NO CHANGES IN SERUM CONCENTRATIONS OF INTERLEUKIN 10 (IL-10) AND INTERFERON γ (IF-γ) BEFORE AND AFTER TREATMENT OF THE THYROID EYE DISEASE (TED)

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Abstract

TED is a severe eye disease leading in rare cases to decrease of sight, optic nerve compression and blindness. Recently, significant progresses in understanding the disease have been done. Nevertheless, the treatment of the disease, especially in its severe form remains challenging. Glucocorticoids (GC) have been the basis of the treatment for a long time. Orbital irradiation (OI) and optical decompression (OD) are also used in managing the severe forms of TED. Somatostatin, intravenous immunoglobulin have been also used, with conflicting results. Regarding the potential for the treatment of TED with cytokine antagonists, controlled clinical studies are not available. Since cytokines play an important role in the pathogenesis of the TED, they seemed to be logical choice for modern TED treatment. It has been shown that both Th1 (interleukin-2, tumor necrosis factor γ, interleukin γ) and Th2 (interleukin-4,-5-,-10) profile T cells are activated in the TED. We therefore measured interleukin-γ, IF-γ and interleukin -10 (IL-10)(Th1 and Th2 pattern) to assess its relationship to the course of the disease. This paper shows that both Th1 (Il-2) and Th2 (If-γ) pathways represented by those two cytokines are not involved (Il-10 before 2,29±5,23 and after treatment 3,77±8,44; IF γ before 0,50±0,24 and after treatment 0,35±0,19). No relationship to the response to treatment was found. GC resulted in positive response in 8/22 patients, OI (12 patients) given after CS therapy, resulted in a response in all patients. Increase in proptosis, loss of visual acuity is spite of CS treatment prompted OD in two patients, who both recovered visual acuity and proptosis fell under 25mm Hertel.

KEY WORDS: thyroid eye disease, treatment, corticosteroids, orbital irradiation, orbital decompression.

Introduction

The pathogenesis of TED is still not quite clear. It has long been proven that there is an increased volume of the extraocular muscles, as well as the increased orbital adipose and connective tissue. There is an increased concentration of glucosoamminogycans and a marked infiltration with immunocompetent cells. T cells (mainly CD4+), macrophages, B lymphocytes have been demonstrated in muscles, fat and connective orbital tissue. The general conception of the immune reactions in TED is as follows: auto reactive T lymphocyte recognize antigen(s) shared by the thyroid and the orbit, lymphocytes infiltrate the tissues (facilitated by locally or circulated adhesion molecules (1, 2), CD4+ T lymphocytes secrete cytokines amplifying the immune reaction by activating CD8+ T lymphocytes or antibody-producing T cells (3). Both Th1 (interleukin-2, tumor necrosis factor α, interleukin γ) and Th2 (interleukin-4,-5,-10) profile T cells are activated. Cytokines influence histocompatibility class II molecules, heat-shock protein-72, stimulate the proliferation of fibroblasts, and stimulate fibroblasts to produce GAGs (4). The predominant data point towards the TSH receptor as the autoantigen in TED (5,6). However, it has been suggested that orbital antigen cross reacting with a thyroid antigen might be located in the eye muscle cells (flavoprotein subunit of mitochondrial succinat dehydrogenase; 7). Other orbital autoantigens have been also implicated: calsequestrin (8), sarcalumenin (9), GS2 – a novel eye muscle protein (9). Most of the anigens (with the notable exception of GS2, a cell-membrane antigen) are intracellular and ubiquitus)(10). In addition, a Fas mediated apoptosis was demonstrated in extraocular muscle tissue in TED patients (11). It has been demonstrated that both Th1 (interleukin-2, tumor necrosis factor α, interleukin γ) and Th2 (interleukin-4,-5,-10) profile T cells are activated in TED. We therefore measured IF γ and IL-10 to assess the Th pattern and its relationship to the course of the disease.

Materials and Methods

It has been shown that both Th1 (interleukin-2, tumor necrosis factor α, interleukin α) and Th2 (interleukin-4,-5,-10) profile T cells are activated in the TED. We therefore measured interleukin γ and interleukin -10 to assess its relationship to the course of the disease. 22 patients with untreated GO, with proptosis of ≥ 25 mm, and/or impaired eye muscle motility (diplopia) were investigated. All were euthyroid (free T4 (fT4) 10–23 pmol/l, total T3 1,3–2,7 nmol/l, thyroid-stimulating hormone (TSH < 4,0 mU/l). Cytokine measurements were performed before and after TED treatment. Four patients had decreased visual acuity, reduction of the amplitude of the visually evoked cortical potential, and a central scotoma. Corticosteroids were given as follows: methylprednisolone 240 mg per day for seven days intravenously, 120mg for 2days, than orally 80, 60, 40, 20 mg every two days (12). Retrobulbar radiotherapy consisted of 10 fractions of 20 Gy, in 2 weeks (13). Clinical Activity Score (CAS) quantified the TED activity (14). The method consists of 10 items, for each item present 1 point is given, with the maximal score of 10. Duration of both TED and GD in months was also noted. Corticosteroid therapy resulted in response of 8/22 patients, OI (12 patients) given after CS therapy, resulted in a response in all patients, while OD was performed in two patients (after a lack of response to CS treatment, with rapid increase in proptosis, and loss of visual acuity). Both OD treated patients recovered visual acuity and proptosis fell under 25 Hertel. fT4 was enzymaticaly determined. Serum samples were kept stored at –20°C until use. All samples were measured in duplicate. Highly sensitive, commercially available ELISA were used to measure serum concentrations, IL-10 (detection limit 0•094 pg/ml), and IF γ (detection limit 30 pg/ml), After descriptive statists was done, *t*-tests and in case of abnormal distribution, the Mann–Whitney *U*-test were used. Multivariate ANOVA test for multiple comparisons was also used. T-tests and in case of abnormal distribution, the Mann–Whitney *U*-test were used. Correlations were calculated with two-tailed Pearson correlation coefficients. Changes in serum cytokine concentrations were evaluated with repeated measurement *F*-tests.

Results

At the start of the disease, free thyroxin was high, TSH low, thyroid ultrasound showed diffuse thyroid enlargement. PTU or MMI resulted in clinical and hormonal remission. Several months after the initiation of the signs of hyperthyroidism, a progression in the ophthalmopathy was observed (Hertel up to 30mm: normal 17 mm) while patients were clinically and hormonally euthyroid. Blood was collected in euthyroid state (with TED signs present, before TED treatment) and after 3 months of treatment (patients without TED and without treatment). CS resulted in response of 8/22 patients, OI (12 patients) given after CS therapy, resulted in a response in all patients. Increase in

Number of patients	Before treatment	After treatment
	IL-10 Reference values (0-6,8)	IF- γ Reference values (0-1,5)
22	2,28±5,23	0,50±0,24
22	3,77±8,44	0,35±0,19

TABLE 1. Serum concentrations of IL-10 and IF- γ in patients with TED (pg/ml)

proptosis, loss of visual acuity is spite of CS treatment prompted OD in two patients, who both recovered visual acuity and proptosis fell under 25mm Hertel. No significant difference was found in the serum levels of IL-10 between the groups of controls (reference values 0-6.8 pg/ml) and patients before treatment (2,28±5,23 and after treatment 3,77±8,44; p<0,005). Serum IF γ (reference values 0-1,5 pg/ml) was also unchanged before and after treatment (before 0,50±0,24 and after treatment 0,35±0,19) (Table 1). The severity of TED was not linked to serum concentrations of IL-10 or to IF γ. The involvement of different cytokines is a subject of active investigation. Jones et al. (15) investigated patients with GD and found increased IL-10 after radiotherapy. Their data showed also reduced IF γ production before treatment. The more prolonged serum concentrations were only found in IF γ. In vitro fibroblasts from patients with TED, were shown that Th1 clones secrete more IF γ. The orbital volume in TED was positively correlated with Il-6 mRNA expression and negatively with Il-4 and Il-10 expression (16). IF γ mRNA was less often detected in orbital fibroblasts than IL-10 mRNA (16). When IF γ was added to orbital fibroblasts inhibited adipocyte differentiation. In active TED (17) significantly higher IF-γ was found. Mysliewec et al. (18) showed up regulation of IL-10 after successful TED treatment. Two thirds of TED regress spontaneously (19). Those patients require either no treatment or only supportive measures (20). Most of the patients present eyelid retraction, followed by proptosis, extra ocular muscular dysfunction. Optic neuropathy is relative rare: only 6% in the series of Barltey (20). The most common complaints in their series were diplopia, pain or discomfort, lacrimation, photophobia or blurred vision. However, severe TED forms can lead to optic nerve compression and loss of sight. Although the definition of severe TED is arbitrary, substantial reduction in visual acuity or marked degree of proptosis are generally held to be sufficient to mark the TED as severe. Soft tissue involvement, although sometimes striking is not a sign of severe TED. Diplopia can markedly hamper the patient's activity. Mourits et al. (21, 14) proposed nowadays well accepted clinical activity score (CAS) with 10 different items reflecting mostly the inflammatory changes. A point is given to each manifestation and the score results: from 0 (no activity) to 10 (highest activity). This score can be a good predictor of treatment response (14). The NO SPECS classification defines the numerical score (ophthalmopathy index) of the disease (N no signs or symptoms, O only signs, S soft tissue involvement, P proptosis, E extraocular muscle involvement, C corneal involvement, S sight loss). The total eye score gave different weight to various items (22). Thyroid Sister Societies (23) revised the classifications mentioned. In general, improvements in the assessment of ocular changes have been done, since the standardization of the assessment is crucial to evaluate the efficiency of the treatment. However, a comprehensive precise and universally accepted evaluation is still lacking. For the purpose of this paper we used the CAS score. For non-severe TED simple measure as change in a sleep position, sunglasses for the photophobia, artificial tears or ointments are used, taping the eyelids during the night. Prisms have been beneficial for the diplopias. β-blocking or guanethidine eye drops are also used with varying success. Smoking withdrawal and reassurance are important. For the initial treatment the majority of the European ophthalmologists preferred glucocorticoid treatment, while 23% chose OI (24). Glucocorticoids have been used for 40 years, given by oral, local or intravenous route. Oral GC is given in higher doses (60-100 mg/day prednisolone for several months). Recurrence is a frequent event, favorable effect is found in 40-100% of patients (25). Intravenous GS methylpredisolone acetate (0,5-1g) at different intervals has also been used in the last 15 years. Some authors found that intravenous GC are more efficient in more severe TED (26). Side effects are a matter of concern: cushingoid facies, diabetes, depression, osteoporosis, peptic ulcer, hirsuitism, cataract, to name only few of them. To limit the number of recurrences GC should be continued for several months. OI is exerting its effect with its nonspecific anti-inflammatory effect and the high sensitivity of the orbital lymphocytes. It seems that OI reduces the production of GAGs, too. High-energy devices (cobalt unit and linear accelerator) allow better collimation, limited side-scatter and low penumbra. The common delivered dose is 20 grays (Gy) per eye (5) in 10 daily doses over a two week period. This is thought to reduce the cataractogenetic effect of irradiation (23). OI is well tolerated, having a transient exacerbation of inflammatory eye signs and symptoms as main side effect. Cataracts, radiation retinopathy are rare. The major concern is the possi-

bility of carcinogenic effect of OI. A theoretical risk of 1.2% for secondary tumors is calculated (27). To date no secondary tumor after OI was reported. A favorable effect of OI can be expected in 60% of patients (1). Some authors used OI with GC (28) the combination seems to be more effective and yields prompt results (28). OD is a very effective method on the proptosis. Marked proptosis and optic nerve compression were indications for OD. However, there is a shift in the opinion for OD indications. Rehabilitate (cosmetic), severe orbital inflammation, glucocorticoid side effects are also accepted as valid indication for OD (29, 30). In general, high incidence of postoperative diplopia (two thirds of the patients) is the major drawback for the OD. It is of note that the unavailability of a skillful orbit surgeon is a major limitation for the indication for OD. Transantral, three-wall coronal orbital surgical approach has been used with various success rates. Cyclosporine, plasmapheresis, somatostatin analogs, intravenous immunoglobulins have been methods of treatment in rare occasions. All of them are lacking sufficient clinical evaluation. In this series of patients, CS resulted in response of 8/22 patients, OI (12 patients) given after CS therapy, resulted in a response in all patients. Rapid increase in proptosis, loss of visual acuity is spite of CS treatment prompted OD in two patients, who both recovered visual acuity and proptosis fell under 25mm Hertel. In addition, no significant difference was found in the serum levels of IL-10 between the groups of controls (reference values 0-6.8 pg/ml) and patients before treatment (2,28±5,23 and after treatment 3,77±8,44; p<0,005). Serum IF γ (reference values 0-1,5 pg/ml) was also unchanged before and after treatment (before 0,50±0,24 and after treatment 0,35±0,19). The severity of TED was not linked to serum concentrations of IL-10 or to IF γ. Obviously, further data should strengthen the use of cytokines as predictors for treatment. Standardization of scores for severity, inflammation in TED is still needed. GC, OI and OD are still mainstay of treatment in TED.

Conclusion

CS treatment is still a mainstream treatment in TED resulting in this series in response in 8/22 patients. OI is often an efficient approach in TED treatment, while OD is required in patients with rapid increase in proptosis and loss of visual acuity is spite of CS and or OI treatment.

Serum levels of IL-10 were not different between the groups of controls and patients. Moreover, its levels were not changed before and after treatment. Therefore, IL-10 was not a predictor of the response of treatment, nor of the severity of the disease. Same pattern of serum concentrations was found for the IF γ, rendering him irrelevant as a marker of severity or a predictor of treatment response. In spite of the intensive research of the role of those cytokines in TED no firm data so far exist to link them to the important clinical aspects of the disease. As well as for the role of different cytokines, as for the treatment of TED standardization of scores for severity and inflammation in TED is essential. A precise clinical staging would clarify not only the role of cytokines but the best treatment approach (GC, OI, OD) for individual patient.

List of Abbreviations

TED	-	thyroid eye disease
CAS	-	clinical activity score
GAGs	-	glucosoamminogycans
Gy	-	Gray
GD	-	Grave's disease
GC	-	glucocorticoids
IL	-	interleukin
IF	-	interferon
OI	-	orbital irradiation
OD	-	ophthalmic decompression
MMI	-	methimasol
NO SPECS	-	N no signs or symptoms, O only signs, S soft tissue involvement, P proptosis, E extraocular muscle involvement, C corneal involvement, S sight loss
PTU	-	propiltiouracil
TNF	-	tumor necrosis factor

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