Soluble CD40 and its ligand CD154 in patients with Graves' ophthalmopathy during combined therapy with corticosteroids and teleradiotherapy

Myśliwiec J[1], Waligórski D[2], Nikołajuk A[1], Górska M[1]*

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

Abstract

Aim: To assess the role of CD40/CD154 interaction in GO pathogenesis and to estimate usefulness of soluble CD40 (sCD40) and CD154 (sCD154) measurements as markers of GO activity.

Material and methods: 61 individuals in 4 groups: 1) 15 euthyroid patients with clinical symptoms of ophthalmopathy (GO) who underwent corticosteroid therapy consisting of intravenous infusions of methylprednisolone (MP) and subsequent treatment with oral prednisone (P) and teleradiotherapy (TR); 2) 14 patients with hyperthyroid GD (GDtox); 3) 22 patients with GD in euthyreosis treated with methimazol (GDeu); 4) 10 healthy volunteers age and sex-matched to group 1-3. The serum samples were collected 24 hours before MP, 24 hours after MP, after TR and at the end of therapy. Serum CD40, CD154 and TPOab were determined by ELISA and TSHRab by RIA.

Results: Serum concentrations of CD40 (in pg/ml) and CD154 (in ng/ml) were increased in GO patients: 84.9 (74.7-93.9) and 4.0 (2.5-7.3) respectively in comparison to controls ($p<0.001$ and $p<0.05$ respectively). Serum CD154 in GO group was elevated as compared to both hyperthyroid and euthyroid GD without clinical ophthalmopathy ($p<0.001$ both). The sCD40/sCD154 quotient was significantly elevated during GO therapy with CS and TR in nonresponders after MP ($p<0.05$) and at the end of the study ($p<0.01$).

Summary: Our data suggest an important role of CD40/CD154 interaction in the pathogenesis of autoimmune process leading to inflammatory infiltration in Graves' ophthalmopathy, however usefulness of sCD40 and sCD154 measurements in prediction of effects of GO treatment and its monitoring needs further investigations.

Key words: sCD40, sCD154, TSHRab, TPOab, Graves' ophthalmopathy.

Introduction

Severe, progressive ophthalmopathy (GO) associated with Graves' disease (GD) is still the most difficult clinical problem in the treatment of this chronic autoimmune disorder [1]. Despite recent progress in understanding of GO pathogenesis it remains a pathogenetic enigma [2]. The characteristic manifestations of progressive GO such as proptosis, extraocular muscle disfunction, periorbital edema or loss of vision are a consequence of an increase in volume of extraocular muscles as well as connective and fatty tissues within a space confined by the orbit bones. The enlargement of retroorbital tissues is predominantly a result of an infiltration of mononuclear cells and accumulation of fibroblast-derived hydrophilic glycosaminoglicans (GAGs). Severe GO need to be intensively treated with immunosuppressive medication, most often by corticosteroids (CS) and teleradiotherapy (TR) [3]. However, the efficacy of both CS and TR is limited and their use entails considerable risk [1]. There have been many attempts to find reliable predictors of response to immunosuppressive treatment, but very few of appeared to be useful in the clinical practice.

CD40 – a member of the TNFα receptor superfamily is expressed on antigen-presenting cells and B and T cells. CD40 ligand (CD40L, CD154) was found on T cells. Ligation of CD40L on T cells by CD40 on B cells or other antigen-presenting cells was shown to be necessary for efficient activation of T-cell effector functions [4,5]. On the other hand binding of CD40 on B cells by its ligand promotes B cell survival and differentiation [6,7]. It has been shown recently that orbital

* CORRESPONDING AUTHOR:
Department of Endocrinology, Diabetology and Internal Diseases,
Medical University of Białystok
ul. M. Skłodowskiej-Curie 24 A, 15-276 Białystok, Poland
Tel. +48 85 7468239, Fax: +48 85 7447611
e-mail: mysjan@poczta.onet.pl (Janusz Myśliwiec)

Received 12.04.2007 Accepted 31.07.2007

Table 1. **Clinical features of the studied patients with Graves' ophthalmopathy**

N	age	sex	initials	GO duration (months)[a]	strumectomy (months)[a]	radioiodine history (months)[a]	smoking history	CAS	response to therapy of GO
1	57	*f*	MR	2	-	3	current	5→1	+
2	68	*f*	GM	4	-	-	current	5→0	+
3	52	*f*	ZJ	8	-	-	quit 12 m ago	5→0	+
4	56	*f*	GE	2	-	4	current	4→0	+
5	49	*f*	KE	2	84	48	current	4→0	+
6	48	*m*	KK	4	-	36	current	5→0	+
7	52	*f*	KH	3	-	8	no	4→1	+
8	53	*f*	FJ	6	48	24, 10	current	4→0	+
9	67	*f*	NT	2	-	172	no	4→1	+
10	66	*f*	SA	6	-	-	current	4→1	+
11	38	*m*	PP	6	-	-	current	5→2	-/+
12	52	*f*	GZ	6	-	-	no	4→2	-/+
13	54	*f*	PA	6	-	-	current	4→4	-
14	52	*f*	ST	6	-	7	current	4→4	-
15	41	*m*	CS	6	-	2	current	5→4	-

[a] – period before GO treatment

fibroblasts express intensively CD40 and its ligation by CD40L stimulates proinflammatory cytokines such as IL-6 and IL-8, GAGs synthesis, cyclooxygenase-2 activity and in consequence PGE2 production [8-10]. CD40/CD154 interaction in GO pathogenesis is suggested an important pathway of T cells induced fibroblast activation and proliferation.

Thus the aim of the study was to assess the role of CD40/CD154 interaction in GO pathogenesis and to estimate usefulness of soluble CD40 (sCD40) and CD154 (sCD154) measurements as markers of GO activity.

Material and methods

The study was carried out in 61 individuals divided into 4 groups:

1) 15 patients with clinical symptoms of ophthalmopathy (GO) (12 females and 3 males, mean age 54.4±8.8 years). Clinical features of subjects included to the study are shown in *Tab. 1*. Patients were euthyroid with thiamazol or levothyroxine (patients 5-9). In all patients Clinical Activity Score of eye changes (CAS) was ≥4 and anamnesis of GO ≤1 year. All of them underwent corticosteroid therapy consisting of intravenous infusions of methylprednisolone (MP) (6 series, 3 grams each time) and subsequent treatment with oral prednisone (P) (30 mg per day for 2 months and then a gradual tapering schedule with reduction of 5 mg per week). During prednisone administration teleradiotherapy (TR) was used in 10 fractions of 2 Gy per day. Magnetic Resonance Imaging (MRI) was used to assess retroorbital muscle volume, a presence of inflammation inside muscle tissue and features of proptosis;

2) 14 patients with hyperthyroid GD (GDtox): 10 females and 4 males aged 41±21 years with duration of the disease from 3-12 months. 3) 22 patients with GD in euthyreosis treated with methimazol (GDeu): 19 females and 3 males in mean age 40±13 years with a duration of the disease from 7-18 months;

4) 10 healthy volunteers age and sex-matched to group 1-3 (ctrl): 8 females and 2 males aged 41±16 years who had either no family history of Graves disease nor other autoimmune diseases. Clinical euthyreosis in groups of 1 and 3 was confirmed by thyrotropin and free thyroxine estimation. No acute infections were observed in patients 3 weeks prior to the study.

The serum samples were collected 24 hours before MP, 24 hours after MP, after TR and at the end of therapy. All the sera were kept frozen at -70°C until used. ELISA commercial kits were used to determine serum levels of CD40 and CD154 (Bender Medsystems, Vienna, Austria; sensitivity respectively 12 pg/ml and 0.062 ng/ml; intra-assay coefficience of variation (CV) 5.5% and 6.8%). To estimate antiperoxidase antibodies (TPOab) AxSYM Anti-TPO kit was used (Abbot Laboratories, USA; normal values <12 IU/ml; CV =9.2%). The serum levels of thyrotropin receptor antibodies (TSHRab) were determined by the RIA method (TRAK kit, BRAHMS, Berlin, Germany; sensitivity 0.9 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 6.0 for Windows XP (StatSoft, Tulsa, USA).

Results

Fig. 1 and *2* show medians and interqurtile ranges of sCD40 (in pg/ml) and sCD154 (in ng/ml) in GO patients: 84.9 (74.7--93.9) and 4.0 (2.5-7.3) respectively, GDtox: 50.5 (30.3-62.9) and 2.9 (1.4-4.8) respectively and GDeu patients: 41.6 (23.4--63.5) and 3.6 (1.5-6.2) respectively in comparison to the control group: 33.0 (18.3-40.6) and 2.2 (1.4-2.9) respectively.

The results of serum CD40 (in pg/ml), CD154 (in ng/ml), TSHRab (in IU/l) and TPOab (in IU/ml) in GO patients who responded to the therapy (satisfactory clinical effect, decrease of CAS ≥1) (n=10) and nonresponders (no clinical effect, no difference in CAS after treatment) (n=5) are shown in *Tab. 2* as median and interquartile range. Also statistical significance

***Table 2*. The medians and interquartile ranges of sCD40 (in pg/ml), sCD154 (in ng/ml), TSHRab (in IU/l) and TPOab (in IU/ml) in patients with Graves' ophthalmopathy in consecutive periods of treatment are shown in table 2 as median and interquartile range**

	before MP	after MP	after TR	after therapy
		sCD40		
responders	82.5 (74.7-91.8)	81.5 (74.3-90.7)	79.6 (74.4-90.5)	98.0 (86.3-102.5)
nonresponders	87.8 (80.1-92.1)	80.4 (69.3-89.3)	98.9 (91.2-110.9)	87.0 (76.5-89.5)
		sCD154		
responders	2.9 (2.3-6.0)	4.9 (3.1-7.2)	5.5 (4.2-6.4)	7.9 *# (3.8-11.1)
nonresponders	5.6 (5.4-7.7)	0.9 ** (0.8-1.9)	6.4 ## (6.1-7.6)	1.0 **•• (0.6-1.1)
		TSHRab		
responders	4.7 (3.4-15.5)	2.2 * (1.2-6.4)	3.1 (1.6-6.4)	2.0 *** (1.2-3.7)
nonresponders	16.8 (15.7-39.5)	3.9 (2.5-13.9)	3.2 (2.3-12.5)	4.6 (2.9-13.4)
		TPOab		
responders	182.5 (44.6-621.0)	60.1 (25.1-64.7)	60.1 (19.6-191.7)	59.5 * (16.9-132.8)
nonresponders	531.6 (349.4-657.0)	210.9 (178.6-235.0)	162.5 (120.3-166.2)	76.9 (64.3-82.5)

*p<0.05 vs I; **p<0.02 vs I; ***p<0.01 vs I; #p<0.05 vs II; ##p<0.02 vs II; •• p<0.02 vs III

***Figure 1*. Serum CD40 in GO (n=15), GDtox (n=14) and GDeu patients (n=22) in comparison to the control group (n=10)**

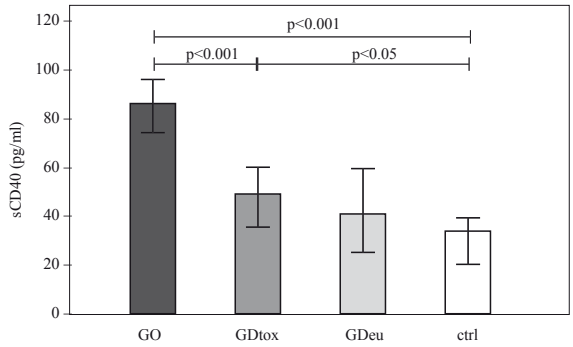


***Figure 2*. Serum CD154 in GO (n=15), GDtox (n=14) and GDeu patients (n=22) in comparison to the control group (n=10)**

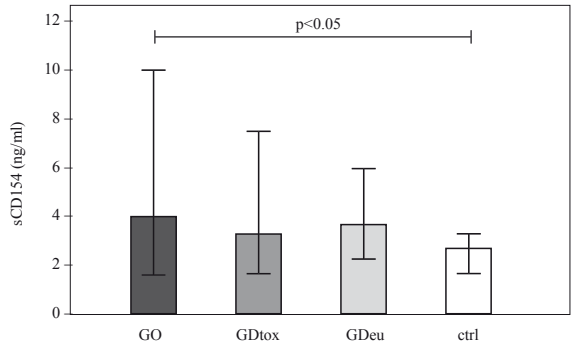


***Figure 3*. The quotient of sCD40/sCD154 in patients with Graves' ophthalmopathy during therapy**

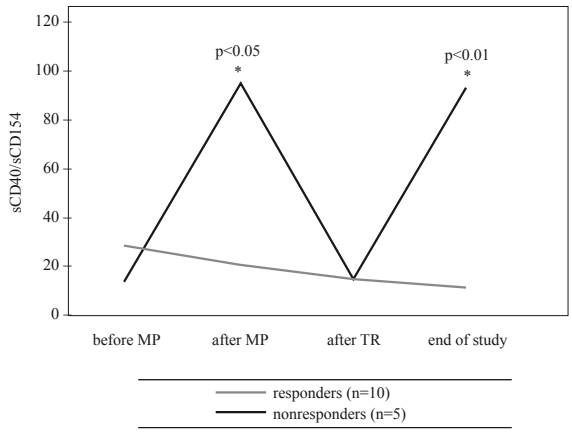
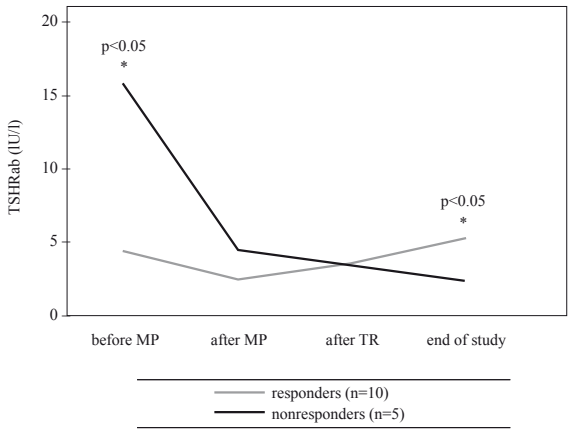


***Figure 4*. The median TSHRab values in patients with Graves' ophthalmopathy during therapy**



of differences in values of studied parameters in consecutive periods of treatment was shown in *Tab. 2*.

Fig. 3 and *4* show differences between the quotient of sCD40/sCD154 and values of TSHRab in GO groups of responders and nonresponders during therapy.

We have found no correlations between sCD40, sCD154, TSHRab and TPOab.

Discussion

TSHR has been hypothesized in GO pathogenesis to be a crucial autoantigen shared by thyroid and orbital tissues [11,12]. TSHRab measurement was found to be useful as a marker of GO activity and as a predictor of relapse in the treatment of Graves' hyperthyroidism [13-15]. Combined determination of TSHRab and TPOab has been recently shown to improve prediction of relapse of Graves' thyrotoxicosis [16]. Lai et al. have confirmed lately the presence of TPO in orbital tissues of GO patiens and suggested modulatory role of the immune responses directed against orbital TPO in clinical expression of GO [17]. Our present data of higher TSHRab in GO nonresponders both at the beginning and at the end of the study confirm previous results of TSRab usefulness in prediction and monitoring of GO therapy [13,14]. Significantly reduced TPOab concentration in responders suggest that TPOab measurement may be helpful as additional marker of immune process activity in GO.

Serum immunoglobulins from Graves' patients were shown to stimulate orbital fibroblasts to produce GAGs and T cell chemoattractants [18,19]. Fibroblasts, functioning as facultative antigen presenting cells, activate T cells via an antigen-dependent mechanism requiring an interaction of class II Major Histocompatibility Complex (MHC) and T cell receptor (TCR) (signal 1) and CD40–CD40L transduction (signal 2) [20]. In the antibody blocking experiments, blockade of class II MHC resulted in signal 2 without signal 1, whereas blockade of CD40 or CD40L costimulation resulted in signal 1 with no signal 2, both of which conditions result in T cell anergy [21,22]. These findings underline the importance of CD40-CD154 signaling pathway in the maintenance of autoimmunity. The candidate gene approach has led to findings of CD40 polymorphism associations with Graves' disease prevalence in some populations [23].

We found increased serum concentrations of CD154 and CD40 in GO patients in comparison to controls. Serum CD154 level in GO group was elevated as compared to both hyperthyroid and euthyroid GD without clinical ophthalmopathy. Moreover the sCD40/sCD154 quotient was significantly elevated during GO therapy with CS and TR in nonresponders suggesting CD40/CD154 pathway activity in ongoing autoimmune inflammatory process and intense infiltration of retrobulbar tissues by immunocompetent cells. An enhanced expression of CD154 on T cells and increased serum concentration of soluble CD154 was detected in patients with active systemic lupus erythematosus and rheumatoid arthritis in correlation with the relevant auto-antibodies and with the clinical disease activity [24,25]. Circulating CD154 may be cleaved from the cell surface of activated T cells and shares properties of the membrane form to stimulate CD40 receptor [26]. Elevated CD154 in the serum was suggested to be derived from the membrane-bound form of CD154 on activated T cells and to reflect autoimmune process intensity in lupus erythematosus [27]. Soluble form of CD40, produced by B cells cocultured with activated T cells, has been shown in vitro to hamper the binding of CD154 to CD40 through competition [28]. Our previous results suggest that soluble CD40 plays an important regulatory role in pathogenesis of Hashimoto's thyroiditis (in press). Contin et al. have demonstrated that soluble CD40 inhibits CD154 ligation onto membrane CD40 and strongly diminishes the production of immunoglobulins by CD154-activated B lymphocytes [29]. On the other hand, increased level of soluble CD40 may reflect more massive T cells infiltration of thyroid gland and/or retrobulbar tissues as degree of surface CD40 expression was shown to closely correlate with intensity of lymphocyte infiltration [30].

In summary, data of the present study suggest an important role of CD40/CD154 interaction in the pathogenesis of autoimmune process leading to inflammatory infiltration in Graves' ophthalmopathy. However, an assessment of usefulness of sCD40 and sCD154 measurements in prediction of effects of GO treatment and its monitoring needs further investigations.

Acknowledgements

This work was supported by AMB Grant 3-50736L (to JM).

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