknown.

IL-17A, a proinflammatory cytokine, is secreted by the CD4+Th17 cells [5]. It is involved in some organ-specific autoimmune diseases such as rheumatoid arthritis, asthma, inflammatory bowel disease, multiple sclerosis, and even organ allograft rejection [6–9], among many others. The main function of IL-17-secreting T cells is to mediate inflammation, by stimulating production of inflammatory cytokines, such as TNF-a, IL-1b, IL-6, and inflammatory chemokines that promote the recruitment of neutrophils and macrophages [10]. Initial evidence suggested that human Th17 cells were regulated by CD4+25+FoxP3+ regulatory T cells, but recent evidence suggests an alternative regulatory mechanism mediated by IL-10 and TGF-b [11, 12].
Ag-specific Th17 cells are induced in HCV-infected patients, but the HCV-NS4 protein also induces the secretion of IL-10 and TGF-b by monocytes [13]. Neutralization of both anti-inflammatory cytokines significantly enhances NS4-specific IL-17 and IFN-gamma production by T cells from HCV-infected donors [14]. Although this may represent a novel immune subversion mechanism by the virus to evade host protective immune responses, it could also be responsible for the thyroid dysfunction. The precise mechanisms of such dysfunction remain obscure.

An initial evaluation of the thyroid function in chronically infected HCV patients referred to the Gastroenterology Service at Mexico’s H.R. “Lic. Adolfo Lopez Mateos”, ISSSTE, showed that a high percentage of patients had subclinical hypothyroidism prior to the initiation of combined treatment. Therefore, our aim was to evaluate the serological status of IL-10, TGF-b and IL-17 in chronically non-interferon treated HCV-infected patients with or without subclinical hypothyroidism.

2. Material and Methods

2.1. Patients. Eighty seven patients attending an HCV outpatient clinic at HR “Lic. Adolfo Lopez Mateos”, ISSSTE, Mexico, were recruited for this study. Written informed consent was obtained from each patient. The study was approved by the local Research and Ethics Committee. All patients tested positive for antibodies to HCV using an enzyme immunoassay (Abbott Diagnostics). They were all infected as determined by consistently positive HCV RNA serum detection results using a qualitative RT-PCR (Amplicor; Roche Diagnostic Systems). None of the patients (25 males and 62 females) had ever received treatment with pegylated interferon and/or ribavirin. Their mean age was 50.1 years old (31–69). A single experienced histopathologist scored liver biopsy specimens from all the patients. All the patients were catalogued as a class A Child-Pugh score and were selected to initiate pegylated interferon treatment. The control group was conformed by 40 individuals (20 males, 20 females; with a mean age of 48 years old) with no history of liver disease, negative to HCV antibodies and normal liver function tests.

2.2. Biochemical Parameters. Blood samples, freshly obtained from each patient one week before treatment was initiated, stood for 5 minutes at room temperature before being centrifuged for 10 minutes at 1800 rpm at 4°C. Plasma and sera was obtained from each patient and aliquots of 0.1 ml were kept at −70°C until use. Plasma levels of IL-2, IL-10, IL-12, IL-17, and TGF-b were determined using commercially available kits purchased from Genzyme and R & D systems (Quantikine) carefully following the manufacturer instructions. The R value for the linear regression of the standard curve had to be 0.95 or higher, a lower value was considered inadequate and the data obtained of such plates was eliminated. In order to activate latent TGF-b to immunoreactive TGF-b platelet-poor plasma samples was incubated for 10 min at room temperature with 1 N HCl and then the sample was neutralized with a solution made of 1.2 N NaOH/0.5 M HEPES for another 10 minutes before the determination was performed. Commercially available kits, also from R & D systems were used to determine antithyroglobulin (TG), antithyroid peroxidase (TPO), anticardiolipin, antiglomerular basement membrane (GBM) and anticyclic citrullinated peptide (CCP) antibodies. The plates were read in a Multiskan Ascent (Thermolab systems, Maltham, MA, USA) microplate reader using a 450 and 490 nm filters. Results represent the mean of duplicates.

Hormone determination (TsH, T3, and free T4) and liver function tests (ALT, AST, Albumin, Immunoglobulin) were performed in the laboratory of the hospital. Subclinical hypothyroidism was defined as a TsH value of ≥4 pg/ml with normal T3 and T4 values.

The results of all these evaluations in the control group were within the established normal values determined in our laboratory (0.25–4.0 μIU/ml for TsH; 0.6–1.9 ng/ml for T3; 0.7–1.8 ng/dl for free T4; 2–40 UI/ml for AST and 2–40 UI/ml for ALT). The results obtained in the control samples for the different cytokines were 6.8 ± 2.7 pg/ml for IL-2; 4.9 ± 1.4 pg/ml for IL-10; 52.7 ± 8.1 pg/ml for IL-12; 6.75 ± 1.14 pg/ml for IL-17, and 398 ± 271 pg/ml for TGF-b1. The normal value for anti-TG is 0–34 IU/ml, 0-12 IU/ml for anti-TPO, >10 IU/ml for anti-cardiolipin, and 0–7 IU/ml for anti-GBM and anti-CCP antibodies.

2.3. Statistical Analysis. Data is expressed as mean ± standard deviation of the mean. Differences between groups were evaluated by Student’s t test, confidence interval at the 95% value, Tukey test and Kruskal-Wallis one way analysis of variance on Ranks, and Holm Sidak method. Data was analyzed with the SPSS 12.0 release. A P < .05 was considered statistically significant.

3. Results

The mean age of male patients was 45.7 years old whereas that of the females was 51.8 years old. The predominant HCV genotype was 1b (42 patients) followed by 1a (18 patients), 2b (17 patients), 2a (9 patients), and one patient with genotype 4. Table 1 shows the clinic-serological parameters of all the patients. Seventy percent of the patients with elevated TsH (6.45 ± 2.21 μIU/ml) (group A) were females; the percentage of female patients in the group with normal TsH values (2.58 ± 0.95 μIU/ml) (group B) was similar (71%). The difference in TsH values between both groups was highly significant (P < .0001) as well as between group A and control group (P < .001; according to the all pairwise multiple comparison procedures-Holm-Sidak method). There were no statistical differences in TsH values related to gender (5.82 ± 1.73 μIU/ml for males and 6.71 ± 2.40 μIU/ml for females in group A versus 2.26 ± 0.76 μIU/ml for males and 2.68 ± 0.99 μIU/ml for females, in group B). Similarly there were no statistical significant differences between both groups in relation to viral load. Although the values of the following markers were statistically different from those of the control group, there
were no statistical significant differences between group A and group B results in relation to AST, ALT, T3, and free T4.

Eight group A patients had elevated anti-TG antibodies (41%). Six of those also had elevated anti-TPO antibodies (33%). One patient with normal anti-TG, anti-TPO, and anti-CCP values had elevated anti-GBM and anti-cardiolipin antibodies. This patient had consistently low platelet counts but had no history of miscarriage or deep vein thrombosis. None of the anti-TPO nor anti-TG positive patients in group A had signs or symptoms of Hashimoto’s thyroiditis. The percentage of autoantibodies in group B was 33% for anti-TG antibodies, and 7% for anti-TPO. None of the patients in group B had elevated values of anti-GBM or anti-CCP, and only one had elevated anti-cardiolipin antibodies. Interestingly, the latter patient was also positive for anti-TPO antibodies and had deep vein thrombosis of the lower extremities.

In relation to the cytokines it was interesting to observe that although the concentrations in HCV+ patients were significantly higher than those found in the control group for all the cytokines, the intergroup differences (group A individuals versus group B) for IL-10 (14.83 ± 2.98 pg/mL versus 15.27 ± 1.70 pg/mL) and IL-12 (271.59 ± 196.43 pg/mL versus 221.13 ± 101.60 pg/mL) were not statistically significant. The cytokines that showed a highly statistically significant difference between group A and group B patients were: IL-2, IL-17, and TGF-b (Table 1). The Kruskal-Wallis analysis of variance at the 0.05 level of significance for IL-2 showed a median value of 611 pg/mL for group A, 195 pg/mL for group B, and 7.36 pg/mL for the control group (P < .001); the significance of these differences between groups A and B with the control was confirmed by the Tukey test (P < .05). As far as IL-17 was concerned, the Kruskal-Wallis analysis of variance showed a median value of 19.4 pg/mL for group A, 18.5 pg/mL for group B, and 8.9 pg/mL for the control group (P < .001); similarly to IL-2, IL-17 results were also confirmed by the Tukey test. There were no differences in IL-17 concentration in both groups in relation to the viral genotype. Finally, TGF-b values passed the normality test and the one way analysis of variance demonstrated a significant difference between groups A and B with the control group (P < .001) but not between group A and group B. It was interesting to observe that TGF-b concentration in group A patients was 38% below that of group B patients.

4. Discussion

HCV infection has been related to various autoimmune disorders including thyroid dysfunction [1–4] and papillary thyroid cancer [15]. Patients with HCV-related mixed cryoglobulinemia also show thyroid dysfunction [3]. In summary, the presence of hypothyroidism in chronically HCV-infected individuals, either under treatment or not, has been well documented [1, 16, 17]. One interesting observation was the high percentage of HCV-infected patients having hypothyroidism (23%) versus an average 12% cited in the literature; this could be secondary to the significantly higher frequency of thyroid microsomal antibodies in Mexican women in comparison with Caucasian or black women [18] thus representing a higher predisposition to autoimmunity against thyroid gland. We found autoimmune thyroid involvement as judged from serum auto-antibodies and Tsh values in 20 of the 87 patients that we analyzed. The percentage of individuals in the subclinical hypothyroidism group with autoantibodies was significantly higher than the percentage found in normothyroid HCV+ patients and control. None of our patients had manifestations of Hashimoto’s disease and only one of the female patients in the hypothyroid group, had a pattern compatible with an antiphospholipids syndrome, an association found in 20% of HCV-infected patients [19]. The percentage of females with autoimmune disease in our study (70%) is identical to that found in a multicenter international study [20, 21]. Nevertheless, it has recently been suggested that the presence of autoantibodies in HCV-infected patients do not appear to be of clinical importance [22]. One patient in each group had elevated anticardiolipin antibodies but none of them suffered stroke, although the patient with deep vein thrombosis is a strong candidate. This association has already been described [23].

The thyroid dysfunction in HCV-infected patients has been associated to interleukin 2 [24–26], a cytokine secreted primarily by Th1 cells. Patients with non-HCV-related diseases being treated with high doses of IL-2 also develop thyroid dysfunction [27, 28]. The increased interleukin 2 serum concentration determined in our non-IL-2 or IFN-treated HCV+ hypothyroid patients, corroborates the above mentioned findings. Interleukin 2 is known to expand Th17 cells in some autoimmune diseases [29].

A key cytokine for IL-2 production and Th1 cell differentiation is IL-12 [30, 31]. Although the core protein of HCV seems to have a suppressive action on IL-12 production at the transcriptional level [32], our results showed that the concentration of IL-12 was similar in both of our HCV-infected groups, but considerably higher than the control group. This suggests that hypothyroidism was not related to interleukin-2 despite the fact that transgenic mice with IL-12 serum concentrations in the 150 pg/ml range develop a moderate primary hypothyroidism [33]. IL-12 is responsible for inducing a Th17 response [5, 34].

The Th17 response is characterized by a serum increase in interleukin-17A, commonly known as IL-17. This cytokine, secreted by the CD4+ T cells known as Th17, is also secreted by some CD8+ T cells, NKT, alpha-beta or gamma-delta T cells, eosinophils, neutrophils, and monocytes [35–37]. Th17 cells are mainly linked to autoimmune diseases, namely, multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, Lyme disease, psoriasis, and uveitis [29, 38]. Of the five different IL-17 receptors (A to E), IL-17 only bind to IL-17RA and C [35, 39]. The pituitary gland and the thyroid, in contrast to hepatocytes, express IL-17RC [40]. The regulation of Th17 cells is basically mediated by CD4+CD25+FoxP3+ T reg cells which are capable of inducing anergy towards self- and alloantigen, thus playing an important role in autoimmunity [41–43]. Nevertheless,
|                | Group A (n = 20) | Group B (n = 67) | Control (n = 40) | P value |
|----------------|-----------------|-----------------|-----------------|---------|
| Females/males  | 14/6            | 48/19           | 20/20           | —       |
| Age, mean ± SD (years) | 49.8 ± 9.7    | 49.8 ± 12.2     | 51.2 ± 14.9     | NS      |
| HCV genotype   |                 |                 |                 |         |
| 1              | 12              | 45              | 0               | —       |
| 2              | 7               | 21              | 0               | —       |
| 4              | 1               |                 |                 | —       |
| Viral load HCV RNA (<50 IU/mL) | 489,731 ± 381,451 | 399,129 ± 319,863 | Negative | NS      |
| ALT (IU/mL)    | 99.75 ± 65.58   | 81.23 ± 55.38   | 25 ± 1.62       | NS      |
| AST (IU/mL)    | 81.37 ± 54.31   | 72.28 ± 54.33   | 32 ± 1.33       | NS      |
| Platelets (k/μL) | 162 ± 73.3     | 206.6 ± 85.1    | 309 ± 95        | NS      |
| Albumine (g/dL) | 4 ± 0.48       | 3.9 ± 0.8       | 4.1 ± 0.4       | NS      |
| TSH (μIU/mL)   |                 |                 |                 |         |
| Total          | 6.45 ± 2.21     | 2.58 ± 0.95     | 2.6 ± 1.1       | < .0001 |
| Males          | 5.82 ± 1.73     | 2.26 ± 0.76     | 2.6 ± 1.1       | < .0001 |
| Females        | 6.71 ± 2.40     | 2.68 ± 0.99     |                 |         |
| T3 (ng/mL)     | 1.4 ± 0.9       | 1.51 ± 0.6      | 1.3 ± 0.5       | NS      |
| T4F (ng/dL)    | 1.18 ± 0.4      | 1.51 ± 0.6      | 0.9 ± 0.3       | NS      |
| Anti-thyroperoxidase antibody (AbTPO)* | 33%            | 7%              | Negative        | < .001  |
| Anti-thyroglobulin antibody (AbTg)* | 41%            | 33%             | Negative        | < .01   |
| Anticyclic Citrullinated Peptides antibody (CCP) | Negative | Negative | Negative | —       |
| Glomerular Basement Membrane (GBM) | Negative | Negative | Negative | —       |
| Cardiolipin    | Negative        | Negative        | Negative        |         |
| IL-2 (pg/mL)   | 643.07 ± 572.66** | 292.89 ± 335.91 | 6.8 ± 2.7       | < .0009 |
| IL-10 (pg/mL)  | 14.83 ± 2.98    | 15.27 ± 1.70    | 4.9 ± 1.4       | NS      |
| IL-12 (pg/mL)  | 271.59 ± 2.98   | 221.13 ± 101.60 | 52.7 ± 8.1      | NS      |
| IL-17 (pg/mL)  | 25.88 ± 23.14** | 14.60 ± 7.83    | 6.75 ± 1.14     | < .001  |
| TGF-β (pg/mL)  | 618.97 ± 221.89** | 850.14 ± 415.64 | 398 ± 271       | .019    |

Group A are the hypothyroid HCV+ patients whereas group B are the normothyroid HCV+ patients.

*Percentage of patients with values above the normal range.

**P < .01 between group A and group B.

Table 1: Clinicoserological findings in hypo- and normothyroidism HCV patients.

a new regulatory mechanism mediated by IL-10 and TGF-β, both of which suppress IL-17 production [11, 12], has been suggested [44]. Our hypothyroid patients had similar amounts of IL-10 than the normo thyroid group but lower TGF-β serum concentration. Therefore, the only abnormality that could explain the high IL-17 serum concentration and the thyroid dysfunction was related to alterations in the FoxP3+ Treg cells, probably through enhanced Stat3 phosphorylation [36, 45]. Nevertheless, recent evidence shows that TGF-beta orchestrates Th17 cell differentiation in a concentration-dependent manner. Low TGF-β concentrations favors Th17 cell differentiation whereas high TGF-β concentrations favors Foxp3+ Treg cell differentiation [43]. The former is accompanied by high levels of IL-17, as we observed in our hypothyroid patients, whereas the latter is accompanied by a tighter regulation of IL-17 secretion, as we observed in our normothyroid patients. Despite TGF-β apparent importance, Th17 differentiation depends basically on the presence of IL-1 and IL-6 [46]. It has recently been shown that the proportion of peripheral Th17 cells in patients with autoimmune thyroid disease is higher than in control subjects [47].

Although there is evidence showing that IL-2 can inhibit human Th17 cell differentiation through a STAT-5 mediated pathway and enhanced TGF-β-induced FoxP3 expression [48, 49], a recent report by Deknuydt et al. [50] show that CD4+CD25+ FOXP3+ T regulatory cells in the presence of IL-2, in an inflammatory microenvironment, can be converted into proinflammatory Th17 cells. This could explain the link between inflammation, high IL-2 and IL-17 concentrations, and autoimmunity in our patients. Interleukin 17 is also involved in human alcoholic liver disease, an entity that shares some features with autoimmune diseases [51].

The viral load in our patients could be definitively considered low and such viral loads have been linked with the development of auto antibodies due to the lowering of the B cell activation threshold or by inducing self-reactivity through a mechanism of molecular mimicry [52, 53].

5. Conclusions

We believe that our results point towards an abnormality in the T regulatory cells, induced by the hepatitis C virus.
The fact that not all the HCV-infected patients develop an full blown autoimmune disease suggests that there are many individual variations that go beyond the viral load, viral genotype, presence of auto antibodies or liver biochemical alterations. We must not forget that the HCV does replicate within the thyroid tissue [54] and that the evolution of the HCV infection is related to distinct immune cell cytokine expression profiles. Recently it has been postulated that a Th17 mediated immune response underpins the association of chronic HCV infection with endocrine disease [55], our results suggest that a Th17 immune response should also be considered.

Acknowledgments

This work was partially supported by Grants IN-202508-3 and IN-210409-3 from DGAPA-UNAM, Mexico.

References

[1] C. Broussolle, M. P. Strineur, F. Bailly, F. Zoulim, and C. Trépo, “Hepatitis C viral infection and thyroid diseases,” Revue de Medecine Interne, vol. 20, no. 9, pp. 766–773, 1999.

[2] G. Indolfi, S. Stagi, E. Bartolini, et al., “Thyroid function and anti-thyroid autoantibodies in untreated children with vertically acquired chronic hepatitis C virus infection,” Clinical Endocrinology, vol. 68, no. 1, pp. 117–121, 2008.

[3] A. Antonelli, C. Ferri, S. M. Ferrari, M. Colaci, and P. Fallahi, “Immunopathogenesis of HCV-related endocrine manifestations in chronic hepatitis and mixed cryoglobulinemia,” Autoimmunity Reviews, vol. 8, no. 1, pp. 18–23, 2008.

[4] M. Marazuela, L. García-Buey, B. González-Fernández, et al., “Thyroid autoimmune disorders in patients with chronic HCV before and during interferon-α therapy,” Clinical Endocrinology, vol. 44, no. 6, pp. 635–642, 1996.

[5] L. Lyakh, G. Trinchieri, L. Prozveza, G. Carra, and F. Gerosa, “Regulation of interleukin-12/interleukin-23 production and the T-helper 1 response in humans,” Immunological Reviews, vol. 226, no. 1, pp. 112–131, 2008.

[6] G. Frisullo, V. Nociti, R. Iorio, et al., “IL17 and IFNy production by peripheral blood mononuclear cells from clinically isolated syndrome to secondary progressive multiple sclerosis,” Cytokine, vol. 44, no. 1, pp. 22–25, 2008.

[7] W. B. van den Berg, P. L. van Lent, L. A. B. Joosten, S. Abdollahi-Roodsaz, and M. I. Koenders, “Amplifying elements of arthritis and joint destruction,” Annals of the Rheumatic Diseases, vol. 66, supplement 3, pp. 45–48, 2007.

[8] T. Kobayashi, S. Okamoto, T. Hisamatsu, et al., “IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn’s disease,” Gut, vol. 57, no. 12, pp. 1682–1689, 2008.

[9] M. Imamura, K. Okunishi, H. Ohtsu, et al., “Pravastatin attenuates allergic airway inflammation by suppressing antigen sensitisation, interleukin 17 production and antigen presentation in the lung,” Thorax, vol. 64, no. 1, pp. 44–49, 2009.

[10] K. H. G. Mills, “Induction, function and regulation of IL-17-producing T cells,” European Journal of Immunology, vol. 38, no. 10, pp. 2636–2649, 2008.

[11] T. Korn, E. Bettelli, M. Oukka, and V. K. Kuchroo, “IL-17 and Th17 cells,” Annual Review of Immunology, vol. 27, pp. 485–517, 2009.

[12] N. Maelan, D. Unutmaz, and D. R. Littman, “The differentiation of human T(H)-17 cells requires transforming growth factor-β and induction of the nuclear receptor RORγt,” Nature Immunology, vol. 9, no. 6, pp. 641–649, 2008.

[13] A. G. Rowan, J. M. Fletcher, E. J. Ryan, et al., “Hepatitis C virus-specific Th17 cells are suppressed by virus-induced TGF-β,” Journal of Immunology, vol. 181, no. 7, pp. 4485–4494, 2008.

[14] M. T. Brady, A. J. MacDonald, A. G. Rowan, and K. H. G. Mills, “Hepatitis C virus non-structural protein 4 suppresses Th1 responses by stimulating IL-10 production from monocytes,” European Journal of Immunology, vol. 33, no. 12, pp. 3448–3457, 2003.

[15] A. Antonelli, C. Ferri, and P. Fallahi, “Thyroid cancer in patients with hepatitis C infection,” Journal of the American Medical Association, vol. 281, no. 17, p. 1588, 1999.

[16] E. J. Bini and S. Mehandru, “Incidence of thyroid dysfunction during interferon alfa-2b and ribavirin therapy in men with chronic hepatitis C: a prospective cohort study,” Archives of Internal Medicine, vol. 164, no. 21, pp. 2371–2376, 2004.

[17] M. Rodriguez-Torres, C. F. Rios-Bedoya, G. Ortiz-Lasanta, A. M. Marxuach-Cuévara, and J. Jiménez-Rivera, “Thyroid dysfunction (TD) among chronic hepatitis C patients with mild and severe hepatic fibrosis,” Annals of Hepatology, vol. 7, no. 1, pp. 72–77, 2008.

[18] S. Kasim and A. Bessman, “Thyroid autoimmunity in type 2 (non-insulin-dependent) diabetic patients of Caucasian, black and Mexican origin,” Diabetologia, vol. 27, no. 1, pp. 59–61, 1984.

[19] D. Sene, J.-C. Piette, and P. Cacoub, “Antiphospholipid antibodies, antiphospholipid syndrome and viral infections,” Revue de Medecine Interne, vol. 30, no. 2, pp. 135–141, 2009.

[20] M. Ramos-Casals, S. Muñoz, F. Medina, et al., “Systemic autoimmune diseases in patients with hepatitis C virus infection: characterization of 1020 cases (The HISPAMEC Registry),” Journal of Rheumatology, vol. 36, no. 7, pp. 1442–1448, 2009.

[21] M. Ramos-Casals, L.-J. Jara, F. Medina, et al., “Systemic autoimmune diseases co-existing with chronic hepatitis C virus infection (The HISPAMEC Registry): patterns of clinical and immunological expression in 180 cases,” Journal of Internal Medicine, vol. 257, no. 6, pp. 549–557, 2005.

[22] E. Kisiel and W. Kryczka, “Antiphospholipid antibodies with HCV infection. Innocent proteins or risk factor?” Przegląd Lekarski, vol. 64, no. 7-8, pp. 521–524, 2007.

[23] I. M. Cojocaru, M. Cojocaru, and S. A. Iacob, “High prevalence of anticardiolipin antibodies in patients with asymptomatic hepatitis C virus infection associated acute ischemic stroke,” Romanian Journal of Internal Medicine, vol. 43, no. 1-2, pp. 89–95, 2005.

[24] Z. Kuloglu, A. Kansu, M. Berberoglu, P. Adiyaman, G. Ocal, and N. Girgin, “The incidence and evolution of thyroid dysfunction during interferon-α therapy in children with chronic hepatitis B infection,” Journal of Pediatric Endocrinology and Metabolism, vol. 20, no. 2, pp. 237–245, 2007.

[25] E. Tartour, M. Schlumberger, T. Dorval, E. Baudin, and W. H. Fridman, “Endocrine involvement in immunotherapy,” Annales d’Endocrinologie, vol. 64, no. 7-8, pp. 521–524, 2007.
carcinoma patients," Oncology Reports, vol. 12, no. 4, pp. 855–859, 2004.

[28] G. Meloni, S. M. Trisolini, S. Capria, et al., “How long can we give interleukin-2? Clinical and immunological evaluation of AML patients after 10 or more years of IL2 administration,” Leukemia, vol. 16, no. 10, pp. 2016–2018, 2002.

[29] A. Amadi-Obi, C.-R. Yu, X. Liu, et al., “TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1,” Nature Medicine, vol. 13, no. 6, pp. 711–718, 2007.

[30] K. A. Smith, “Interleukin-2: inception, impact, and implications,” Science, vol. 240, no. 4836, pp. 1169–1176, 1988.

[31] G. Trinchieri, “Interleukin-12 and the regulation of innate resistance and adaptive immunity,” Nature Reviews Immunology, vol. 3, no. 2, pp. 133–146, 2003.

[32] A. Cecere, F. Marotta, B. Vangieri, L. Tancredi, and A. Gattoni, “Progressive liver injury in chronic hepatitis C infection is related to altered cellular immune response and to different cytokine profile,” Panminerva Medica, vol. 46, no. 3, pp. 171–187, 2005.

[33] H. Kimura, S.-C. Tzou, R. Rocchi, et al., “Interleukin (IL)-12- driven primary hypothyroidism: the contrasting roles of two Th1 cytokines (IL-12 and interferon-γ),” Endocrinology, vol. 146, no. 8, pp. 3642–3651, 2005.

[34] K. I. Happel, P. J. Dubin, M. Zheng, et al., “Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae,” Journal of Experimental Medicine, vol. 202, no. 6, pp. 761–769, 2005.

[35] S. L. Gaffen, J. M. Kramer, J. J. Yu, and F. Shen, “The IL-17 cytokine family,” Vitamins and Hormones, vol. 74, pp. 255–282, 2006.

[36] Z. Chen and J. J. O’Shea, “Regulation of IL-17 production in human lymphocytes,” Cytokine, vol. 41, no. 2, pp. 71–78, 2008.

[37] J. K. Kolls and A. Lindén, “Interleukin-17 family members and inflammation,” Immunity, vol. 21, no. 4, pp. 467–476, 2004.

[38] C. T. Weaver, R. D. Hatton, P. R. Mangan, and L. E. Harrington, “IL-17 family cytokines and the expanding diversity of effector T cell lineages,” Annual Review of Immunology, vol. 25, pp. 821–852, 2007.

[39] J. F. Wright, F. Bennett, B. Li, et al., “The human IL-17F/IL-17A heterodimeric cytokine signals through the IL-17RA/IL-17RC receptor complex,” Journal of Immunology, vol. 181, no. 4, pp. 2799–2805, 2008.

[40] D. Ge and Z. You, “Expression of interleukin-17RC protein in normal human tissues,” International Archives of Medicine, vol. 1, article 19, 2008.

[41] L. A. Schubert, E. Jeffery, Y. Zhang, F. Ramsdell, and S. F. Ziegler, “Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation,” Journal of Biological Chemistry, vol. 276, no. 40, pp. 37672–37679, 2001.

[42] L. Xu, A. Kitani, I. Fuss, and W. Strober, “Cutting edge: regulatory T cells induce CD4+CD25-Foxp3- T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-β,” Journal of Immunology, vol. 178, no. 11, pp. 6725–6729, 2007.

[43] L. Zhou, J. Lopes, M. M. W. Chong, et al., “TGF-β-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORyt function,” Nature, vol. 453, no. 7192, pp. 236–240, 2008.

[44] J. R. Wilczynski, M. Radwan, and J. Kalinka, “The characterization and role of regulatory T cells in immune reactions,” Frontiers in Bioscience, vol. 13, no. 6, pp. 2266–2274, 2008.

[45] Z. Chen, A. Laurence, Y. Kanno, et al., “Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 21, pp. 8137–8142, 2006.

[46] E. V. Acosta-Rodriguez, G. Napolitani, A. Lanzavecchia, and F. Sallusto, “Interleukins 18 and 20 but not transforming growth factor-β are essential for the differentiation of interleukin 17-producing human T helper cells,” Nature Immunology, vol. 8, no. 9, pp. 942–949, 2007.

[47] T. Nanba, M. Watanabe, N. Inoue, and Y. Iwatan, “Increases of the Th1/Th2 cell ratio in severe Hashimoto’s disease and in the proportion of Th17 cells in intractable Graves’ disease,” Thyroid, vol. 19, no. 5, pp. 495–501, 2009.

[48] A. Awasthi, G. Murugaiyan, and V. K. Kuchroo, “Interplay between effector Th17 and regulatory T cells,” Journal of Clinical Immunology, vol. 28, no. 6, pp. 660–670, 2008.

[49] I. I. Ivanov, L. Zhou, and D. R. Littman, “Transcriptional regulation of Th17 cell differentiation,” Seminars in Immunology, vol. 19, no. 6, pp. 409–417, 2007.

[50] F. Deknuydt, G. Bioley, D. Valmori, and M. Ayoub, “IL-1β and IL-2 convert human Treg into Th(H)17 cells,” Clinical Immunology, vol. 131, no. 2, pp. 298–307, 2009.

[51] G. M. Thiele, T. L. Freeman, and L. W. Klassen, “Immunologic mechanisms of alcoholic liver injury,” Seminars in Liver Disease, vol. 24, no. 3, pp. 273–287, 2004.

[52] L. Bai, Z.-R. Feng, H.-Y. Lu, et al., “Prevalence of antinuclear and anti-liver-kidney-microsome type-1 antibodies in patients with chronic hepatitis C in China,” Chinese Medical Journal, vol. 122, no. 1, pp. 5–9, 2009.

[53] L. Sansonno, F. Anna Tucci, S. Sansonno, et al., “B cells and HCV: an infection model of autoimmunity,” Autoimmunity Reviews, vol. 9, no. 2, pp. 93–94, 2009.

[54] J. Bartolomé, E. Rodríguez-Iñigo, P. Quadros, et al., “Detection of hepatitis C virus in thyroid tissue from patients with chronic HCV infection,” Journal of Medical Virology, vol. 80, no. 9, pp. 1588–1594, 2008.

[55] A. Antonelli, C. Ferri, S. M. Ferrari, et al., “Endocrine manifestations of hepatitis C virus infection,” Nature Clinical Practice Endocrinology and Metabolism, vol. 5, no. 1, pp. 26–34, 2009.
