Serum Monocyte Chemoattractant Protein-1 Is Increased in Chronic Autoimmune Thyroiditis

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Chemokines are a large family of cytokines, which may be involved in the pathogenesis of a wide variety of inflammatory or autoimmune conditions. The role of chemokines in chronic autoimmune thyroiditis is unknown. We sought to examine the role of CC chemokines in chronic autoimmune thyroiditis. We measured serum levels of CC chemokines, including monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein 1a and 1b (MIP-1a and MIP-1b) in 32 women with chronic autoimmune thyroiditis in comparison with 2 control groups (33 apparently healthy women and 43 women with benign cold thyroid nodules) by enzyme-linked immunosorbent assay (ELISA). We found a 45% increase in serum MCP-1 levels in women with chronic autoimmune thyroiditis compared with either of the 2 control groups ($P = .01$). There was no difference in either serum MIP-1a ($P = .69$) or MIP-1b ($P = .81$) levels between women with chronic autoimmune thyroiditis and controls. Among women with chronic autoimmune thyroiditis, women with a family history of hypothyroidism had a 59% increase in serum MCP-1 levels compared with women with no family history of hypothyroidism ($P = .02$). Serum MCP-1 levels were associated with serum levels of antithyroid peroxidase ($r = .2$, $P = .03$) (anti-TPO Ab) and antithyroglobulin ($r = .2$, $P = .04$) antibodies (anti-TG Ab). There was no association between serum MCP-1 levels and serum free thyroxine index ($P = .57$), triiodothyronine (T_3) ($P = .47$) or thyroid-stimulating hormone (TSH) ($P = .47$) levels. Serum MCP-1 is increased in women with chronic autoimmune thyroiditis, especially in the presence of a family history of hypothyroidism, indicating a possible pathogenetic role for MCP-1 in this condition.

CHEMOKINES ARE A large family of cytokines with diverse functions.[1,2] Based on structural criteria, chemokines are divided into 4 subfamilies, including CXC, CC, C, and CX_3C subfamilies.[1,2] Chemokines may be produced by a large variety of cells and may regulate immune responses.[3-5] CXC chemokines generally attract neutrophils, and CC chemokines generally attract monocytes, T lymphocytes, and natural killer (NK) cells.[6,7]

Although expression of CC chemokines, including monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein 1a and 1b (MIP-1a and MIP-1b) has been documented in several inflammatory and autoimmune conditions, including atherosclerosis, asthma, inflammatory bowel disease, and rheumatoid arthritis, their pathogenetic role in these conditions has not been fully elucidated.[1,8-15]

Autoimmune thyroid disease (AITD), including chronic autoimmune thyroiditis and Graves' disease, is characterized by the presence of diffuse mononuclear cell infiltration, which varies with the type and stage of AITD.[16-18] In chronic autoimmune thyroiditis, CD4 (helper) and CD8 (cytotoxic) T and B lymphocytes, plasma cells, NK cells, and macrophages are present in the thyroid and may lead to hypothyroidism.[16-19] Nonchemokine cytokines, including several interleukins (IL-2, IL-4, IL-6, IL-8), interferon gamma and tumor necrosis factor-α (TNF-α), are expressed in thyroid glands with chronic autoimmune thyroiditis, and serum IL-5 levels are increased in sera of patients with AITD.[20-22]

The role of CC chemokines in the pathogenesis of AITD has not been established. Previous data suggest that both MIP-1a and MIP-1b are expressed in the thyroid glands of patients with Graves' disease.[23] In addition, MCP-1 expression by thyroid cells in culture has been reported.[24] There have been no data on tissue expression or serum levels of CC chemokines in chronic autoimmune thyroiditis.

In the present study, we measured serum levels of CC chemokines, including MCP-1, MIP-1a and MIP-1b, in women with chronic autoimmune thyroiditis and 2 control groups (apparently healthy women and women with benign cold thyroid nodules).

MATERIALS AND METHODS

Patients

A total of 32 women with chronic autoimmune thyroiditis, who had a history of hypothyroidism, diffuse goiter, or both, and increased levels of serum thyroid-specific autoantibodies (antithyroid peroxidase [anti-TPO] or antithyroglobulin [anti-TG]) were consecutively recruited at the outpatient Endocrine Unit, Evgenidion Hospital, Athens, Greece. Thirty-three apparently healthy women with no clinical or laboratory evidence of thyroid disease and 43 women with history of single cold nodules and benign cytologic findings on fine needle aspiration biopsy were recruited as 2 separate control groups. Women in both control groups had normal serum thyroid hormone levels (free T_4 index [FTI] and total triiodothyronine [T_3]), and low or undetectable levels of serum thyroid-specific autoantibodies.

Exclusion Criteria

Women currently pregnant or with acute or chronic systemic diseases, including cardiovascular, pulmonary, gastrointestinal, hepatic, renal, or infectious disease or injury, were excluded from the study.

The study was approved by the Institutional Committee on Human Research. All study subjects provided written informed consent. Blood samples were obtained by phlebotomy, promptly spun, and sera frozen

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Submitted February 12, 2002; accepted March 19, 2002.

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0026-0495/02/5111-0047$35.00/0
doi:10.1053/meta.2002.34717

Table 1. Demographic and Clinical Characteristics of the Study Population

	Chronic Thyroiditis	Cold Nodule	Healthy Women	*P* Value
Age (yr)	43 ± 3	44 ± 2	34 ± 3	<.01
Height (m)	1.59 ± 0.01	1.61 ± 0.01	1.61 ± 0.01	.43
Weight (kg)	68.6 ± 2.6	69.5 ± 2.1	67.3 ± 2.9	.83
L-thyroxine therapy (%)	15/32 (47)	21/43 (49)	0/33 (0)	<.01
Cigarette smoking (%)	6/32 (19)	16/43 (37)	10/33 (30)	.17
Family history of hypothyroidism (%)	6/32 (19)	3/43 (7)	3/33 (9)	.25
Goiter or thyroid nodule (%)	19/32 (59)	43/43 (100)	0/33 (0)	<.01

at -70°C until assayed. Samples were batch processed to minimize interassay variation.

Assays

Serum levels of CC chemokines, including MCP-1, MIP-1a and MIP-1b, were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Quantikine; R & D Systems, Minneapolis, MN). The performance characteristics of the ELISAs were as follows: MCP-1: sensitivity 5.0 pg/mL, intra-assay coefficient of variation (CV) up to 5.9%, interassay CV up to 5.9%; MIP-1a: sensitivity 6.0 pg/mL, intra-assay CV up to 2.8%, interassay CV up to 6.9%; MIP-1b: sensitivity 4.0 pg/mL, intra-assay CV up to 9%, interassay CV up to 9.7%.

Radioimmunoassay (RIA) was used to measure serum levels of total T_4 (Amerlex-M T_4; Ortho Clinical Diagnostics, Amersham, UK) and total T_3 (Amerlex-M T_3, Ortho Clinical Diagnostics). Immunoradiometric assay (IRMA) was used to measure serum TSH levels (Incstar, Stillwater, MN) and serum anti-TPO (DiaSorin, Srl, Saluggia, Vercelli, Italy) and anti-TG (DiaSorin, Srl, Saluggia) antibody (Ab) levels, according to the manufacturers' instructions. The performance characteristics of the RIAs and IRMAs were as follows: total T_4: sensitivity 0.03 μg/dL, intra-assay CV up to 3.3%, interassay CV up to 4.7%; total T_3: sensitivity 0.1 ng/mL, intra-assay CV up to 3.7%, interassay CV up to 4.9%; TSH: sensitivity 0.03 μU/mL, intra-assay CV up to 3.3%, interassay CV up to 5.7%; anti-TPO Ab: sensitivity 1.0 U/mL, intra-assay CV up to 6.1%, interassay CV up to 9.1%; anti-TG Ab: sensitivity 5.0 U/mL, intra-assay CV up to 4.4%, interassay CV up to 11.6%.

The T_3 resin uptake (T_3RU) was measured as previously described and was used to estimate the free thyroxine index (FTI) by using the formula FTI = $T_4 \times (T_3RU)/100$.[25] The intra-assay CV of the T_3RU was 1.2% and the interassay CV was 1.4%.

An ELISA reader (SPECTRAmax 340PC^{384}; Molecular Devices, Sunnyvale, CA) was used to measure all ELISA plates, and a gamma counter (COBRA, Hewlett Packard, Palo Alto, CA) was used to count all RIA and IRMA tubes. Standard curves were plotted according to the manufacturers' instructions and used to estimate sample hormone concentrations.

Statistics

Data are presented as mean ± 1 standard error. As continuous variables were approximately normally distributed, data were analyzed using analysis of variance (ANOVA) and analysis of covariance (ANCOVA) followed by posthoc comparisons by means of Fisher's PLSD test (Statview v. 5, SAS Institute, Cary, NC). Additional analyses were performed using the Student's *t* test, the χ^2 test, and linear regression analysis as appropriate (Statview v. 5, SAS Institute). All statistical analyses were 2-tailed and *P* values less than .05 were considered significant.

RESULTS

The demographic and clinical characteristics of the study population are shown in Table 1. Women with chronic autoimmune thyroiditis or women with a single cold nodule were older than healthy women ($P = .01$). However, there was no difference in age between women with chronic autoimmune thyroiditis and women with a single cold nodule ($P = .62$). There was no difference in height ($P = .43$), weight ($P = .83$), current cigarette smoking ($P = .17$), or family history of hypothyroidism ($P = .25$) between study groups. Approximately 47% of women with chronic autoimmune thyroiditis and 49% of women with a single cold nodule and normal baseline thyroid function were receiving L-thyroxine therapy. Approximately 59% of women with chronic autoimmune thyroiditis had a goiter on physical examination.

Serum indices of thyroid function and serum thyroid-specific autoantibody levels are shown in Table 2. There was no difference in serum total T_4 ($P = .14$), FTI ($P = .30$), total T_3 ($P = .92$), or TSH ($P = .15$) between the 3 groups. By selection, both anti-TPO Ab ($P < .0001$) and anti-TG Ab ($P < .0001$) levels were significantly higher in women with chronic autoimmune thyroiditis.

Serum levels of CC chemokines, including MCP-1, MIP-1a

Table 2. Serum Indices of Thyroid Function and Thyroid-Specific Autoantibody Levels of the Study Population

	Chronic Thyroiditis	Cold Nodule	Healthy Women	*P* Value
Total T_4 (μg/dL)	9.3 ± 0.3	9.5 ± 0.3	8.8 ± 0.3	.14
FTI	2.6 ± 0.1	2.7 ± 0.1	2.4 ± 0.1	.30
Total T_3 (ng/mL)	110 ± 4	112 ± 4	113 ± 4	.92
TSH (μU/mL)	2.5 ± 0.5	1.5 ± 0.2	1.9 ± 0.2	.15
Antithyroid peroxidase Ab (U/mL)	296 ± 61	3 ± 1	3 ± 1	<.0001
Antithyroglobulin Ab (U/mL)	295 ± 55	24 ± 4	22 ± 4	<.0001

Abbreviations: Ab, antibodies; T_4, thyroxine; T_3, triiodothyronine; TSH, thyroid-stimulating hormone; FTI, free thyroxine index.

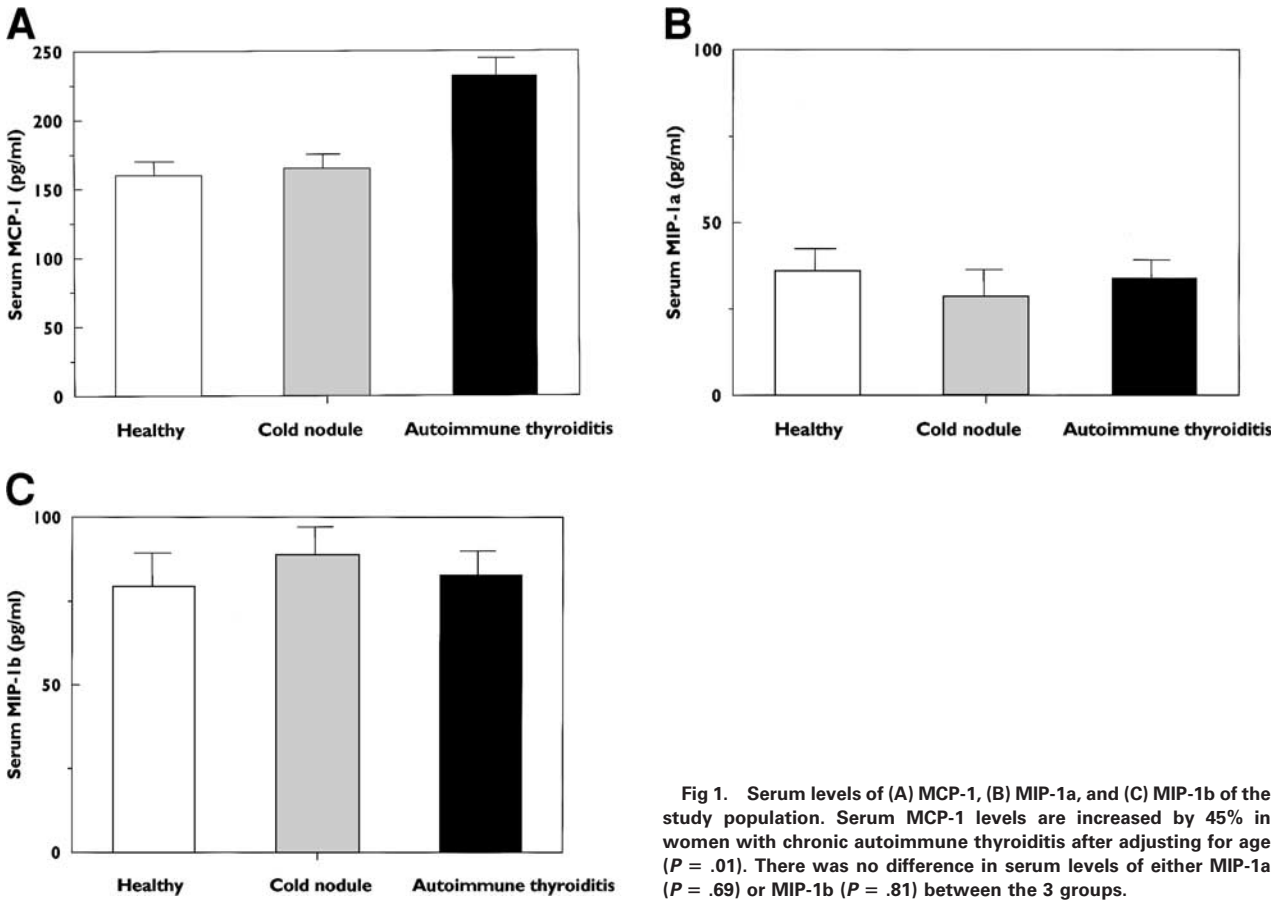

Fig 1. Serum levels of (A) MCP-1, (B) MIP-1a, and (C) MIP-1b of the study population. Serum MCP-1 levels are increased by 45% in women with chronic autoimmune thyroiditis after adjusting for age ($P = .01$). There was no difference in serum levels of either MIP-1a ($P = .69$) or MIP-1b ($P = .81$) between the 3 groups.

and MIP-1b, are shown in Fig 1. There was a 45% increase in serum MCP-1 levels ($P = .01$, Fig 1A) in women with chronic autoimmune thyroiditis (MCP-1: 232 ± 22 pg/mL) compared with either of the 2 control groups (MCP-1: 159 ± 20 pg/mL in healthy controls and 165 ± 19 pg/mL in women with cold nodules). After adjusting for age, the difference in serum MCP-1 levels remained statistically significant ($P = .01$).

There was no significant difference in serum MIP-1a ($P = .69$, Fig 1B) and MIP-1b ($P = .81$, Fig 1C) levels between women with chronic autoimmune thyroiditis (MIP-1a: 34 ± 6 pg/mL, MIP-1b: 83 ± 5 pg/mL) in comparison to either healthy controls (MIP-1a: 36 ± 3 pg/mL, MIP-1b: 79 ± 7 pg/mL), or women with cold nodules (MIP-1a: 29 ± 3 pg/mL, MIP-1b: 88 ± 14 pg/mL).

Women with chronic autoimmune thyroiditis and family history of hypothyroidism showed a 59% increase ($P = .02$, Fig 2) in serum MCP-1 levels (MCP-1: 332 ± 40 pg/mL) compared with women with chronic autoimmune thyroiditis and no family history of hypothyroidism (MCP-1: 209 ± 22 pg/mL).

Using univariate regression analysis, there was a positive association between serum MCP-1 levels and serum levels of anti-TPO Ab ($r = .2$, $P = .03$) or anti-TG Ab ($r = .2$, $P = .04$). However, there was no association between serum MCP-1 levels and serum FTI ($P = .57$), serum T_3 ($P = .47$), or serum TSH ($P = .47$) levels.

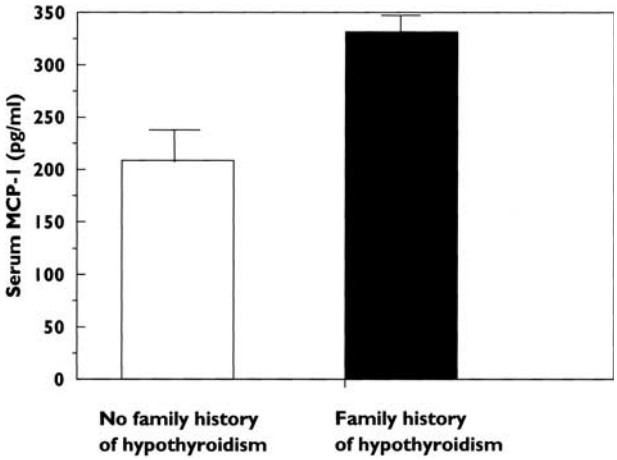

Fig 2. Serum MCP-1 levels in women with chronic autoimmune thyroiditis with or without a family history of hypothyroidism showing a 59% increase ($P = .02$) in serum MCP-1 levels among women with a family history of hypothyroidism.

DISCUSSION

In the present study, we measured serum levels of CC chemokines in women with chronic autoimmune thyroiditis in comparison to 2 control groups and found an increase in serum MCP-1 levels in the former group, but no difference in serum MIP-1a or MIP-1b levels. The difference in serum MCP-1 levels remained significant after adjusting for age.

Although we found no association between serum MCP-1 levels and serum indices of thyroid function, we could not determine the effect of L-thyroxine therapy on serum MCP-1 levels, as our study was cross-sectional in design.

We also found increased serum MCP-1 levels in women with chronic autoimmune thyroiditis and a family history of hypothyroidism. It is thus tempting to speculate that MCP-1 secretion may be genetically determined, at least in part, in women with chronic autoimmune thyroiditis.

An increase in MCP-1 level may have a role in the pathogenesis of chronic autoimmune thyroiditis, as this chemokine has chemoattractant properties for T lymphocytes, NK cells, and macrophages, which are all present in the thyroid gland of patients with this condition.[17,18,26] The association between serum MCP-1 level and serum levels of antithyroid-specific Ab also suggests that MCP-1 may affect immune responses in chronic autoimmune thyroiditis. Previous data support the hypothesis that MCP-1 is involved in regulating immune responses in animal models of nonthyroid autoimmune disease.[1,27]

Our study suggests an association between serum MCP-1 levels and chronic autoimmune thyroiditis. As our data do not demonstrate a cause-and-effect relationship, the role of MCP-1 in the pathogenesis of chronic autoimmune thyroiditis requires further investigation.

Both macrophages and thyroid follicular cells may secrete MCP-1 and, therefore, either cell type could be the source of MCP-1 detected in the serum of women with chronic autoimmune thyroiditis.[24,28-30] However, our study did not directly examine this hypothesis.

In conclusion, we found that serum MCP-1 levels are increased in women with chronic autoimmune thyroiditis, particularly in the presence of a family history of hypothyroidism, suggesting that MCP-1 may have a pertinent pathogenetic role. However, further studies are required to fully elucidate the role of MCP-1, as well as other chemokines, in the pathogenesis of chronic autoimmune thyroiditis.

ACKNOWLEDGMENT

The authors would like to thank Dr John Ladias (Division of Experimental Medicine, Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA) for helpful advice and assistance with the performance of the chemokine assays.

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