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Soluble Interleukin-2 Receptor Is a Thyroid Hormone-Dependent Early-Response Marker in the Treatment of Thyrotoxicosis

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Thyrotoxic patients exhibit increased levels of immune activation molecules (soluble interleukin-2 receptor [sIL-2R], intercellular adhesion molecule-1 [ICAM-1], and endothelial-leukocyte adhesion molecule-1 [ELAM-1]) in serum, although the clinical significance of these measurements remains unclear. In a randomized 4-week study, we have recently shown that in the treatment of hyperthyroidism, the combination of cholestyramine and methimazole (MMI) resulted in faster lowering of serum thyroid-hormone levels than did MMI alone. Stored serial serum samples from patients participating in this randomized treatment trial were analyzed for sIL-2R, soluble ICAM-1 (sICAM-1), and soluble ELAM-1 (sELAM-1). The levels of all three molecules were elevated in patients with hyperthyroidism. Although the levels of sICAM-1 and sELAM-1 remained elevated through the 4-week follow-up period in both groups of patients, the sIL-2R levels (normal levels, 1.0 to 4.2 ng/ml) decreased significantly in the 10 patients who received cholestyramine in addition to MMI (week 0, 14.2 ± 1.5 ng/ml; week 2, 10.8 ± 1.2 ng/ml; week 4, 8.9 ± 1.5 ng/ml). In eight patients who received MMI alone, sIL-2R decreased less rapidly (week 0, 12.3 ± 1.4 ng/ml; week 2, 12.3 ± 1.3 ng/ml; week 4, 10.9 ± 1.3 ng/ml). sICAM-1 and sELAM-1 were elevated at baseline but did not decrease during therapy. In the former group, free thyroxine and free triiodothyronine decreased faster. These data show that levels of sIL-2R in serum, but not those of sICAM-1 and sELAM-1, may be of clinical use in the early follow-up evaluation of medically treated patients.

Interleukin-2 receptor (IL-2R) is expressed on the surface membranes of activated immune cells, and a soluble form (sIL-2R) is detectable in serum (10). IL-2R concentrations are elevated in the sera of patients with several autoimmune disorders, including systemic lupus erythematosus (1), juvenile rheumatoid arthritis (1, 6), systemic sclerosis (11), and chronic active hepatitis (10), and may reflect disease activity.

Patients with Graves' disease have been reported to have elevated serum sIL-2R levels, thought initially to reflect an activated immune system (2). Subsequent cross-sectional studies, in which patients with hyperthyroidism of both autoimmune and nonautoimmune origin were studied, reported increased sIL-2R levels for both conditions (5, 7–9). Furthermore, sIL-2R levels correlated with levels of free thyroxine (FT_4) and free triiodothyronine (FT_3) in serum but not with the presence of circulating antithyroid antibodies (thyroid binding inhibitory immunoglobulin, thyroid-stimulating antibody, antithyroglobulin, or anti-thyroid peroxidase).

Lymphocyte adhesion molecules play important roles in the pathogenesis of autoimmunity and inflammation (13). In two recent studies, circulating levels of soluble forms of adhesion molecules were measured in the sera of patients with thyroid diseases. In one, increased concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) in serum were detected in patients with Graves' disease, particularly those with ophthalmopathy, but not in individuals with non-Graves' hyperthyroidism (3). Increased levels of soluble endothelial-leukocyte adhesion molecule-1 (sELAM-1) in serum were noted in patients with ophthalmopathy but not in patients with hyperthyroidism alone (3). In contrast, sICAM-1 and sELAM-1 have been reported by others to be elevated in patients with nonautoimmune hyperthyroidism (16).

Lack of prospective controlled studies has prevented determination of the potential clinical utility of these immune response-related molecules as auxiliary markers of thyroid disease. In the present study, serum samples from patients with active Graves' hyperthyroidism who participated in a controlled prospective therapeutic protocol were collected and the levels of sIL-2R, sICAM-2, and sELAM-1 were examined. Patients received either methimazole (MMI) alone or MMI along with cholestyramine. The latter regimen has been shown to reduce circulating thyroid hormone levels faster (14, 15). We assumed that any of the tested soluble markers reflecting disease activity would decrease during treatment. We found that sIL-2R levels dropped significantly in patients receiving both MMI and cholestyramine, suggesting a role for thyroid hormone in the activation of immune cells and the release of sIL-2R in the serum. sICAM-1 and sELAM-1 levels did not decrease during therapy.

MATERIALS AND METHODS

Subjects. Nineteen patients with newly diagnosed Graves' hyperthyroidism gave informed consent to participate in an Institutional Review Board-approved human-use protocol. Serum samples from patients were positive for thyroid-stimulating and/or -inhibitory immunoglobulins. Patients were divided into two groups matched for age, gender, and severity of disease. Group 1 patients (seven women and two men) received 30 mg of MMI daily in a single dose. Group 2

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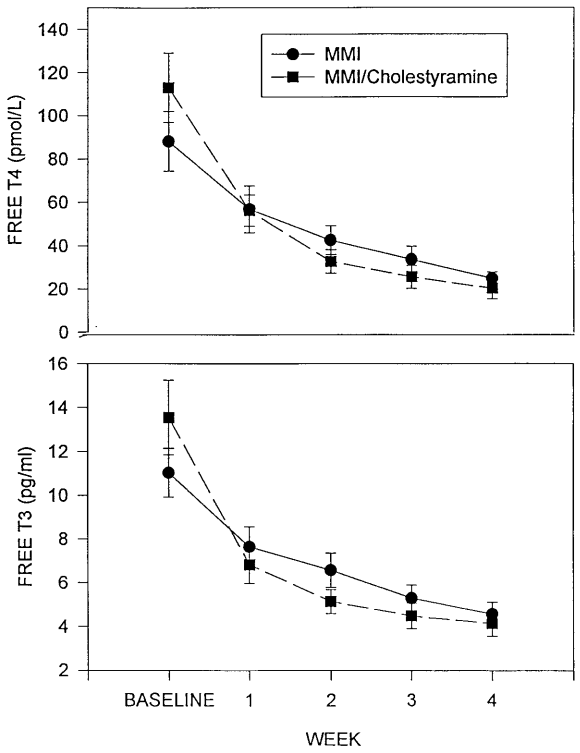

FIG. 1. Serum FT_4 and FT_3 levels in group 1 (MMI) and group 2 (MMI-cholestyramine) patients during the first 4 weeks of therapy. FT_3 levels decreased significantly more in group 2 than in group 1 ($P < 0.013$ by MANOVA) at weeks 1 to 3. Serum FT_4 levels did not show a significant group-versus-time effect ($F = 2.23$; $P = 0.075$).

patients (eight women and two men) received, in addition to MMI, 4 g of cholestyramine powder four times daily, to inhibit the enterohepatic recirculation of thyroid hormones. All patients received 50 mg of atenolol daily. Cholestyramine was taken at least 2 h before or after other medications to minimize potential interference with drug absorption. Disease severity was defined according to initial levels of total T_4 in serum, measured in micrograms per deciliter, as mild (13 to 17), moderate (17.1 to 21.5), or severe (≥21.6) (normal range, 4.5 to 12.5). Stored serum samples from a study reported in abstract form were used for the current investigation (15). These samples were aliquots that had been stored at −70°C and had undergone one freeze-thaw. Adequate samples were available from 19 of the 22 original subjects.

Methods. Serum FT_4 levels were measured by the Free T_4 Equilibrium Dialysis Kit (Nichols Diagnostics, San Juan Capistrano, Calif.); the normal range is 10.3 to 30.6 pmol/liter. Serum FT_3 levels were measured by solid-phase radioimmunoassay at Hazleton Laboratories (Vienna, Va.) by using the Diagnostic Products Corp. (Los Angeles, Calif.) assay kit. The normal range is 1.4 to 4.4 pg/ml.

sIL-2R was measured by enzyme-linked immunosorbent assay (ELISA) (Endogen, Cambridge, Mass.). In brief, serum samples or standards were incubated at room temperature for 2 h, washed three times, and incubated for 10 to 20 min with TMB substrate solution. The enzyme reaction was stopped by adding 4 N sulfuric acid, and the color was read at 450 nm. Normal values are 1.7 ± 0.7 ng/ml (mean ± standard deviation). sICAM-1 and sELAM-1 ELISAs were performed in a similar manner with kits obtained from Bender MedSystems (Vienna, Austria). Normal values are 230 ± 47 and 31.6 ± 12.8 ng/ml, respectively. Control serum samples for the present study, stored under similar conditions, were not available, so the normal values given above were obtained from the assay kits. Since prior studies have shown sIL-2R, sICAM-1, and sELAM-1 to be elevated in untreated hyperthyroid patients, and since the significant findings in the present study pertain to changes during treatment, it was believed that the conclusions drawn would be valid without a concurrent control group. Furthermore, control serum samples for sICAM-1 and sELAM-1, obtained at a similar time for other studies by two of the authors (G.C.T. and P.P.S.), had levels of 300 ± 136 and 34.4 ± 16.0 ng/ml ($n = 69$), respectively.

Statistical analysis. A multiple-analysis-of-variance (MANOVA) repeated-measures procedure was employed to examine levels of thyroid hormones and immune response markers in serum, looking for a group-by-time effect. Since every subject received antithyroid drug treatment, a time effect should occur; if

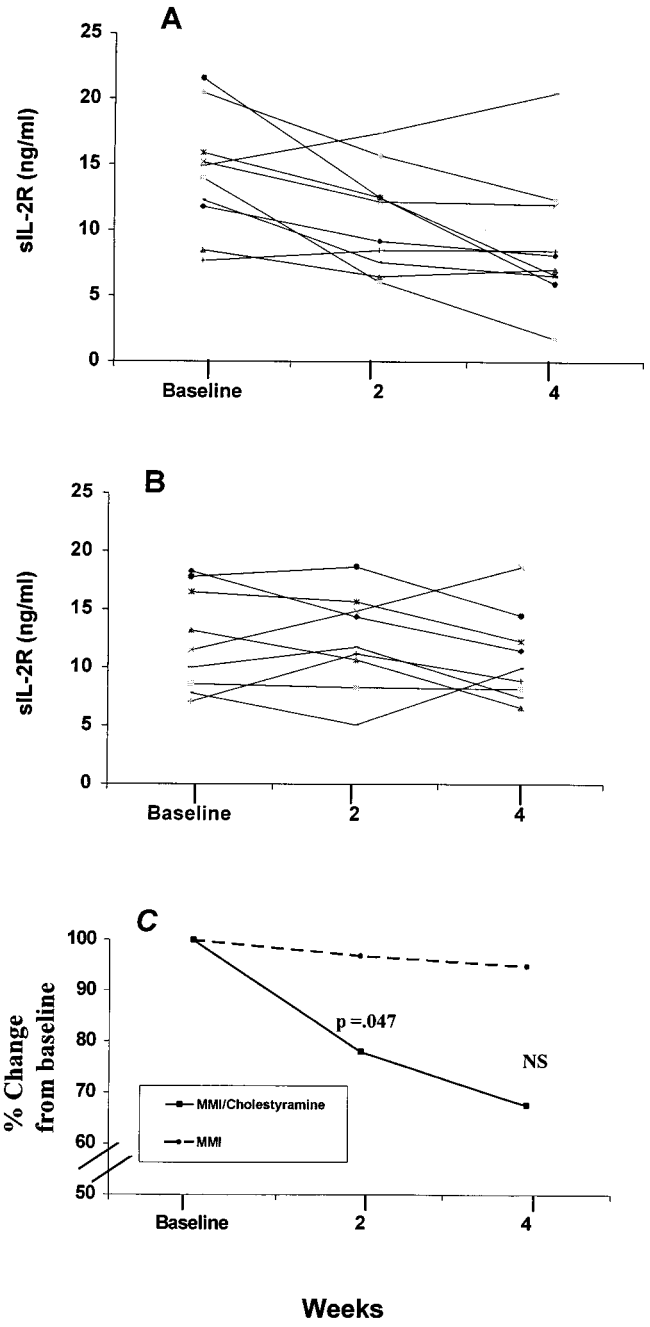

FIG. 2. Individual values for sIL-2R at baseline and at 2 and 4 weeks of treatment are depicted for group 2 (MMI-cholestyramine) (A) and group 1 (MMI) (B) subjects. There was no significant difference in baseline values between groups. (C) Percentage change from baseline for both groups.

groups were matched properly, a group effect should not occur. At each time point, the percent changes in FT_4, FT_3, sIL-2R, sELAM-1, and sICAM-1 were calculated and compared to baseline. *t* Tests were performed to determine whether the rate of change differed between groups. The SPSS-PC program (SPSS Inc., Chicago, Ill.). was used, with significance at a *P* value of <0.05.

RESULTS

The two groups of patients with Graves' disease did not differ at the beginning of the study with regard to gender distribution, age, or severity of disease. FT_4 ($P = 0.2$) and FT_3

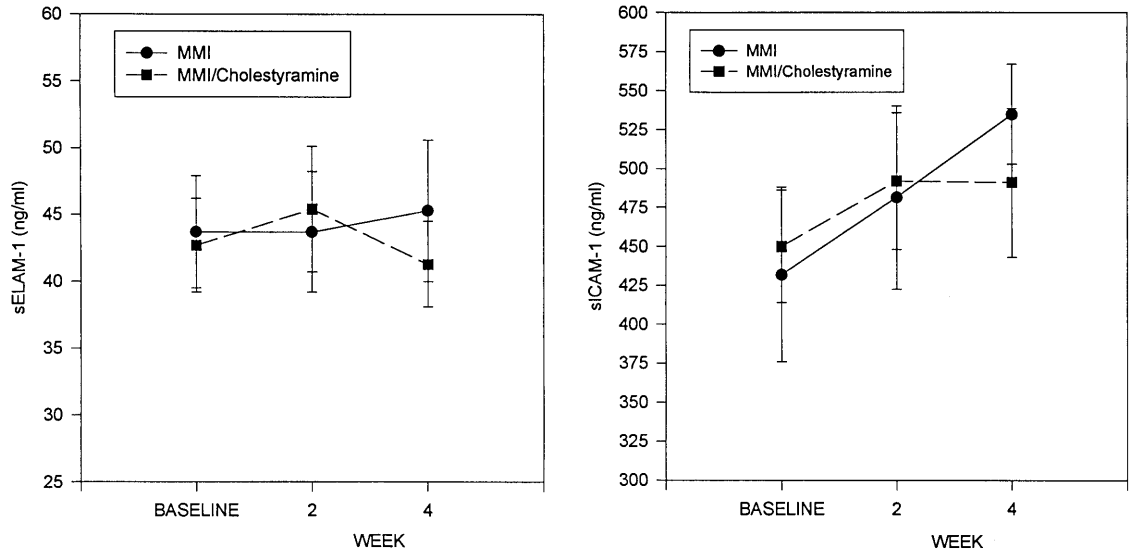

FIG. 3. sELAM-1 and sICAM-1 concentrations were increased in both group 1 and group 2 patients and did not decrease with lowering of thyroid hormone levels.

($P = 0.25$) did not differ at baseline (Fig. 1). The levels of FT_3 in serum were reduced significantly in both groups ($F = 3.42$; $P = 0.013$), but the levels in group 2 (MMI-cholestyramine) were lower than those in group 1 (MMI alone) at weeks 1 to 3. By week 4, there was no difference in serum FT_3 levels between the two groups. Serum FT_4 levels barely failed to demonstrate a statistically significant group-versus-time effect ($F = 2.23$; $P = 0.075$), indicating no significant difference in the rate of lowering of FT_4 levels between the two groups. However, in the original 22 subjects, the rate of lowering of FT_4 levels was greater in group 2. Both total T_4 and total T_3 levels in serum (data not shown) decreased significantly. The reduction was greater in group 2 for TT_4 at all times, and for TT_3 at weeks 1 to 3.

sIL-2R concentrations at 0, 2, and 4 weeks of therapy are depicted in Fig. 2. There was a significant 24% reduction at 2 weeks in patients taking both MMI and cholestyramine, with a further decrease at 4 weeks. The difference at 4 weeks was not significant, due to wide variance. In patients receiving MMI alone, reduction in sIL-2R was not observed until the 4th week of treatment.

We next considered whether levels of soluble forms of adhesion molecules in serum also reflect changes in levels of thyroid hormones in serum. We measured the concentrations of sICAM-1 and sELAM-1 in the serum samples and found them to be elevated in both study groups prior to therapy (Fig. 3). This finding is in agreement with those of earlier reports (3, 16). Neither adhesion molecule showed reduced levels in serum 2 or 4 weeks after the initiation of treatment. Although there was a trend toward an increase in sICAM-1 in the MMI group, this change was not statistically significant. Therefore, sICAM-1 and sELAM-1 levels, in contrast to sIL-2R levels, did not reflect a change in thyroid hormone levels during the duration of this study.

DISCUSSION

The present study documented that the addition of cholestyramine to MMI, as the initial therapy of patients with Graves' hyperthyroidism, produces a more rapid and greater reduction in T_4 and T_3 levels than does MMI alone (14, 15). In addition, in accordance with prior reports, levels of sIL-2R, sICAM-1, and sELAM-1 in serum were found to be increased in nontreated patients. This study documents that sIL-2R levels in serum reflect the levels of thyroid hormones in serum. Chow et al. (2) initially described increased levels of sIL-2R in serum in hyperthyroid patients with diffuse goiter; sIL-2R levels returned to normal after a mean of 3.6 months of therapy with antithyroid drugs. In addition, a correlation between sIL-2R and FT_3 or total T_3 in serum has been noticed (2, 5, 7–9), but no association between serum sIL-2R levels and the presence of any type of antithyroid antibody was observed (7–9). Subsequent reports have described elevated sIL-2R in patients with nonautoimmune hyperthyroidism, due either to toxic multinodular goiter (5, 9) or toxic adenoma (5, 7, 8). Furthermore, when T_3 was given orally to healthy subjects and euthyroid Graves' disease patients, there was a similar increase in sIL-2R levels in both groups (9).

While most studies suggest that thyroid hormone levels are influencing sIL-2R, few have prospectively examined the temporal relationship. Nakanishi et al. (9) found a reduction in sIL-2R after 3 months of therapy, and recently, Escobar-Morreale et al. (4) observed a reduction of approximately 35% in sIL-2R after 4 weeks of therapy and a 60% reduction by 12 weeks. In our study, sIL-2R was not reduced at 2 weeks but was significantly lower by 11% after 4 weeks of MMI alone. The reason for the lesser reduction compared to the report of Escobar-Morreale et al. (4) may be that our patients received a smaller dose of MMI. In contrast, the patients in our study who received cholestyramine with MMI had 24 and 37% reductions in sIL-2R at 2 and 4 weeks, respectively. These results provide further evidence that the concentration of sIL-2R is thyroid hormone dependent and that it is a relatively early circulating response marker of thyroid hormone action.

Adhesion molecules promote cell-cell communication and enhance the immune response. ICAM-1 binds to CD11a/CD18, increasing the effector function of T lymphocytes during inflammation. Elevation of sICAM-1 has been reported for several inflammatory conditions, including systemic sclerosis (11) and lupus (12). DeBellis et al. (3) have shown that

sICAM-1 levels are elevated in hyperthyroid Graves' disease patients, with the highest levels found in individuals who have ophthalmopathy. Euthyroid patients with eye involvement had higher levels than hyperthyroid patients without eye involvement. sELAM-1 levels were also increased, but the increases were related only to ophthalmopathy and not to hyperthyroidism. Neither adhesion molecule was increased in the sera of patients with toxic adenomas. These authors proposed that sICAM-1 might reflect orbital and thyroidal inflammation, while sELAM-1 might reflect only the former.

In our study, both sICAM-1 and sELAM-1 were elevated in untreated Graves' disease. Neither, however, returned towards normal at 2 or 4 weeks of therapy. In contrast, Wenisch et al. (16) found an increase in those two adhesion molecules in hyperthyroid patients due either to Graves' disease or toxic nodular goiter. ELAM-1 levels normalized after 4 weeks of therapy, while sICAM-1 levels returned partially to normal after 8 weeks of MMI. The decrease in sELAM-1 in the latter study might be explained by the larger dose of MMI (40 mg/day versus 30 mg/day in our study). However, both FT_4 and FT_3 were reduced substantially by 2 weeks in both studies. While agreement is not uniform, our results suggest that neither of these molecules is of value in monitoring the early course of treatment. Whether they are independent of thyroid hormone regulation, as suggested by our results but not by Wenisch et al. (16), requires further studies.

We cannot exclude the possibility that the more rapid effects on sIL-2R in the MMI-cholestyramine group are due to differences in the enterohepatic circulation of sIL-2R versus sICAM-1 and sELAM-1. However, the reduction in sIL-2R soon after FT_3 decreased, and the reports of others that sIL-2R decreases in patients not receiving cholestyramine, is consistent with a thyroid hormone-dependent effect.

In summary, elevated levels of sIL-2R (but not of sICAM-1 or sELAM-1) in Graves' disease decreased significantly within 2 weeks in response to reduction in levels of thyroid hormones. These observations suggest that thyroid hormones contribute to the production and/or release of IL-2R and that IL-2R can be used as an indicator of tissue response to treatment. Measurement of sIL-2R may be useful in characterizing the various tissue responses in patients with thyroid hormone resistance. The production and release of sIL-2R and the two adhesion molecules in patients with Graves' disease are controlled, at least in part, by different mechanisms.

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