Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 63; Numer/Number 2/2012
ISSN 0423–104X

Serum interleukin-16 and RANTES during treatment of Graves' orbitopathy with corticosteroids and teleradiotherapy

Stężenie interleukiny 16 i RANTES w surowicy pacjentów z orbitopatią Gravesa w trakcie leczenia kortykosteroidami i teleradioterapii

Janusz Mysliwiec[1], Iwona Palyga[2], Agnieszka Nikolajuk[1], Aldona Kowalska[2], Maria Gorska[1]

[1]Department of Endocrinology, Diabetology and Internal Diseases, Medical University of Bialystok, Poland
[2]Department of Endocrinology, Holycross Cancer Centre, Kielce, Poland

Abstract

Introduction: To assess the usefulness of circulating IL-16 and RANTES measurements as markers of Graves' orbitopathy (GO) activity and to estimate the role of these cytokines in GO pathogenesis.

Material and methods: 42 individuals were divided into four groups: Group 1 comprised 15 euthyroid patients with clinical symptoms of GO who underwent corticosteroid therapy consisting of intravenous infusions of methylprednisolone (MP) and teleradiotherapy (TR); Group 2 comprised ten patients with hyperthyroid GD (Gtx); Group 3 comprised ten patients with GD in euthyreosis (Geu); and Group 4 comprised seven healthy volunteers age- and sex-matched to Groups 1–3. Serum samples were collected 24 hours before the first dose of MP, 24 hours after the first dose of MP, before TR, and at the end of therapy. Serum IL-16 and RANTES were determined by ELISA and TSH-Rab by RIA.

Results: Serum IL-16 levels in patients with GO were significantly elevated at the end of therapy: 346 pg/mL (257–538) compared to IL-16 values before treatment: 250 ng/mL (211–337) and to the control group. RANTES serum concentrations did not significantly differ between studied groups, and immunosuppressive treatment did not influence its level. A negative correlation between TSH-Rab and RANTES was found in all studied groups ($R = –0.32$, $p < 0.01$).

Conclusions: Our data suggests that IL-16 may exert an immunoregulatory effect in Graves' orbitopathy. Serum measurements of both IL-16 and RANTES may be clinically useful; however, establishing their place in the diagnostics and treatment monitoring of GO needs further research. **(Pol J Endocrinol 2012; 63 (2): 92–96)**

Key words: *cytokines, chemokines, TSH-Rab, Graves' disease, orbitopathy*

Streszczenie

Wstęp: Ocena przydatności oznaczania krążącej IL-16 i RANTES jako wskaźników aktywności GO oraz określenie roli tych cytokin w patogenezie GO.

Materiał i metody: 42 osoby w 4 grupach: 1 — 15 pacjentów z klinicznymi objawami orbitopatii w eutyreozie (GO), którzy poddali się leczeniu kortykosteroidami przy zastosowaniu podawanego dożylnie metylprednizolonu (MP) i teleradioterapii (TR); 2 — 10 pacjentów z chorobą Gravesa w nadczynności (Gtx); 3 — 10 pacjentów z chorobą Gravesa w eutyreozie (Geu); 4 — 7 zdrowych ochotników dobranych pod względem płci i wieku do grup 1.–3. Próbki krwi pobrano 24 h przed MP, 24 h po 1. dawce MP, przed TR i po zakończeniu leczenia. Stężenia IL-16 i RANTES w surowicy oznaczono metodą ELISA, a TSH-Rab — metodą RIA.

Wyniki: Stężenie IL-16 w surowicy u pacjentów z GO było istotnie wyższe po zakończeniu terapii — 346 pg/ml (257–538) w porównaniu z wartością IL-16 przed leczeniem — 250 ng/ml (211–337) i w odniesieniu do grupy kontrolnej. Stężenie RANTES w surowicy nie różniło się istotnie między badanymi grupami i leczenie immunosupresyjne nie wpłynęło na jej wartość. Wykazano ujemną korelację między TSH-Rab i RANTES we wszystkich badanych grupach ($R = –0,32$; $p < 0,01$).

Wnioski: Uzyskane dane wskazują, że IL-16 może wywierać immunomodulujący wpływ na przebieg orbitopatii. Zarówno oznaczenia IL-16, jak i RANTES w surowicy mogą być przydatne klinicznie, jednak ustalenie ich miejsca w diagnostyce i monitorowaniu leczenia GO wymaga dalszych badań. **(Endokrynol Pol 2012; 63 (2): 92–96)**

Słowa kluczowe: *cytokiny, chemokiny, TSH-Rab, choroba Gravesa, orbitopatia*

Introduction

Graves' orbitopathy (GO) is the most challenging clinical problem in the treatment of the common but poorly understood autoimmune syndrome, Graves' disease (GD). In the pathogenesis of GD, involving both thyroid and orbital connective tissue, a crucial role is attributed to thyrotropin receptor (TSH-R) as a main autoantigen [1, 2]. The presence of circulating antibodies against TSH-R (TSH-Rab) is a characteristic feature of GO and

Prof. Janusz Mysliwiec MD, PhD, Department of Endocrinology, Diabetology and Internal Diseases, Medical University of Bialystok, ul. Skłodowskiej-Curie 24 A, 15–276 Białystok, Poland, tel.: +48 85 746 82 39, fax: +48 85 744 76 11; e-mail: mysjan@poczta.onet.pl

a positive correlation has been found between TSH-Rab and the activity of GO [3, 4].

However, another autoantigen, namely insulin-like growth factor 1 receptor (IGF-1R), has been recently shown to play an important role in GO development. Immunoglobulins of patients with Graves' disease can induce orbital fibroblasts from patients with GO to produce hyaluronan and to up-regulate the synthesis of interleukin-16 (IL-16) and RANTES [5,6]. These effects are absent in fibroblasts from normal controls and are mediated through IGF-1R [7].

Interleukin-16 is a pleiotropic cytokine that is a natural ligand of CD4 [8]. It is a known chemoattractant for CD4+ T cells, monocytes, eosinophils and dendritic cells, with preferential chemoattractant activity for the Th1 subset of CD4+ T cells [9]. IL-16 has been shown to induce the high affinity IL-2R receptor (CD25) on CD4+ T cells that imparts responsiveness to IL-2 [10].

Regulated upon activation normal T cell expressed and secreted, also known as CCL5 (RANTES), was originally recognised as a product of activated T cells [11]. Now widely established as an inflammatory chemokine, CCL5 mediates chemotactic activity in T cells, monocytes, dendritic cells, natural killer cells, eosinophils, and basophils [12]. High serum concentrations of CCL5 have been associated with rapid progression of radiographic changes in rheumatoid arthritis [13].

Severe GO requires intensive treatment with immunosuppressive medication, most often by corticosteroids (CS) and teleradiotherapy (TR) [14]. However, the efficacy of both CS and TR is limited, and their use entails considerable risk. There have been many attempts to find reliable predictors of response to immunosuppressive treatment, but very few have proved useful in clinical practice.

Thus the aim of this study was to assess the usefulness of circulating IL-16 and RANTES measurements as markers of GO activity, and to assess the role of these cytokines and IGF-1R in GO pathogenesis.

Material and methods

The study was carried out in 42 individuals divided into four groups.

Group 1 (the GO group) comprised 15 GD patients with clinical symptoms of orbitopathy (ten females and five males, mean age 48 ± 12 years). Clinical features of subjects included in the study are shown in Table I. All these patients were euthyroid with thiamazol (patients 1, 3, 5, 9, 10, 11), levothyroxine (patients 4, 7, 12) or without treatment (patients 2, 6, 8, 13, 14, 15). In all patients, 7-item Clinical Activity Score of eye changes (CAS) was ≥ 3 and anamnesis of GO ≤ 1 year. All of them underwent 12 weeks of corticosteroid therapy consisting of intravenous infusions of methylprednisolone (MP): 6 × 500 milligrams and subsequently 6 × 250 milligrams. In the period of minor MP dose administration, teleradiotherapy (TR) (ten fractions of 1 Gy per day) was used in ten individuals (patients 6–15) with high activity of inflammation assessed in Magnetic Resonance Imaging (MRI). The serum samples were collected 24 hours before the first dose of MP, 24 hours after the first dose of MP, before TR (after the 6th dose of MP), and at the end of therapy.

Group 2 (the Gtx group) comprised ten patients with hyperthyroid untreated Graves' disease without GO symptoms: eight females and two males aged 54 ± 14 years with duration of the disease from 3–12 months. Thyrotoxicosis was confirmed with TSH, free thyroxin and triiodothyronin serum concentration measurement.

Group 3 (the Geu group) comprised ten patients with Graves' disease without GO symptoms in euthyreosis (at least three months) treated with thiamazol: seven females and three males, mean age 48 ± 18 years with a duration of the disease from 7–18 months.

Group 4 (the Ctrl group) comprised seven healthy volunteers age- and sex-matched to Groups 1–3: five females and two males aged 48 ± 13 years who had no family history of Graves' disease or of any other autoimmune disease.

Clinical euthyreosis in Groups 1 and 3 was confirmed by thyrotropin, free thyroxine and free triiodothyronin estimation. No acute infections were observed in patients three weeks prior to the study.

All the sera were kept frozen at –70°C until used. ELISA commercial kits were used to determine serum levels of IL-16 and RANTES (Quantikine, R&D Systems, Minneapolis, MN, USA; sensitivity respectively 6.2 pg/mL and 2.0 pg/mL; intra-assay coefficient of variation [CV] 4.8% and 2.4%). The serum levels of thyrotropin receptor antibodies (TSHRab) were determined by the RIA method (TRAK kit, Brahms, Berlin, Germany; sensitivity 0.3 IU/L; CV 7.0%).

The statistical significance was estimated by Mann-Whitney test. To evaluate relationships between variables, Spearman's test was performed using Statistica v. 9.0 for Windows XP (StatSoft, Tulsa, OK, USA).

Results

Figure 1 shows medians, interquartile and total ranges of IL-16 (in pg/mL). In the Ctrl group, IL-16 median was 280 pg/mL, in the Geu group, it was 302, in the Gtx group, it was 244, and in the GO group before treatment, it was 250. In GO 24 hours after the first dose of MP (GO1), it was 311, in GO after the 6th dose of MP (GO2), it was 348, and in GO at the end of therapy (GO3), it was 346 pg/mL. We found statistical significance in IL-16 values between GO3 *vs* the controls ($p < 0.02$) and

Table I. *Clinical features of studied patients with Graves' orbitopathy*
Tabela I. *Kliniczne cechy pacjentów z orbitopatią Gravesa poddanych analizie*

N	Age	Sex	Initials	GO duration (months)[a]	Strumectomy (months)[a]	Radioiodine (months)[a]	Smoking history	CAS	GO activity after therapy (MRI)
1	51	m	NJ	1	–	–	Current	4 → 1	–
2	25	m	MP	8	–	–	Current	3 → 1	–
3	50	f	WB	3	–	–	Current	3 → 0	–
4	52	f	TA	3	–	24	Current	3 → 1	–
5	48	f	ZM	8	–	–	Current	3 → 0	–
6	36	f	KE	4	–	–	No	3 → 1	–
7	76	m	WT	2	–	8	No	3 → 1	–
8	60	f	LH	6	11	–	Current	4 → 1	–
9	50	f	PB	6	–	–	Current	3 → 0	–
10	50	m	SD	12	–	–	Current	3 → 1	–
11	30	f	SK	3	–	6	Current	3 → 2	+
12	49	f	JJ	2	–	84	Current	4 → 1	+
13	41	f	SG	4	–	–	Current	4 → 2	+
14	52	f	CK	6	–	24	Current	3 → 0	+
15	51	f	WK	3	–	–	Current	3 → 1	+

[a]Period before GO treatment

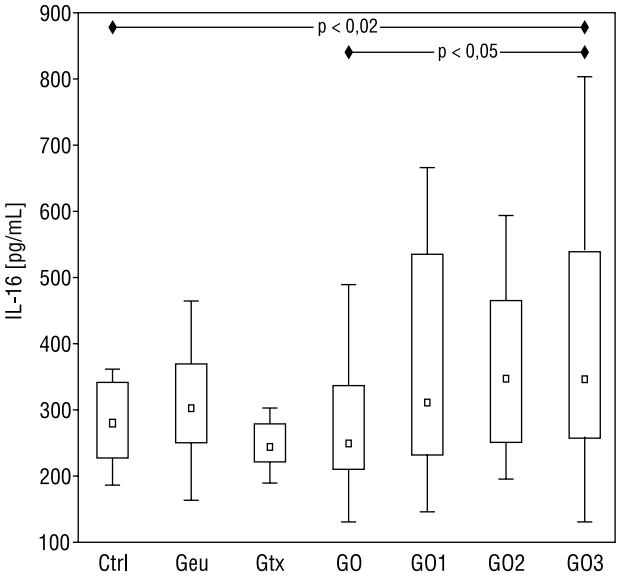

Figure 1. *Medians, interquartile and total ranges of IL-16 (in pg/mL) in the control group (n = 7), in patients with euthyroid Graves' disease (n = 10), in thyrotoxic Graves' patients (n = 10), and in individuals with Graves' orbitopathy (n = 15) before the treatment, after first methylprednisolone dose (GO1), after the 6th methylprednisolone dose (GO2), and at the end of therapy (GO3)*

Rycina 1. *Mediany, zakres kwartylowy i całkowity wartości IL-16 (w pg/ml) w grupie kontrolnej (n = 7), u pacjentów z chorobą Gravesa w eutyreozie (n = 10), u pacjentów z chorobą Gravesa w nadczynności tarczycy (n = 10) i u osób z orbitopatią Gravesa (n = 15) przed leczeniem, po 1. dawce metylprednizolonu (GO1), po 6. dawce metylpredizolonu (GO2) i po zakończeniu terapii (GO3)*

between IL-16 values in GO patients at the end of therapy (GO3) and basal values in this group (GO) ($p < 0.05$).

RANTES (in ng/mL) medians, interquartile and total ranges in the controls were 106 (65 [75–133] 160); in the Geu group they were 80 (47 [59–97] 125); in the Gtx group they were 67 (35 [55–92] 94); in the GO group before treatment they were 69 (27 [51–89] 119); in the GO group 24 hours after the first dose of MP (GO1) they were 74 (43 [64–86] 108); in the GO group after the 6th dose of MP (GO2) they were 81 (44 [47–96] 125); and in the GO group at the end of therapy (GO3) they were 80 (30 [54–115] 152). No statistical significance was found in RANTES serum concentrations between the studied groups, and immunosuppressive therapy in GO patients did not influence its value significantly.

Figure 2 shows medians, interquartile and total ranges of TSH-Rab (in IU/L). In the Ctrl group TSH-Rab median was 0.5 IU/L; in the Geu group it was 2.3; in the Gtx group it was 5.5; in the GO group before treatment it was 4.4; in the GO group 24 hours after the first dose of MP (GO1) it was 5.3; in the GO group after the 6th dose of MP (GO2) it was 3.8; and in the GO group at the end of therapy (GO3) it was 1.9. We found statistically significant differences in TSH-Rab values between Geu, Gtx and GO compared to Ctrl (for all relationships $p < 0.001$) and between TSH-Rab values in the GO group at the end of therapy (GO3) and basal values in this group (GO) ($p < 0.05$).

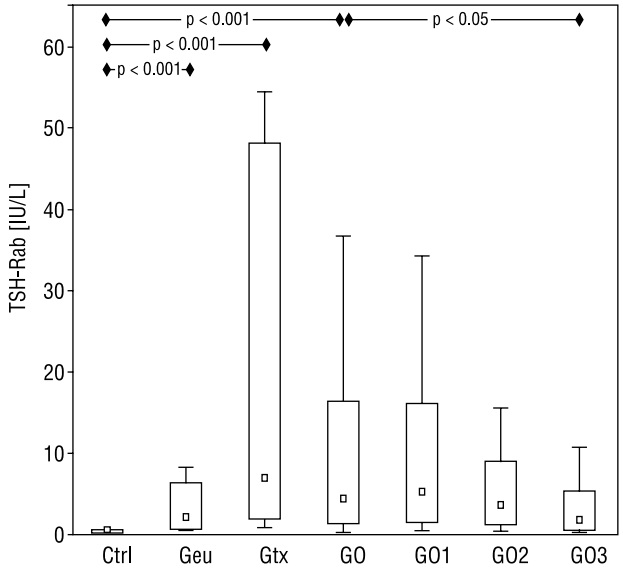

Figure 2. *Medians, interquartile and total ranges of TSH-Rab (in IU/L) in the control group (n = 7), in patients with euthyroid Graves' disease (n = 10), in thyrotoxic Graves' patients (n = 10), and in individuals with Graves' orbitopathy (n = 15) before the treatment, after first methylprednisolone dose (GO1), after the 6th methylprednisolone dose (GO2), and at the end of therapy (GO3)*

Rycina 2. *Mediany, zakres kwartylowy i całkowity wartości TSH-Rab (w IU/l) w grupie kontrolnej (n = 7), u pacjentów z chorobą Gravesa w eutyreozie (n = 10), u pacjentów z chorobą Gravesa w nadczynności tarczycy (n = 10) i u osób z orbitopatią Gravesa (n = 15) przed leczeniem, po 1. dawce metylprednizolonu (GO1), po 6. dawce metylpredizolonu (GO2) i po zakończeniu terapii (GO3)*

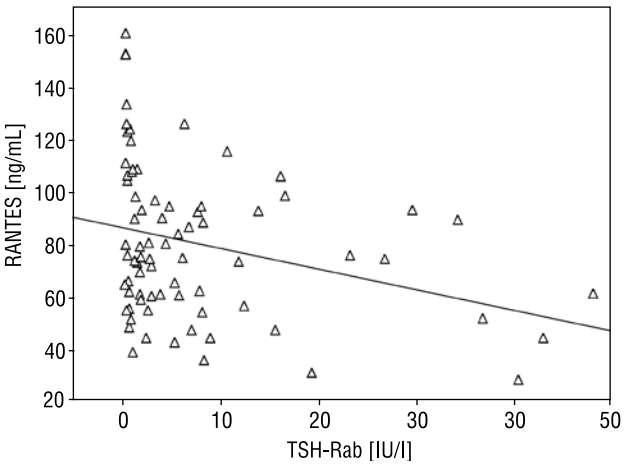

Figure 3. *Negative correlation between TSH-Rab (IU/l) and RANTES (ng/mL) in all studied groups (R = –0.32, p < 0.01)*

Rycina 3. *Ujemna korelacja pomiędzy TSH-Rab (IU/l) i RANTES (ng/ml) we wszystkich badanych grupach (R = –0,32, p < 0,01)*

The results of serum IL-16, RANTES (in ng/mL) and TSH-Rab in GO patients who responded to the therapy (no activity signs in MRI after treatment) (n = 10) and non-responders (signs of activity in MRI after treatment) (n = 5) did not differ significantly.

We found a negative correlation between RANTES (in ng/mL) and TSH-Rab (IU/L) (Figure 3).

Discussion

In our previous study, we showed the importance of CD40/CD40L interaction in GO development [15]. Data from our present study suggests that IL-16, a chemokine secreted in consequence of signal transduction via the CD40/CD40L pathway, may exert immunoregulatory properties in the autoimmune process in patients with GO. Immunosuppressive therapy with corticosteroids and teleradiotherapy led to a significant increase in serum IL-16. Recent studies suggest that the presence of IL-16 may contribute to the recruitment and induction of the peripheral expansion of CD4+ immunoregulatory T (Treg) cells at sites of inflammation [16]. Treg cells have been classified into three major groups: CD4+CD25+ cells, Tr1 cells, and Th3 cells [17]. These cells are distinct in terms of cytokine production and suppressive function. CD4+CD25+ cells generated in the thymus, also termed 'natural Treg cells', secrete both IL-10 and TGFß and have been shown by *in vitro* studies to be suppressive through cell-cell contact [18]. IL-16 has been recently shown to have chemoattractant activity for a T cell CD25+CTLA-4+FoxP3+ subpopulation, and is capable of suppressing the proliferation and production of Th2 cytokines of autologous T cells [16].

This is consistent with previous reports demonstrating the preferential effect of IL-16 on Th1 cell migration and the selective inhibition of Th2 cytokines [19]. Interestingly the diseases in which anti-IL-16 antibodies treatment is beneficial are generally classified as Th1-mediated, whereas IL-16 treatment is beneficial in Th2-mediated diseases. Recent data suggests the potential of IL-16 to recruit CD4+ T cells that are primarily Th1, including a preferential effect on Treg cells that have the ability to inhibit Th2 cell activation without affecting Th1 cell activity. We have not found significant alterations in RANTES serum concentrations during immunosuppressive therapy in GO patients, but, based on a negative correlation between RANTES and TSH-Rab, it could be speculated that, similarly to IL-16, RANTES may chemoattract Treg as immunomodulating factors.

TSH-Rab measurement has been found to be useful as a marker of GO activity and as a predictor of relapse in the treatment of Graves' hyperthyroidism [3, 4]. Our present TSH-Rab measurements are in line in these findings: we observed a gradual decrease of TSH-Rab concentration during immunosuppressive therapy. These clinical observations make the hypothesis of a pivotal role of TSH-R very attractive, although direct evidence is lacking. IGF-1R may be another important autoantigen in GO. Tsui et al.

suggested physical and functional relationships between IGF-1R and TSH-R based on colocalisation studies and the finding that monoclonal antibodies specifically blocking IGF-1R inhibit thyrotropin-induced kinase signalling [20]. Orbital fibroblasts in GO patients express higher levels of IGF-1R than normal fibroblasts [5]. Moreover, stimulation of orbital fibroblasts from GO patients to synthesise IL-16 and RANTES-chemoattractants of CD4+ cells by immunoglobulins γ fraction of serum samples obtained from GD patients has been shown to be mediated by IGF-1R- dependent pathway [6, 7].

In our study, IL-16 and RANTES serum concentrations were not elevated in GO patients compared to other groups.

Summary

In summary, our data suggests that IL-16 may exert an immunoregulatory influence in Graves' orbitopathy. Assessing the clinical usefulness of IL-16 and RANTES measurements, and defining the role of these cytokines and IGF-1 receptor in the pathogenesis of Graves' orbitopathy, needs further research.

Acknowledgements

This work was supported by UMB Grant 3-50691 (to JM).

References

1. Jyonouchi SC, Valyasevi RW, Harteneck DA, Dutton CM, Bahn RS. Interleukin-6 stimulates thyrotropin receptor expression in human orbital preadipocyte fibroblasts from patients with Graves' orbitopathy. Thyroid 2001; 11: 929–934.
2. Ludgate M, Crisp M, Lane C. The thyrotropin receptor in thyroid eye disease. Thyroid 1998; 8: 411–413.
3. Mysliwiec J, Kretowski A, Stepien A, Mironczuk K, Kinalska I. Thyrotropin receptor antibodies detected by the human recombinant TBII assay-a surrogate marker for autoimmune activity in Graves' orbitopathy? Med Sci Monit 2002; 8: MT159–162.
4. Eckstein AK, Plicht M, Lax H et al. Clinical results of anti-inflammatory therapy in Graves' orbitopathy and association with thyroidal autoantibodies. Clin Endocrinol (Oxf) 2004; 61: 612–618.
5. Smith TJ, Hoa N. Immunoglobulins from patients with Graves' disease induce hyaluronan synthesis in their orbital fibroblasts through the self antigen, IGF-1 receptor. J Clin Endocrinol Metab 2004; 89: 5076–5080.
6. Pritchard J, Horst N, Cruikshank W, Smith TJ. Igs from patients with Graves' disease induce the expression of T cell chemoattractants in their fibroblasts. J Immunol 2002; 168: 942–950.
7. Pritchard J, Han R, Horst N, Cruikshank W, Smith TJ. Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the IGF-1 receptor pathway. J Immunol 2003; 170: 6348–6354.
8. Rand TH, Cruikshank W, Center DM, Weller PF. CD4-mediated stimulation of human eosinophils: lymphocyte chemoattractant factor and other CD4-binding ligands elicit eosinophilic migration. J Exp Med 1999; 173: 1521–1528.
9. Lynch EA, Heijens CA, Horst NF, Center DM, Cruikshank W. Cutting edge: IL-16/CD4 preferentially induces Th1 cell migration: requirement of CCR5. J Immunol 2003; 171: 4965–4968.
10. Parada NA, Center DM, Kornfeld H et al. Synergistic activation of CD4+ T cells by IL-16 and IL-2. J Immunol 1998; 160: 2115–2120.
11. Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 1990; 347: 669–671.
12. de la Rosa G, Longo N, Rodriguez-Fernandez JL, Puig-Kroger A, Pineda A, Corbi AL. Migration of human blood dendritic cells across endothelial cell monolayers: adhesion molecules and chemokines involved in subset-specific transmigration. J Leukoc Biol 2003; 73: 639–649.
13. Boiardi L, Macchioni P, Meliconi R, Pulsatelli L, Facchini A, Salvarani C. Relationship between serum RANTES levels and radiological progression in rheumatoid arthritis patients treated with methotrexate. Clin Exp Rheumatol 1999; 17: 419–425.
14. Consensus statement of the European Group on Graves' orbitopathy (EUGOGO) on management of GO. Eur J Endocrinol. 2008; 158: 273–285.
15. Mysliwiec J, Waligorski D, Nikolajuk A, Gorska M. Soluble CD40 and its ligand CD154 in patients with Graves' orbitopathy during combined therapy with corticosteroids and teleradiotherapy. Adv Med Sci 2007; 52: 104–109.
16. McFadden C, Morgan R, Rahangdale S et al. Preferential migration of T regulatory cells induced by IL-16. J Immunol 2007; 179: 6439–6445.
17. Cottrez F and Groux H. Specialization in tolerance: innate CD4+CD25+ versus acquired Tr1 and Th3 regulatory T cells. Transplantation 2004; 77: S12–S15.
18. DiPaolo RJ, Glass DD, Bujwaard K, Shevach EM. CD4+CD25+ T cells prevent the development of organ-specific autoimmune disease by inhibiting the differentiation of autoreactive effector T cells. J Immunol 2005; 175: 7135–7142.
19. Hawrylowicz CM. Regulatory T cells and IL-10 in allergic inflammation. J Exp Med 2005; 202: 1459–1463.
20. Tsui S, Naik V, Hoa N et al. Evidence for an association between thyroid-stimulating hormone and insulin-like growth factor 1 receptors: a tale of two antigens implicated in Graves' disease. J Immunol 2008; 181: 4397–4405.