

Th1 and Th2 Chemokine Serum Levels in Systemic Sclerosis in the Presence or Absence of Autoimmune Thyroiditis

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ABSTRACT. Objective. We evaluated contemporarily serum alpha and beta chemokines in patients with newly diagnosed systemic sclerosis (SSc) in the presence or absence of autoimmune thyroiditis (AT).

Methods. Serum levels of CXCL10 and CCL2 chemokines, prototypes of the 2 major subclasses (Th1 and Th2), were measured in patients with newly diagnosed SSc with (n = 40; SSc-II) or without (n = 50; SSc-I) AT, in comparison with 50 normal controls (control-I) and 40 AT controls without SSc (control-II) (sex- and age-matched).

Results. Serum CXCL10 levels were significantly higher in control-II, SSc-I, and SSc-II than in control-I (150 ± 131 , 196 ± 137 , 254 ± 98 , 83 ± 42 pg/ml, respectively; $p < 0.001$ for all). SSc-I had serum CXCL10 levels significantly higher than control-II ($p = 0.03$), and significantly lower than SSc-II ($p = 0.04$). SSc-II had serum CXCL10 levels significantly higher than control-II ($p = 0.002$). Serum CCL2 levels were significantly higher in SSc-I and SSc-II than in control-I (378 ± 192 , 403 ± 131 , 316 ± 113 pg/ml, respectively; $p = 0.03$ and $p < 0.01$, respectively). SSc-II had serum CCL2 levels significantly higher than control-II (327 ± 123 pg/ml; $p = 0.04$).

Conclusion. Our study demonstrates high serum levels of both CXCL10 (Th1) and CCL2 (Th2) chemokines in patients with SSc, and suggests a prevalence of Th1 immune response in the early phase of the disease. A further increase of serum CXCL10, but not of CCL2, is observed in SSc patients with AT. (First Release Aug 15 2008; J Rheumatol 2008;35:1809–11)

Key Indexing Terms:

CXCL10 (IP-10)

AUTOIMMUNE THYROIDITIS

CCL2 (MCP-1)

AbTg

SYSTEMIC SCLEROSIS

AbTPO

A high prevalence of autoimmune thyroiditis (AT) has been demonstrated in patients with systemic sclerosis (SSc)¹. The pathogenetic base of this association is under debate, and chemokines may play a role in this process². Our aim was to measure serum levels of CXCL10 (Th1; alpha) and CCL2 (Th2; beta) prototype chemokines of the 2 major subclasses (CXC; CC) in patients with newly diagnosed SSc in presence or absence of AT.

MATERIALS AND METHODS

Fifty SSc patients without AT (SSc-I; Table 1) and 40 SSc patients with AT (SSc-II; Table 1) classified according to the American College of Rheumatology preliminary criteria^{3,4}, consecutively referred to the Rheumatology Unit-University of Modena (from 1999 to 2004) were recruited into the study. Only patients at the first diagnosis of SSc, without

other non-thyroidal autoimmune disorders, not treated, in whom complete thyroid investigations excluded (SSc-I) or demonstrated (SSc-II) the presence of AT, were enrolled. Clinico-serological features of SSc were (respectively, in SSc-I and SSc-II)^{3,4}: (1) skin sclerosis: diffuse, 10%, 12%; intermediate, 19%, 18%; limited, 71%, 70%; (2) visceral involvement: peripheral vascular system, 84%, 86%; gastrointestinal system, 43%, 45%; lung, 43%, 47%; joint/tendons, 11%, 13%; heart, 22%, 26%; kidney, 6%, 7%; and (3) prevalence of autoantibodies³: antinuclear, 89%, 90%; antinuclear, 31%, 33%; anti-topoisomerase I, 35%, 34%.

Two control groups were included, extracted from a random sample of the general population from the same geographic area⁵, coupled by sex and age with SSc patients, in whom a complete clinical examination excluded the presence of other autoimmune or inflammatory disorders, or any kind of immunomodulant therapy; and in whom complete thyroid investigations excluded (control-I; Table 1) or demonstrated (control-II; Table 1) the presence of AT. The thyroid evaluation consisted of thyroid ultrasonography and color-flow Doppler⁵ and determination of serum thyroid stimulating hormone (TSH) [reference range (rr) 0.3–3.6 μ U/ml], free triiodo-thyronine (FT3) (rr 1.45–3.70 pg/ml), free thyroxine (FT4) (rr 0.7–1.8 ng/dl), anti-thyroglobulin (AbTg) (rr < 100 UI/ml), and anti-thyroid peroxidase (AbTPO) (rr < 100 UI/ml) antibodies⁵.

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 measurement. Serum CXCL10 and CCL2 levels were assayed by a quantitative sandwich immunoassay using a commercially available kit (R&D Systems, Minneapolis, MN, USA), with a sensitivity ranging from 0.41–4.46 pg/ml and < 5.0 pg/ml, respectively^{5,6}.

All subjects gave their informed consent to our study, which was approved by the local Ethical Committee.

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RESULTS

SSc-I and SSc-II were not significantly different in relation to the SSc clinical phenotype. The demographic and clinical thyroid features of patients and controls are reported in Table 1.

Serum CXCL10 levels were significantly higher in SSc-I, and SSc-II versus controls (Table 1; Figure 1). Serum CCL2 levels were significantly higher in SSc-I and SSc-II versus controls (Table 1; Figure 2). Serum CXCL10 and CCL2 levels were not associated with any clinical features of SSc in patients SSc-I and SSc-II.

Serum CXCL10 levels were significantly increased in patients SSc-II with a thyroid hypoechoic pattern versus those without a hypoechoic pattern [227 ± 110 vs 291 ± 164 ; $p = 0.04$; analysis of variance (ANOVA)], and in controls with a thyroid hypoechoic pattern versus those without a hypoechoic pattern (187 ± 139 vs 107 ± 121 ; $p = 0.03$; ANOVA); no other association was found with the other clinical features of AT. By contrast, serum CCL2 levels were not significantly associated with any of the clinical features of AT in SSc-II and control-II. Finally, no correlation between CXCL10 and CCL2 serum levels could be demonstrated.

DISCUSSION

In our patients with SSc, characterized by relatively short disease duration, high serum levels of both CXCL10 (Th1) and CCL2 (Th2) were found; however, high levels of CXCL10 (\geq mean + 2 SD of control-I; > 167 pg/ml) were found more frequently (47% SSc-I; 80% SSc-II) than high levels of CCL2 (> 542 pg/ml) (17% SSc-I, 15% SSc-II), suggesting a prevalence of Th1 immune response in early

phase of the disease, even if our study lacks a longitudinal evaluation. Our findings are fully consistent with the model in murine sclerodermatous graft-versus-host disease, in which increased expression of interferon- γ inducible chemokines were observed in early disease (Th1-like), with progression to a later noninflammatory (Th2-like) stage⁷. Indeed, high levels of CCL2 have been found^{8,9} in long-standing SSc, suggesting that the disease progresses from early inflammatory (Th1-like) to later noninflammatory (Th2-like) stages, when the fibrosis prevails over inflammation, and CCL2 shows profibrotic effects^{10,11}. However, serum levels do not necessarily reflect tissue levels, where disease is driven by a microenvironment of inflammatory mediators.

Regarding AT, our results obtained in a large cohort of newly diagnosed AT patients with SSc demonstrate increased CXCL10 with respect to SSc without AT, while CCL2 serum levels are not different between SSc-I and SSc-II, suggesting that Th2-CCL2 chemokine is not associated to AT in patients with SSc. These results are fully consistent with those obtained in a large cohort of newly diagnosed patients with AT who demonstrate increased CXCL10, especially in hypothyroid patients with a more aggressive disorder and normal CCL2 serum levels^{1,5,6,12,13}. Moreover, our results are in agreement with findings¹⁴ that showed type-1 activation in the peripheral lymphocytes of patients with SSc and coexistent AT.

The association of autoimmune disorders is a well known phenomenon^{1,14}, and chemokines may play a role in this process². The Th1 prevalence present both in SSc and AT, under the combined action of genetic and environmental conditions, may involve different organs in the same subject,

Table 1. Thyroid status of control subjects (control-I), autoimmune thyroiditis controls (control-II), SSc patients without or with autoimmune thyroiditis.

| | Control-I | Control-II Thyroiditis | SSc-I Without Thyroiditis | SSc-II With Thyroiditis | p |
|-------------------------------|---------------|----------------------------|------------------------------|-----------------------------|----------|
| N | 50 | 40 | 50 | 40 | |
| Age, yrs | 53 \pm 12 | 55 \pm 14 | 52 \pm 14 | 53 \pm 11 | NS |
| Sex, M/F | 6/44 | 4/36 | 6/44 | 4/36 | NS |
| Thyroid volume, ml | 11 \pm 9 | 14 \pm 13 | 10 \pm 9 | 9 \pm 9 | NS |
| Hypoechoic, % | 0 | 78 | 0 | 71 | 0.0001 |
| Hypervascular, % | 0 | 45 | 0 | 37 | 0.0001 |
| Serum TSH, μ U/ml | 1.3 \pm 0.8 | 1.7 \pm 1.8 | 1.2 \pm 0.7 | 3.1 \pm 2.1* | 0.006 |
| AbTPO, UI/ml | 10 \pm 10 | 168 \pm 371** | 9 \pm 7 | 120 \pm 412** | 0.0001 |
| AbTg, UI/ml | 10 \pm 10 | 197 \pm 306** | 13 \pm 11 | 178 \pm 503** | 0.0007 |
| AbTR, UI/ml | 0 | 0 | 0 | 0 | < 0.0001 |
| AbTPO positivity, % | 0 | 82 | 0 | 75 | 0.0001 |
| AbTg positivity, % | 0 | 78 | 0 | 63 | 0.0001 |
| Subclinical hypothyroidism, % | 0 | 5 | 0 | 18 | 0.004 |
| CXCL10, pg/ml | 83 \pm 42 | 150 \pm 131 [†] | 196 \pm 137 ^{††} | 254 \pm 98* | < 0.0001 |
| CCL2, pg/ml | 316 \pm 113 | 327 \pm 123 | 378 \pm 192 [†] | 403 \pm 131 ^{††} | 0.03 |

AbTPO: antithyroperoxidase antibody; AbTg: antithyroglobulin antibody; TSH: thyroidstimulating hormone; AbTR: antithyrotropin-receptor. * $p \leq 0.05$ vs control-I or vs autoimmune thyroiditis control-II, or vs SSc-I; ** $p \leq 0.05$ vs control-I; [†] $p < 0.05$ vs control-I or vs autoimmune thyroiditis control-II; ANOVA for continuous variables; chi-square for categorical variables. SSc: systemic sclerosis. NS: nonsignificant.

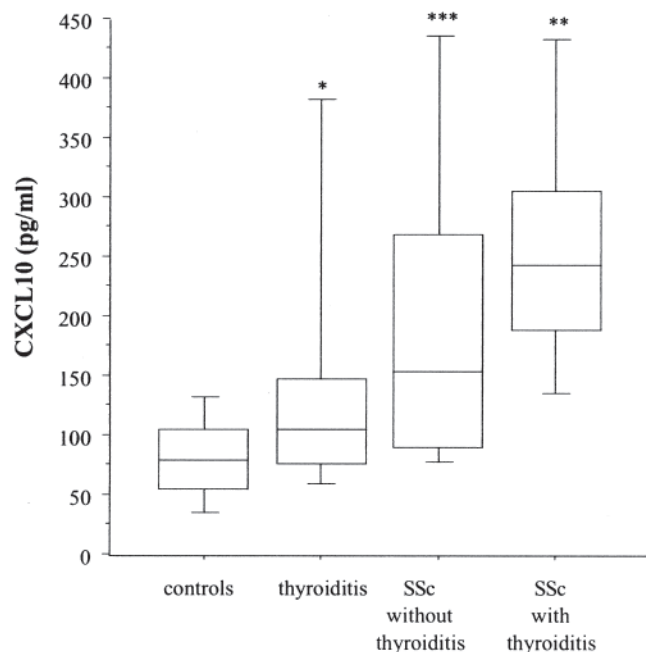


Figure 1. Serum CXCL10 levels are significantly higher in control-II (thyroiditis) (*), SSc without thyroiditis (***), and SSc with thyroiditis (**) than in control-I (controls) ($p < 0.001$, for all). SSc without thyroiditis have serum CXCL10 levels significantly higher than control-II ($p = 0.03$) and significantly lower than SSc with thyroiditis ($p = 0.04$) (ANOVA). SSc with thyroiditis have serum CXCL10 levels significantly higher than control-II ($p = 0.002$). Box indicates the lower and upper quartiles, center line is the median value; 2.5% and 97.5% values.

with the appearance of multiple immune mediated disorders, leading to different clinical disorders².

Our study demonstrates high serum levels of both CXCL10 (Th1) and CCL2 (Th2) chemokine in patients with SSc, and suggests a prevalence of Th1 immune response in the early phase of the disease. A further increase of serum CXCL10 is observed in SSc patients with AT, suggesting a superimposed Th1 response originating in the thyroid.

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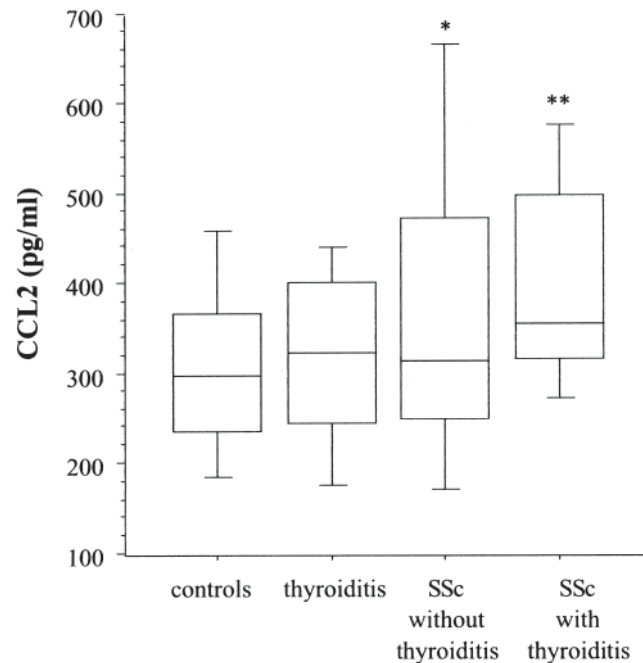


Figure 2. Serum CCL2 levels are significantly higher in SSc without thyroiditis (* $p = 0.03$), and SSc with thyroiditis (** $p < 0.01$) than in control-I (controls). SSc with thyroiditis have serum CCL2 levels significantly higher than control-II (thyroiditis) ($p = 0.04$) (ANOVA). Serum CCL2 levels are not significantly different (a) between controls and control-II and (b) between SSc without thyroiditis and SSc with thyroiditis.

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