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α-Chemokine CXCL10 and β-chemokine CCL2 serum levels in patients with hepatitis C–associated cryoglobulinemia in the presence or absence of autoimmune thyroiditis

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Abstract

Chemokines have been identified to play an important role in endocrine autoimmune disease and hepatitis C chronic infection. To our knowledge, no study has evaluated serum levels of CXCL10 and CCL2 in patients with "mixed cryoglobulinemia and hepatitis C virus chronic infection" (MC) in the presence or absence of autoimmune thyroiditis (AT). Serum CXCL10 and CCL2 were assayed in 60 patients with MC, in 45 patients with "MC with AT" (MC + AT), and in controls (60 without [control 1] and 45 with AT [control 2]). CXCL10 was significantly higher (1) in control 2 than in control 1 ($P < .001$), (2) in MC than in control 1, and (3) in MC + AT than in controls 1 and 2 and in MC ($P = .002$). A high CXCL10 level (>mean + SD control 1; >167 pg/mL) was present in 7% control 1, 21% control 2, 49% MC, and 78% MC + AT ($P < .0001$). CCL2 was significantly higher in MC and in MC + AT than in control 1 or in control 2 ($P < .01$). A high CCL2 level (>mean + SD control 1; >730 pg/mL) was present in 2% control 1, 1% control 2, 18% MC, and 21% MC + AT ($P < .0001$). The study demonstrates high CXCL10 and CCL2 serum levels in patients with MC; CXCL10 in MC + AT is significantly higher than that in MC. Future studies in larger series will be needed to evaluate the potential usefulness of serum CXCL10 and CCL2 determination as a prognostic marker in the follow-up of MC patients, also in relation to the presence of AT.

1. Introduction

Chemokines are a group of peptides (more than 50) of low molecular weight that induce the recruitment of leukocytes to inflammation sites, which have been classified into 4 major families [1].

Two of these families have been extensively studied, namely, the CC and CXC chemokines. Chemokines from the CC family are chemoattractant to T lymphocytes, monocytes, and natural killer cells. The prototype CC chemokine is monocyte chemoattractant protein 1, which is a crucial factor for the development of adaptive Th2 responses by directing the differentiation of Th0 cells to Th2 in vitro [2,3].

CXC chemokines attract neutrophils [1]. Among CXC chemokines, CXCL10 displays a strong chemoattractant activity for Th1 lymphocytes secreting interferon (IFN)γ; and it is a reliable marker of aggressive Th1-mediated autoimmune disease [4].

More recently, chemokines have been identified to play an important role in endocrine autoimmune disease; and particular attention has been raised by studies demonstrating both CC and CXC chemokines overexpression in Hashimoto thyroiditis and in early phases of Graves disease (GD) and Hashimoto thyroiditis [5-11].

The role of circulating α- and β-chemokines in hepatitis C–associated cryoglobulinemia has been less extensively studied. Regarding CXCL10, there is no study to our knowledge; with regard to CCL2, only 1 study suggests that CCL2 may play a major role in modulating the inflamma-

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tory process observed in cryoglobulinemic glomerulonephritis [12].

However, other studies in hepatitis C chronic infection (HCV+) reported that CXCL10 is expressed in hepatocytes in chronic hepatitis C patients, is high in serum of HCV patients, and correlates with histologic severity and lobular inflammation [13-15]. Regarding serum CCL2, discordant results have been found in HCV+: high serum levels of CCL2 have been found in HCV patients by Narumi et al [16], but not by Panasiuk et al [17].

We have previously demonstrated a high frequency of autoimmune thyroid disorders in cryoglobulinemic patients [18]; the immunologic base of this association remains to be investigated.

To our knowledge, no study evaluated serum levels of CXCL10 and CCL2 in patients with "mixed cryoglobulinemia and hepatitis C virus chronic infection" (MC) in the presence or absence of autoimmune thyroiditis (AT). The aim of the study is to evaluate serum levels of CXCL10 and CCL2 in a series of MC patients in the presence or absence of AT, and to relate CXCL10 levels to the clinical phenotype of these patients.

2. Patients and methods

The diagnosis of MC was based on the presence of serum mixed (type II or III) cryoglobulins and the classic clinical triad—purpura, weakness, arthralgias—and on the exclusion of other well-known systemic disorders, such as immunorheumatic, neoplastic, and infectious diseases [19,20]. The HCV infection was systematically evaluated in all patients, and HCV-negative patients were excluded. Only patients with MC were included, without hepatocellular carcinoma and/or liver cirrhosis (identified by histology, laboratory evidence of liver failure, and/or ultrasound-proven portal hypertension) [21,22]. The presence of Sjogren syndrome, skin ulcers, peripheral neuropathy, and renal and liver involvement in MC patients was evaluated as previously described [23]. Routine blood chemistry was carried out by standard methods [18]. No MC patient had had plasma exchange treatment in the last year before the study.

A thyroid screening included history; physical examination; thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), antithyroglobulin (AbTg), and antithyroid peroxidase (AbTPO) antibodies measurements; and neck ultrasonography.

All study subjects gave their informed consent to the study, which was approved by the local ethical committee.

2.1. Patients with mixed cryoglobulinemia without AT

Sixty MC patients consecutively referred to the rheumatology unit were recruited into the study between 1999 and 2005. Only patients with MC in whom a thyroid screening excluded the presence of associated thyroid autoimmune disorders, a well-known cause of high serum CXCL10 [6], were included in this group. The main demographic and clinicoserologic features of patients with mixed cryoglobulinemia without AT (MCo) are reported in Table 1. Among them, 26 had been previously treated with IFNα for an average of 6.9 months (range, 1-13), at a mean dosage of 9.9 million units/wk (range, 3-7); the time elapsed from the last course of IFNα treatment ranged from 4 to 82 months (mean, 45). No statistically significant difference was observed in the main demographic and clinicoserologic features of MCo patients treated (MCo/IFN+) or untreated (MCo/IFN−) with IFNα.

At the time of the study, 39 MCo patients were taking low doses of corticosteroids, 7 had previously been on corticosteroids, and 14 had never been treated with corticosteroids.

2.2. Patients with mixed cryoglobulinemia and AT

Forty-five MC patients consecutively referred to the rheumatology unit were recruited into the study between 1999 and 2005. Only patients with MC in whom a thyroid screening revealed the presence of associated thyroid autoimmune disorders (MC + AT) were included in this group.

The main demographic and clinicoserologic features of MC + AT patients are reported in Table 1. Among them, 18 had been previously treated with IFNα for an average of 6.7 months (range, 1-11), at a mean dosage of 6 million units/wk (range, 3-6); the time elapsed from the last course of IFNα treatment ranged from 4 to 79 months (mean, 46). No statistically significant difference was observed in the main demographic and clinicoserologic features of MC + AT

Table 1
Clinical characteristics of 60 patients with HCV-related MCo and 45 with MC + AT

	MCo	MC + AT
	n = 60	n = 45
Age (y)	61 ± 10	62 ± 13
Male/female	12/48	9/36
Disease duration with MC (y)	13 ± 10	10 ± 12
Purpura	85%	88%
Weakness	91%	94%
Arthralgias	94%	96%
Arthritis	14%	11%
Sjogren syndrome	52%	47%
Peripheral neuropathy	76%	71%
Renal involvement [a]	17%	12%
Aminotransferases elevation and/or histologic activity [b]	71%	79%
Cryocrit (%)	4.3 ± 9.4	4.7 ± 9.3
C3 (normal, 60-130 mg/dL)	83 ± 36	84 ± 33
C4 (normal, 20-55 mg/dL)	11 ± 10	12 ± 11
Autoantibodies [c]	24%	27%

No significant differences were observed in the above-mentioned characteristics in the 2 groups.

[a] Serum creatinine >1.5 mg/dL and/or proteinuria >0.5 g/24 h.

[b] Increase of the liver enzyme (ALT) and/or histologic alterations.

[c] Presence of antinuclear and/or antimitochondrial and/or anti–smooth muscle and/or antiextractable nuclear antigen antibodies.

patients treated (MC + AT/IFN+) or untreated (MC + AT/IFN−) with IFNα.

At the time of the study, 27 MC + AT patients were taking low doses of corticosteroids, 5 had previously been on corticosteroids, and 13 had never been treated with corticosteroids.

2.3. Controls

Two control groups were included.

2.3.1. Control 1

The first control group (control 1) consisted of 60 subjects (Table 2), extracted from a random sample of the general population within the same geographic area [7,24] without HCV infection or other liver disorders, coupled by sex and age (a well-known confounding factor [25]) with MCo patients, in whom a complete thyroid workup was available, and excluded the presence of thyroid or autoimmune disorders, or any kind of immunomodulant therapy.

2.3.2. Control 2

The second control group (control 2) consisted of 45 subjects (Table 2), extracted from a random sample of the general population within the same geographic area [7,24], coupled by sex and age (a well-known confounding factor [25]) with MC + AT patients, without HCV infection or other liver disorders, in whom a complete thyroid workup was available, and demonstrated the presence of thyroid autoimmune disorders, but excluded the presence of other autoimmune disorders and any kind of immunomodulant therapy.

In all patients and controls, a blood sample was collected in the morning, after overnight fasting; and serum was kept frozen until CXCL10 and CCL2 measurements.

2.4. Immunologic studies

Cryocrit was measured as the percentage of packed cryoglobulins after cold centrifugation of the serum. Cryoglobulin composition was determined by including the presence in cryoprecipitates of monoclonal or polyclonal immunoglobulin M–rheumatoid factor (ie, MC type II or MC type III). The C3 and C4 fractions were measured as previously described [26]. Antinuclear, anti–smooth muscle, and antimitochondrial autoantibodies were detected by current techniques [26]: a titer >1:40 was considered positive. Antiextractable nuclear antigen antibodies, including anti-Scl70, anti-Sm, -RNP, -SSA/SSB, -PCNA, -SL and -Jo1 specificities, were detected by counterimmunoelectrophoresis according to Bunn et al [27].

2.5. Virologic studies

Anti-HCV antibodies and HCV RNA were determined on serum clotted and centrifuged at 37°C and stored at −70°C. Antibodies against HCV (anti-HCV) were detected by an enzyme-linked immunoassay (Chiron ELISA HCV, Third Generation; Emeryville, CA). A recombinant-based immunoblot assay (Chiron RIBA HCV, Third Generation Assay) was used to investigate the specificity of anti-HCV seropositivity. The presence of HCV RNA in the serum was investigated by a polymerase chain reaction technique as previously described [28,29]. Amplification of HCV complementary DNA was performed using a "nested" polymerase chain reaction, with primers located in the 5′ noncoding region [28]. The analysis of amplification products was performed by both ethidium bromide staining and hybridization with a radiolabeled oligonucleotide probe internal to the amplified sequence.

Table 2
Thyroid status of control 1, control 2, patients with MCo, and patients with MC + AT

	Control 1	Control 2	MCo	MC + AT	*P*
n	60	45	60	45	
Age (y)	61 ± 10	62 ± 13	60 ± 12	63 ± 11	NS
Male/female	12/48	9/36	12/48	9/36	NS
Thyroid volume (mL)	11 ± 10	14 ± 11	12 ± 12	11± 10	NS
Hypoechoic (%)	0	76	0	79	.0001
Hypervascular (%)	0	47	0	49	.0001
Serum TSH (μU/mL)	1.1 ± 0.8	1.7 ± 1.6	1.3 ± 0.9	3.1 ± 2.5 *	.001
AbTPO (IU/mL)	10 ± 8	167 ± 321 §	11 ± 8	171 ± 387 §	.0001
AbTg (IU/mL)	11 ± 9	156 ± 215 §	10 ± 11	187 ± 432 §	.0001
TRAb (IU/mL)	0	0	0	0	NS
AbTPO positivity (%)	0	72	0	78	.0001
AbTg positivity (%)	0	69	0	68	.0001
Subclinical hypothyroidism (%)	0	5	0	20	.001
CXCL10 (pg/mL)	92 ± 53	151 ± 133 †	283 ± 151 †, ‡	354 ± 138 *, †	<.0001
CCL2 (pg/mL)	386 ± 172	385 ± 141	509 ± 323 ‡	520± 388 ‡	<.01

TRAb indicates antithyrotropin-receptor antibody.

* $P < .05$ or less vs control 1, or vs AT control 2, or vs MCo.

† $P < .05$ or less vs control 1.

‡ $P < .05$ or less vs control 1 or vs AT control 2.

§ $P < .05$ or less vs control 1 and vs MC.

2.6. Ultrasonography of the neck and fine-needle aspiration

Thyroid ultrasonography was performed both in patients and controls. Neck ultrasonography was performed by the same (blinded) operator using an Esaote AU5 (Florence, Italy) with a sectorial 7.5-MHz transducer. Thyroid volume was calculated using the ellipsoid formula, as described [30-32]. The presence of hypoechoic and dyshomogeneous echogenicity was arbitrarily rated at 3 levels (0 = normal echogenicity, 1 = slight hypoechoic and dyshomogeneous pattern, and 2 = severely hypoechoic and dyshomogeneous pattern) to evaluate structural abnormalities of thyroid tissue associated with thyroid autoimmunity [33,34]. The presence of thyroid nodules was recorded; and nodules with a diameter >10 mm were submitted to ultrasonography-guided fine-needle aspiration, which was performed by the same operator, using the freehand method as already described [30,33].

2.7. Thyroid blood flow

Thyroid blood flow (TBF) by color-flow Doppler was studied in all patients [35]. The TBF pattern was defined as followed: (*a*) *normal* (or *type 0*) when TBF was limited to peripheral thyroid arteries; (*b*) *type I* when TBF was mildly increased; (*c*) *type II* when TBF was clearly increased; and (*d*) *type III* when TBF was markedly increased [35].

2.8. Laboratory evaluation

Laboratory evaluation included measurement of serum levels of TSH (reference range, 0.3-3.6 μU/ml), FT3, FT4, AbTg, and AbTPO. Circulating FT3 and FT4 were measured by commercial radioimmunoassay kits (AMERLEX-MAB FT3/ FT4 Kit; Amersham, Buckinghamshire, UK). Serum TSH (DiaSorin, Stillwater, MN), AbTPO, and AbTg (ICN Pharmaceuticals, Costamesa, CA) were evaluated by immunoradiometric assay methods. For AbTg and AbTPO, positivity was set at >100 IU/mL and >100 IU/mL, respectively. Values are given as mean ± SD for normally distributed variables. Anti–TSH-receptor autoantibodies were measured in patients with the use of a radioreceptor assay (Radim, Pomezia, Italy) (reference range, 0-1 IU/mL).

2.9. Chemokines assay

Serum CXCL10 levels were assayed by a quantitative sandwich immunoassay using a commercially available kit (R&D Systems, Minneapolis, MN) with a sensitivity ranging from 0.41 to 4.46 pg/mL. The intra- and interassay coefficients of variation were 3.0% and 6.9%. Serum CCL2 levels were assayed by a quantitative sandwich immunoassay using a commercially available kit (R&D Systems) with a sensitivity of less than 5.0 pg/mL. The intra- and interassay coefficients of variation were 4.7% and 5.8%.

2.10. Data analysis

Values are given as mean ± SD for normally distributed variables. Mean group values were compared by using 1-way analysis of variance (ANOVA) for normally distributed variables; otherwise, the Mann-Whitney *U* test was used. Proportions were compared by the χ^2 test. Post hoc comparisons on normally distributed variables were carried out using the Bonferroni-Dunn test. Univariate analysis was performed by simple regression.

3. Results

The MCo and MC + AT patients were not significantly different in relation to the clinical phenotype of cryoglobulinemia (Table 1). The demographic and clinical thyroid features of patients and controls are reported in Table 2. As expected, MC + AT patients and control 2 showed significantly higher thyroid autoantibodies levels, as well as hypoechogenicity and hypervascularity of the thyroid gland, and subclinical hypothyroidism in comparison with control 1 and MC patients.

3.1. Serum CXCL10

Serum CXCL10 levels were significantly (Table 2) higher in control 2 than in control 1 ($P < .001$). The MCo patients had significantly higher serum CXCL10 levels than control 1 (Fig. 1A) or control 2 (Table 2). The MC + AT patients had higher serum CXCL10 levels than control 1 and 2 (Fig. 2A) and than MCo ($P = .002$) (Table 2). Serum CXCL10 levels were not associated with any of the clinical features of cryoglobulinemia in patients with MCo and MC + AT (data not shown).

To better define the role of increased serum chemokines in AT, CXCL10 was studied in relation to clinical features of AT (age, sex, thyroid volume<6 mL, thyroid hypoechoic pattern or hypervascularity, AbTg or AbTPO positivity, subclinical hypothyroidism) in MC + AT patients and control 2. Serum CXCL10 levels were significantly increased in patients with MC + AT with a hypoechoic thyroid pattern with respect to those without a hypoechoic pattern (392 ± 151 vs 304 ± 117, $P = .03$, ANOVA) and hypothyroidism (397 ± 151 vs 303 ± 121, $P = .04$, ANOVA), and in control 2 with a thyroid hypoechoic pattern with respect to those without a hypoechoic pattern (187 ± 152 vs 107 ± 117, $P = .03$, ANOVA).

By defining a high CXCL10 level as a value at least 2 SD greater than the mean value of the control group (>167 pg/mL), 7% of control 1, 21% of control 2, 49% of MCo, and 78% of MC + AT had high CXCL10 levels ($P < .0001$, χ^2).

3.2. Serum CCL2

Serum CCL2 levels were similar (Table 2) in control 1 and control 2. The MCo patients had significantly higher serum CCL2 levels than control 1 (Fig. 1B). The MC + AT patients had higher ($P < .01$) serum CCL2 levels than control 2 (Fig. 2B) and had similar levels to MCo (Table 2). Serum CCL2 levels were not associated with any of the clinical features of cryoglobulinemia in patients with MCo and MC + AT (data not shown).

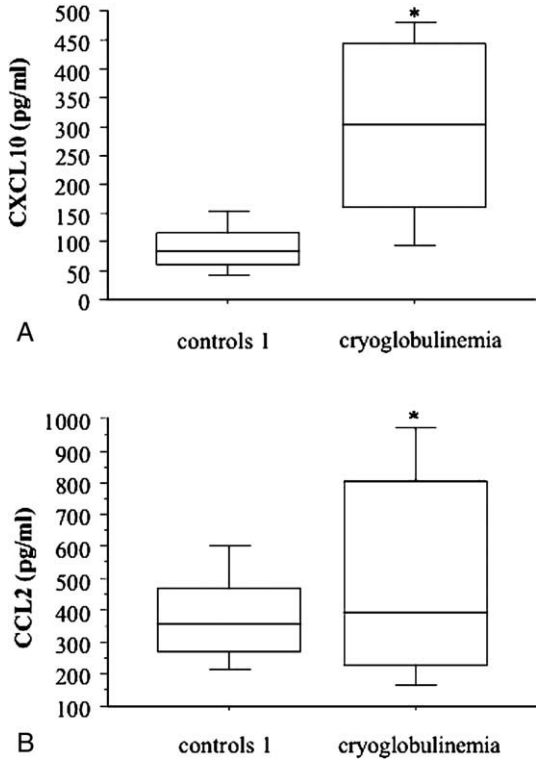

Fig. 1. A, Patients with cryoglobulinemia without AT (cryoglobulinemia) have serum CXCL10 levels significantly higher than control subjects without thyroiditis (controls 1) ($P < .0001$, ANOVA). B, Patients with cryoglobulinemia without AT (cryoglobulinemia) have serum CCL2 levels significantly higher than control subjects (controls 1) without thyroiditis ($P < .01$, ANOVA). The box indicates the lower and upper quartiles, and the central line is the median value; the horizontal lines at the end of the vertical lines are the 2.5% and 97.5% values.

To better define the role of increased serum chemokines in AT, CCL2 was studied in relation to clinical features of AT (age, sex, thyroid volume <6 mL, thyroid hypoechoic pattern or hypervascularity, AbTg or AbTPO positivity, subclinical hypothyroidism) in MC + AT patients and control 2; but no significant association was found (data not shown).

By defining a high CCL2 level as a value at least 2 SD greater than the mean value of the control group (>730 pg/mL), 2% of control 1, 1% of control 2, 18% of MCo, and 21% of MC + AT had high CCL2 ($P < .0001$, χ^2).

No significant association was observed in relation to the presence or absence of active or previous treatments, or in relation to the duration of the disease and serum CXCL10 or CCL2. No association was found between serum CXCL10 or serum CCL2 levels by simple regression.

4. Discussion

Our study is the first to demonstrate high serum levels of CXCL10 and CCL2 chemokines in patients with MCo and MC + AT with respect to control 1 and control 2. Serum CXCL10 levels of MC + AT patients were significantly higher than those of MCo patients or control 2, whereas no significant difference was observed in CCL2 between MCo and MC + AT.

Our data demonstrate high serum CXCL10 in MCo patients, with values similar to those found in HCV chronic infection without cirrhosis, and suggest that, in MCo patients, CXCL10 serum levels are mainly sustained by the liver HCV chronic infection [36-39].

In fact, CXCL10 serum levels in our patients were similar to those observed in another study in HCV patients (332 ± 222 pg/mL) before IFN treatment [39].

It has been previously reported that CXCL10 messenger RNA was expressed in hepatocytes in chronic hepatitis C patients, as demonstrated by in situ hybridization [16]. CXCL10 is specifically produced by hepatocytes in inflammatory areas and recruits T cells to the hepatic lesions in chronic viral hepatitis [13,37].

Furthermore, it has been shown that expression of the chemokine CXCL10 by hepatocytes in chronic hepatitis C virus infection correlates with histologic severity and lobular inflammation.

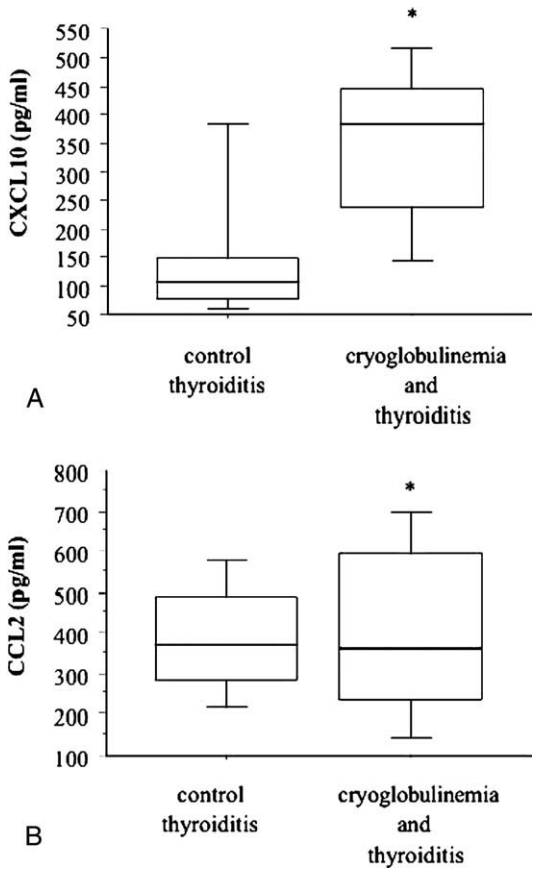

Fig. 2. A, Patients with cryoglobulinemia and thyroiditis have serum CXCL10 levels higher than control thyroiditis (control 2) ($P < .0001$, ANOVA). B, Patients with cryoglobulinemia and thyroiditis have serum CCL2 levels higher than control thyroiditis ($P < .01$, ANOVA). Data are displayed as box and whisker plots.

Harvey et al [13] sought to define the cellular source of CXCL10 in the liver by immunohistochemistry to correlate CXCL10 expression with the histologic markers of inflammation. CXCL10 was expressed by hepatocytes but not by other cell types within the liver, and the most intense immunoreactivity was evident in the areas of lobular inflammation. These findings suggest that CXCL10 may be induced by HCV within hepatocytes and may be important in the pathogenesis of chronic HCV infection, as recruitment of inflammatory cells into the lobule is an important predictor of disease progression [13-15].

It is noteworthy that, because of the inclusion criteria of the patients, only patients without cirrhosis and thyroid autoimmune disorders (which may influence CXCL10 serum levels) were present in the MCo group to avoid bias due to patient selection. Our data demonstrate high serum CXCL10 in MCo patients, with values similar to those found in HCV chronic infection without cirrhosis, and suggest that, in MC patients, CXCL10 serum levels are mainly sustained by the liver HCV chronic infection.

The MC + AT patients have significantly increased serum CXCL10 than MCo patients. These data suggest that, in the presence of thyroiditis, the further increase of serum CXCL10 levels may be due to the thyroiditis itself and suggest a predominance of the Th1 immune response in the presence of thyroiditis in MC patients.

Recent experimental evidence has demonstrated that CXCL10 plays an important physiopathological role in the initial phases of autoimmune thyroid disorders. Increased expression of CXCL10 and CXCL9 was also observed in thyroid tissue specimens obtained from subjects affected by GD and AT by immunohistochemistry, in infiltrating inflammatory cells, and also in thyrocytes [40]. Furthermore, human thyrocytes in primary culture produce large amounts of CXCL10 when stimulated by IFNγ [7]. Serum CXCL10 levels have been found to increase in both GD and AT, with an inverse correlation between circulating CXCL10 levels and disease duration in GD [41] and with a strong association with hypothyroidism and thyroid hypoechogenicity in AT [5,6]. In GD, it has been shown that removal of the thyroid itself by surgery [11] or by radioiodine [10] reduces CXCL10 serum levels, suggesting that the intrathyroidal lymphocytes and/or thyrocytes [7] may be the source of CXCL10. Therefore, we can speculate that a superimposed Th1 response is active in the thyroid of patients with MC + AT, explaining the higher levels of CXCL10 in MC + AT with respect to MCo.

These data are in agreement with our results in patients with MC + AT, confirming higher levels of CXCL10 in the presence of hypothyroidism and thyroid hypoechogenicity and thus also suggesting that, in these patients, CXCL10 may be regarded as a marker of a more aggressive thyroiditis. In our study, we have not found any relation between serum CXCL10 levels and the duration of cryoglobulinemia, probably because of the long mean duration (>10 years) of the disease in our patients.

Regarding CCL2, our study shows significantly higher serum levels in MCo and MC + AT patients with respect to controls 1 and 2, without any significant influence of the presence or absence of AT both in controls and in MCo patients, suggesting that the increase of CCL2 in MC patients is specifically associated to the cryoglobulinemia itself, but not to AT. No study until now evaluated CCL2 in MC patients; however, high serum levels of CCL2 have been found in HCV patients by Narumi et al [16], but not confirmed by Panasiuk et al [17]. High serum CCL2 has also been found in other types of vasculitis [42,43]. Furthermore, these results are fully consistent with those obtained in a large cohort of newly diagnosed AT patients without HCV infection that demonstrate normal CCL2 serum levels in AT [6]. Our data suggest that CCL2 may be a marker of Th2 immune activation in patients with MC, irrespective of the presence of AT.

Experimental evidence has demonstrated that, in vivo, cytokines act in distinct combinations that determine the final effect on the target cells. The patterns of chemokines secreted by Th1 and Th2 cells constitute paradigmatic combinations that may change in relation to the clinical phase of the disease [44]. Therefore, the simultaneous assessment of different chemokines may be potentially of great interest [45]. In fact, we have recently shown that increased serum CXCL10 levels in patients with GD are associated mainly with the active phase of GD and are in relation not with the hyperthyroidism itself, but mainly with the autoimmune response [8,9].

However, no correlation between serum CXCL10 and CCL2 levels in AT patients was found in our study group. Increased CXCL10 and normal CCL2 levels in AT are consistent with the recently proposed immunoregulatory characteristics of AT [6].

Longitudinal studies evaluating serum CXCL10 and CCL2 levels in large series of MC patients will be necessary to evaluate if serum CXCL10 measurement could represent an easily assayable marker for clinical management of MC patients.

More recently, it has been shown that high plasma CXCL10 levels correlate with a poor outcome of antiviral therapy in patients with hepatitis C. In fact, a low baseline CXCL10 level was significantly associated with a low baseline viral load, rapid viral response, and sustained viral response in HCV patients treated with IFN [39,46-48].

Regarding CCL2, Panasiuk et al [17] reported an increase of serum CCL2 in HCV patients in the course of IFNα treatment and proposed that CCL2 concentrations may be a prognostic marker of the efficacy of IFNα therapy in patients with chronic hepatitis C.

Because IFN is a well-known effective therapy for MC, future studies will be necessary to evaluate whether serum CXCL10 and CCL2 levels may be associated with sustained virologic response to IFN in MC patients [19,20].

In conclusion, our study is the first to demonstrate higher serum levels of CXCL10 and CCL2 chemokines in patients

with MC than in controls. Serum CXCL10 levels in MC patients with AT are significantly higher than those of MC patients without thyroiditis. Future studies in larger series will be needed to evaluate the potential usefulness of serum CXCL10 and CCL2 determination as a prognostic marker in the follow-up of MC patients, also in relation to the presence of AT.

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