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Decreased serum level of IL-7 in patients with active Graves' disease

Ewelina Motylewska [a,1], Martyna Nieć [a,1], Agnieszka Siejka [b,2], Jan Komorowski [b,2], Hanna Ławnicka [a,1], Jacek Świętosławski [c,3], Henryk Stępień [a,*]

[a] Department of Immunoendocrinology, Chair of Endocrinology, Medical University of Lodz, Sterlinga 3, 91-425 Lodz, Poland
[b] Department of Clinical Endocrinology, Chair of Endocrinology, Medical University of Lodz, Sterlinga 3, 91-425 Lodz, Poland
[c] Department of Neuroendocrinology, Medical University of Lodz, Sterlinga 3, 91-425 Lodz, Poland

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ABSTRACT

Background: Graves' disease (GD) is a common autoimmune disease which is one of the major causes of hyperthyroidism. Interleukin 7 (IL-7) has been recently reported to play an important role in various autoimmune diseases, but its role in the pathogenesis of GD has not been assessed. The aim of this study was to evaluate the levels of IL-7 and the soluble form of its receptor (sIL-7R) in the serum of GD patients, and to identify their association with disease activity.

Methods: A total of 37 GD patients were enrolled into the experimental group and 16 individuals into the control group. All patients were further classified into three subgroups: a GD-active group (hyperthyroidism and TRAb (thyroid stimulating hormone receptor antibody) >7.5 U/L) ($N = 15$), a GD-inactive group (euthyreosis and TRAb < 1 U/L) ($N = 8$), and other GD patients (euthyreosis and TRAb > 1 U/L) ($N = 14$). Concentrations of IL-7 and sIL-7R were assayed with ELISA. Additionally, the relationship between IL-7 and sIL-7R serum concentrations with disease activity (free triiodothyronine [FT3], free thyroxine [FT4], thyroid stimulating hormone [TSH] and TRAb) was also analyzed.

Results: The serum concentrations of IL-7 in GD-active patients were significantly lower than those of the control group as well as the GD-inactive and GD-other groups. The serum level of IL-7 in GD patients negatively correlated with FT4 and TRAb concentrations. Moreover, no significant difference was observed in the serum level of sIL-7R in GD patients compared to the control group.

Conclusions: These observations suggest that IL-7 may play a role in the pathogenesis of GD and may be associated with its clinical activity. To this end, the serum level of IL-7 could be an additional diagnostic biomarker predictive of the disease and could be particularly valuable for TRAb-negative GD patients.

1. Introduction

Interleukin (IL)-7 is a cytokine of IL-2 family, which also includes IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 [1]. Human IL-7 is a 25 kDa glycoprotein [2] which was first discovered in 1988 as a factor that promoted the growth of murine B cell precursors in a bone marrow culture system [3]. IL-7 secretion has been detected mainly in stromal cells, including epithelial cells in thymus and bone marrow [1]. In addition, intestinal epithelial cells, keratinocytes, hepatic tissue, peripheral blood dendritic cells, follicular dendritic cells, endothelial cells, smooth muscle cells and fibroblasts have also been shown to produce IL-7 [2].

The IL-7 receptor (IL-7R) consists of an α chain (IL-7Rα), shared with thymic stromal lymphopoietin (TSLP), and a common γ chain (γc), which is shared by the receptors for IL-2, IL-4, IL-7, IL-9 and IL-15 [4]. Both chains of IL-7R are needed for IL-7 signaling transduction [1,2]. IL-7 signaling, initiated with receptor heterodimerization, involves a number of tyrosine kinase pathways associated with the cytoplasmic tail of the receptor. These include the Janus kinase/signal transducer and activator of transcription (Jak/STAT) pathway, phosphatidylinositol 3-kinase (PI3-kinase) and Src family tyrosine kinases pathway [1,2,5]. Besides the cell-associated form of the IL-7 receptor, human plasma-soluble forms such as sIL-7Rα and sγc have also been detected, with the levels of sIL-7Rα being higher than those of sγc [6]. Plasma sγc in normal individuals is found mainly as a complex bound to sIL-7Rα. sIL-7Rα/sγc heterodimers were shown to be less numerous but to have binding sites with higher affinity than the sIL-7Rα monomer [6]. Considering the fact that these soluble proteins can bind IL-7 in a similar manner as their membrane

* Corresponding author. Tel./fax: +48 42 6324854.
E-mail address: henryk.stepien@umed.lodz.pl (H. Stępień).
[1] Tel./fax: +48 42 6324854.
[2] Tel./fax: +48 42 6331931.
[3] Tel./fax: +48 42 6644309.

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counterparts, they may also be involved in the critical role played by IL-7 in immune system.

IL-7 plays a fundamental role in T cell development, peripheral T cell homeostasis and metabolism, and immune tolerance. IL-7 provides a survival and proliferation signal in the early stages of T cell development ($CD4^{-}CD8^{-}$ double negative cells). These are followed by suppression of IL-7 signal transduction in the preselection of double positive thymocytes and its restoration after positive selection [1]. Although IL-7 is best known for its effects on developing T cell populations, it also modulates peripheral T cell functions via multifactorial mechanisms. First, IL-7 potently inhibits programmed cell death of mature T cells through maintaining a favorable balance of bcl-2 family members including Bcl-2 itself and Mcl-1 on the positive side, and Bax, Bad and Bim on the negative side [2]. IL-7 also costimulates T cell activation by enhancing proliferation and cytokine production [5]. The combination of enhanced costimulation and programmed cell death inhibition by IL-7 is likely responsible for the role of IL-7 in facilitating memory T cell differentiation [5]. Moreover, IL-7 enhances the cytolytic function of mature T cells and appears to enhance T cell–APC (antigen presenting cell) interactions [4,5]. Although IL-7 does not seem to be indispensable for human B-cell development, immature human B cells do proliferate in response to IL-7, and IL-7 treatment leads to the expansion of immature B cells in mice [5]. Additionally, other immune cells such as dendritic cells, peripheral blood monocytes, lymphoid tissues inducer (LTi) cells and innate lymphoid cells (ILC) respond to IL-7 [7]. IL-7 involves maintenance of glucose metabolism and possibly in pH regulation in dependent cells [2]. There is also increasing evidence that aberrant expression and dysfunction of IL-7 or other components in IL-7 signaling may be implicated in autoimmune diseases including rheumatoid arthritis, type 1 diabetes, multiple sclerosis and systemic lupus erythematosus, Sjögren's syndrome and inflammatory bowel disease [1,7].

Graves' disease (GD) is an autoimmune disorder characterized by the presence of autoantibodies that bind to and stimulate the thyroid stimulating hormone receptor (TSHR), resulting in hyperthyroidism and goiter. Extrathyroidal manifestations of GD include ophthalmopathy, dermopathy and acropachy [8]. The autoimmune response in GD is believed to result from a complex interaction between genetic susceptibility and environmental factors [8,9]. Although the detailed mechanisms are not fully understood, they are known to involve the destruction of the balance of pro- and anti-inflammatory cytokines, thyroid lymphocytic infiltration and B cell activation resulting in the production of TRAb (thyroid stimulating hormone receptor antibody), which has been considered to play a crucial role in the autoimmune process [8–11]. However, the role of IL-7 in the pathogenesis of GD has not yet been evaluated.

The aim of the study was to determine for the first time the serum concentration of IL-7 and sIL-7R in 37 patients with GD and 16 healthy controls. Additionally, the correlations between the levels of serum IL-7 and sIL-7R, and those of thyroid hormones and TRAb, were analyzed.

2. Subjects and methods

2.1. Individuals and samples

GD patients in different stages of the disease ($N = 37$; 7 males and 30 females; aged 25–82 years; mean age 50.9 years) were enrolled in the study. The diagnosis of GD was based on clinical criteria and confirmed by measurements of thyroid function and thyroid antibody level. Clinical evaluation included the patient's history as well as the presence of typical symptoms and signs of hyperthyroidism. The laboratory diagnosis included serum free triiodothyronine (FT3) and free thyroxine (FT4) concentrations, serum TSH concentration and TRAb. The exclusion criteria involved patients with other coexisting autoimmune diseases, severe systemic diseases or infectious diseases. The criteria for group division in GD patients included serum concentration of TRAb and thyroid hormones (FT3 and FT4). All 37 GD patients were further classified into three subgroups: the GD-active group (hyperthyroidism and high levels of TRAb > 7.5 U/L [12]) ($N = 15$), the GD-inactive group (euthyreosis and negative results for TRAb < 1 U/L) ($N = 8$), and other GD patients who did not meet the criteria of these two main groups (euthyreosis and positive results for TRAb > 1 U/L) ($N = 14$).

Sixteen healthy volunteers (8 males and 8 females; aged 19–74 years; mean age 50.5 years) were also recruited as a control group. The subjects within the control group were evaluated for any history of thyroid or other autoimmune diseases, as well as their present thyroid function and autoantibody level: FT3, FT4, TSH, thyroperoxidase antibodies (TPOAb), thyroglobulin antibodies (TGAb) or TRAb. Euthyreosis and negative results in autoantibody tests were confirmed in all subjects within the control group.

The study protocol was approved by the Ethics Committee of the Medical University of Lodz and written consent form was obtained from all subjects.

2.2. Sample preparation

Peripheral blood samples from all subjects were collected by venipuncture during a fasting state in the morning and centrifuged after clotting. The sera were stored at −70 °C before the serum levels of IL-7, sIL-7R and biochemical markers were measured. Repeated freeze–thawing was avoided.

The serum concentration of TSH was measured by automated electro-chemiluminescence binding assay (ECLIA) (COBAS e 411; Roche) according to the manufacturers' protocol (reference range: 0.27–4.2 μIU/mL; sensitivity range: 0.005–100 μIU/mL).

The serum concentrations of FT3 and FT4 were assessed by automated chemiluminescent magnetic immunoassay (CMIA) (ARCHITECT i 1000SR; Abbott) according to the manufacturers' protocol (reference range for FT3: 1.71–3.71 pg/mL; sensitivity range for FT3: 0–30.0 pg/mL; reference range for FT4: 0.7–1.48 ng/dL; sensitivity range for FT4: 0–6.0 ng/dL).

The serum concentrations of TPOAb and TGAb were measured by automated enzymatic chemiluminescent assays (IMMULITE 1000; Siemens) according to the manufacturers' protocol (reference range for TPOAb: <35 IU/mL; sensitivity range for TPOAb: 7–1000 IU/mL; reference range for TGAb: <40 IU/mL; sensitivity range for TGAb: 10–3000 IU/mL).

The serum concentration of TRAb was measured using a radio receptor assay (RRA) manufactured by BRAHMS GmbH according to the manufacturers' protocol (reference range: <1 IU/L negative, 1–1.5 IU/L borderline, >1.5 IU/L positive; sensitivity range: 0–40 IU/L).

The mean values of TSH, FT3, FT4 and TRAb level in GD patients and the control group are shown in Table 1.

2.3. The serum concentration of IL-7

The serum concentrations of IL-7 were tested in all 37 GD patients and 16 controls using commercially available ELISA kits (Quantikine® HS ELISA, Human IL-7 Immunoassay, R&D Systems; Sensitivity: 0.1 pg/mL; Assay Range for serum: 0.25–16 pg/mL; Intra-assay precision: 6.5%; Inter-assay precision: 11.8%) according to the manufacturer's instructions. Briefly, the standards and samples were incubated in a 96-well microplate precoated with an anti-IL-7 antibody. The optical density (O.D.) at 450 nm was measured using an automated microplate reader (METERTECH E960) and was positively correlated with IL-7 concentration. The

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