Novel Therapies for Thyroid Autoimmune Diseases

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Summary

C-X-C chemokine receptor (CXCR)3 and its interferon(IFN)γ-dependent chemokines (CXCL10, CXCL9, CXCL11) are implicated in the immune-pathogenesis of autoimmune thyroiditis (AT), **Graves** disease (GD) and **Graves** Ophthalmopathy (GO). In tissue, recruited Th1 lymphocytes produce IFNγ, enhancing the tissue secretion of IFNγ-inducible chemokines, initiating and perpetuating the autoimmune process. Patients with AT (with hypothyroidism), and with GO and GD, particularly in the active phase, have high IFNγ-inducible chemokines. Peroxisome proliferator-activated receptor(PPAR)γ or -α agonists and methimazole exert an immune-modulation on CXCR3 chemokines in AT, GD and GO. Other studies are ongoing to evaluate new molecules acting as antagonists of CXCR3, or blocking CXCL10, in **Hashimoto** thyroiditis (HT), GD and GO.

Recently, novel molecules targeting the various agents involved in the pathogenesis of GO, such as rituximab, have been proposed as an alternative to corticosteroids. However, randomized and controlled studies are needed to generalize these interesting results.

Keywords: autoimmune thyroiditis; **Graves** disease; **Graves** Ophthalmopathy; chemokines; CXCR3; CXCL10; corticosteroids; rituximab; teprotumumab; tocilizumab.

EXPERT COMMENTARY

Many progresses have been made in the knowledge of the immune-pathogenesis of autoimmune thyroid disorders. Th1 lymphocytes are very important in the autoimmune process, such as IFNγ and the IFNγ dependent chemokines. CXCR3 and its IFNγ dependent chemokines (CXCL10, CXCL9, CXCL11) are important in the immune-pathogenesis of autoimmune thyroiditis (AT), **Graves** Ophthalmopathy (GO), and **Graves** disease (GD). Under the stimulation of IFNγ, the IFNγ-induced chemokines are secreted by thyrocytes, orbital fibroblasts or preadipocytes. Th1 lymphocytes are recruited in tissues, hence IFNγ is further produced, enhancing the tissue secretion of IFNγ-inducible chemokines, perpetuating the autoimmune process. The presence of elevated IFNγ-inducible chemokines levels in peripheral fluids is a marker of Th1 orientated immune-response. High circulating levels of IFNγ-inducible chemokines have been shown in patients with AT (with hypothyroidism), and in GD and GO patients (in the active phase).

Attempts have been made to modulate the autoimmune process in autoimmune thyroid diseases (AITD). Peroxisome proliferator-activated receptor (PPAR)γ or -α agonists exert a modulatory role on CXCR3 chemokines in autoimmune thyroid disorders. Also methimazole has as immune-modulatory effect on CXCR3 and its chemokines in GD. Cortiscosteroids have been shown to inhibit the production of IFNγ and the IFNγ dependent chemokines in AITD, too. Other studies are ongoing to evaluate the use of new molecules antagonists of CXCR3, or that block CXCL10, in **Hashimoto** thyroiditis (HT), GD and GO.

In recent years, novel molecules which target the different agents involved in the immune-pathogenesis of GO have been proposed as an alternative to corticosteroids. A randomized trial with rituximab suggests good efficacy in patients with active GO. However discordant results have been reported, too. Promising results have been observed with small antagonists of TSH-R molecules (that interact with the receptor in thyrocytes and fibroblasts), with the anti-IGF-1 receptor monoclonal antibody teprotumumab, and with tocilizumab (an antibody anti-soluble IL-6 receptor) in GO. However, randomized and controlled studies are needed to generalize these interesting results.

Five-year view:

Studies are evaluating the use of new molecules antagonists of CXCR3, or that block CXCL10, in HT, GD and GO.

The possibility that new selective PPARγ or PPARα agonists exert anti-inflammatory action, without undesirable secondary effects, in HT, GD and GO, will be explored.

Small antagonists of TSH-R molecules (that interact with the receptor in thyrocytes and fibroblasts), which have been shown effective in vitro, will be tested for the therapy of GD and GO.

The results of a randomized trial show the efficacy of rituximab in patients with active GO, when compared with intravenous methylprednisolone. However, because of other discordant results, other studies are needed to evaluate rituximab in GO patients with different phenotype and clinical history.

Teprotumumab, a monoclonal anti-IGF-1R antibody, is evaluated in a randomized controlled trial in GO.

On the base of the results of a recent study with tocilizumab (monoclonal antibody anti-soluble-IL-6 receptor) that have shown efficacy in inactivating GO, randomized studies will be conducted.

Key issues

1- Many progresses have been made in the knowledge of the immune-pathogenesis of autoimmune thyroid disorders. Th1 lymphocytes are very important in the pathogenesis of AITD.

2- IFNγ, IFNγ dependent chemokines (CXCL9, CXCL10, CXCL11) and their receptor (CXCR3) are important in the pathogenesis of AT, GD and GO.

3- High circulating levels of IFNγ-inducible chemokines have been shown in patients with AT (with hypothyroidism), and in GD or GO patients (in the active phase).

4- PPARγ or -α agonists, MMI and corticosteroids have been shown to exert a modulatory role on CXCR3 chemokines in AT, GO and GD.

5- Studies are evaluating the use of new molecules antagonists of CXCR3, or that block CXCL10, in HT, GD and GO.

6- The results of a randomized trial show the efficacy of rituximab in patients with active GO. However, because of other discordant results, other studies are needed.

7- Small antagonists of TSH-R molecules (that interact with the receptor in thyrocytes and fibroblasts), which has been shown effective *in vitro*, will be tested for the therapy of GO and GD.

8- Teprotumumab, a monoclonal anti-IGF-1R antibody, is evaluated in a randomized trial in GO.

9- On the base of the results of a recent study with tocilizumab (a monoclonal anti-soluble-IL-6 receptor) that have shown efficacy in inactivating GO, randomized studies will be conducted.

Introduction

The prevalence of autoimmune thyroid diseases (AITD) is about 5% [1]. The most common AITD are **Hashimoto** thyroiditis (HT) or **Graves** disease (GD), clinically characterized by hypothyroidism and thyrotoxicosis, respectively [2].

An increased incidence of AITD have been shown [3] from the mid-1990s, associated with a progressive reduction in age at presentation and female/male ratio.

The reasons of the change of AITD epidemiology are not known. **Rizzo *et al.* [3], that collected 8397 fine needle aspiration cytology (FNAC) between 1988 and 2007, demonstrated that the frequency of HT increased from 1996 (+350% over 1995).** Only 1 man was present till 1995, but there were 22 men in 2005-2007. These results suggested that only environmental changes are able to explain the strong incidence modifications occurred in that short lapse of time.

Many studies have demonstrated that: a) the risk for women is higher than for men (5/1, female/male); b) the hypothyroidism linked to HT is more frequent with aging; c) the frequency of antithyroid antibodies is increasing with age; d) a significant geographic variability in the prevalence of AITD is known; e) the prevalence of AITD is higher in iodine-sufficient areas than iodine-deficient ones [4].

Other environmental possible risk factors shown by recent studies include vitamin D deficiency, age and of course sex [5].

AITD are usually of low severity, but in some cases are able to affect importantly the quality-of-life (QOL), and cause substantial medical costs. Cognitive function is one of the most important parameter of the QOL and the available literature about this has been reviewed [6], reporting conflicting results on the association between subclinical hypothyroidism and cognitive and health related QOL (HRQOL) impairment.

However, a lowering in HRQOL in patients with thyroid autoimmune diseases, regardless of hypothyroidism or hyperthyroidism, has been shown in most of the studies [6].

Graves Ophthalmopathy (GO) is a debilitative condition causing facial disfigurement and impairing visual function, with a negative impact on patients' employment, and psychosocial function [7], characterized by inflammation in the orbital tissues (eye muscles and connective tissue), and tissue remodeling owing to an altered production of preadipocytic fibroblasts and increased adipogenesis [8]. The supposed mechanism of GO pathogenesis is an immunological cross-reactivity among antigens expressed into the thyroid or the orbit, in which the coexpression of thyroid-stimulating hormone receptor (TSH-R) and insulin growth factor-1 receptor (IGF-1R) in orbital fibroblasts has been shown to be determinant [9].

However another study demonstrated the existence of IGF1R-antibodies in humans but do not support the hypothesis that the IGF1R-antibodies contribute to GO pathogenesis [10].

Furthermore, euthyroid GD is an important model for ophthalmopathy in the absence of thyroid autoimmunity. Recent studies have shown that TSH receptor antibodies measured in the Thyroid-Stimulating Immunoglobulin (TSI) assay was not detected in any patient with euthyroid GD defined as ophthalmopathy, suggesting that these antibodies might not be the cause of ophthalmopathy in GD either [11].

Other candidate antigens in GO are collagen XIII, flavoprotein sub-unit SDH and particularly calsequestrin although antibodies and T cell reactivity against these proteins are likely secondary to the disease [12].

According to epidemiological data, the mechanisms that cause the autoimmune attack in the thyroid are linked to an interaction among environmental triggers and genetic susceptibility, causing the failure of immune-tolerance and the development of the autoimmune disease [4].

AITD are predominant in female gender, and estrogens seem to be important in the appearance of AITD, such as the immunological modifications associated with pregnancy and postpartum. It has been hypothesized that the presence of cells from one subject to another genetically distinct (microchimerism) during pregnancy, could be an important endogenous factors linked to AITD [13].

Environmental risk factors (such as radiation, iodine, drugs, smoking, stress and viruses) have been identified, that are able to activate the development of AITD in susceptible individuals [4]. The prevalence of AITD is higher in areas with iodine sufficiency, and iodine supplementation is associated with an increase of AITD (overall in areas previously iodine deficient) [14].

Cigarette smoking is a risk factor for GD and GO, while it decreases the risk of overt hypothyroidism [15].

Specific selenoproteins are expressed by the thyroid tissue, and selenium deficiency is important in the development of thyroid autoimmunity, while selenium supplementation protects from AITD [16].

Many studies have evaluated the importance of viruses in the occurrence of AITD, reporting controversial results [17].

However more recently, the association between HCV infection and AITD has been reported in adults, and in children [18-20].

A high frequency of autoimmune thyroiditis (AT) and hypothyroidism, in the female gender, in the presence of high anti-thyroperoxidase (AbTPO) levels, characterizes

the thyroid disorders observed in HCV infection [4]. Moreover, an increased prevalence of serum AbTPO, and/or anti-thyroglobulin antibodies (AbTg), and subclinical hypothyroidism have been shown in patients with mixed cryoglobulinemia and hepatitis C (MC+HCV) [21, 22]. More recently, it has been demonstrated the presence of HCV in the thyroid tissue in HCV patients, and that HCV is able to infect human thyroid cells (ML1), suggesting that HCV infection of thyrocytes is determinant in the association between HCV and AITD [23].

It has been reported an association of AITD with interferon (IFN)-α therapy in HCV patients: in fact, 40% of HCV patients present thyroid disorders (destructive thyroiditis, or AT) during IFN-α therapy. IFN-α induces thyroiditis through direct toxic effects on the thyroid cells, or immune stimulation. However, HCV and IFN-α could synergistically cause AITD [24].

Genetic susceptibility to AITD has been evidenced by: 1- the familial clustering of the disease (25% of AITD in siblings of AITD patients); 2- sibling risk ratio of approximately 17 in AITD; 3- a higher prevalence of thyroid autoantibodies in siblings of AITD patients. Furthermore, a concordance rate for AITD of 0.5 for monozygotic, and the heritability of GD of about 80%, have been shown by twin studies, while that of thyroid autoantibodies is approximately 70% [13].

Various genes are significantly associated with the risk of AITD and the presence of thyroid antibodies, and among them about 70% (of the ones whose function is known) are involved in T cells function. This suggests the importance of T lymphocytes in the pathogenesis of AITD [25].

In fact, chronic AT may occur also in the absence of circulating anti-thyroid antibodies [26].

A recent publication shows that two single neucleotide polymorphisms in the calsequestrin gene are linked to ophthalmopathy in patients with GD [27].

An association between AITD and other autoimmune disorders has been reported. Polyglandular autoimmune syndromes are organ specific autoimmune disorders characterized by failure of several endocrine glands and non endocrine organs, caused by an autoimmune attack **[28]**.

Type 1 diabetes, GD, HT, Addison's disease, vitiligo, alopecia, hypogonadism were shown respectively in 61%, 33%, 33%, 19%, 20%, 6%, and 5%, of these patients. A common genetic susceptibility is the base of the association of AITD and Type 1 diabetes in these patients. Many studies suggest that patients with AITD and polyglandular autoimmune syndromes should be followed regularly, in order to evaluate the appearance of new circulating organ-specific antibodies, and of other clinical diseases [29].

Patients with systemic rheumatological diseases [Sjögren's syndrome (SS), scleroderma, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), sarcoidosis] show frequently thyroid autoantibodies and dyfunctions [4, 30-32]. Also in these patients the presence of a common genetic susceptibility have been demonstrated: for example in Caucasian patients the histocompatibility antigens (HLA) of the haplotypes HLA-B8 and DR3 are associated both with AITD, such as with primary SS [33]. In SLE patients: a- the association with AITD has been reported in 5q14.3-q15 (the major *locus* of susceptibility for SLE, found in AITD, too) patients; b- the frequency of HLA-B8 and DR3 is significantly higher in patients with AITD and SLE than in controls [34]. Also environmental factors could be important in the association of AITD and systemic autoimmune disorders [4].

Several studies have shown an association of AITD with papillary thyroid cancer (PTC) [35]. The link of the association between PTC and AITD has been suggested to be the increase of TSH (that is a growth factor for PTC) in HT, or the autoimmune inflammatory process, in different studies. In a recent report of 13738 patients [36], the frequency of PTC, TSH levels and thyroid autoantibodies were evaluated. The results showed the frequency of PTC was mainly associated with the increased TSH levels [36] in HT. On the contrary, other studies have found that both thyroid autoimmunity, and a high TSH, are independent risk factors for PTC [37]. The increased frequency of PTC in AITD patients is clinically important; in fact, about 20% of PTC patients may develop an aggressive disease [35].

On the base of the above mentioned studies, it is suggested that AITD patients should be followed for the appearance of thyroid dysfunctions, or nodules, or other systemic autoimmune disorders during the disease course [4].

Th1 chemokines in HT

During AITD, inflammatory immune-cells infiltrate the thyroid and secrete autoantibodies to thyroglobulin and thyroperoxidase, leading to the destruction of the follicles and the appearance of hypothyroidism. **Th1 lymphocytes, as IFNγ and the IFNγ dependent chemokines (CXCL9, -10, -11), play a critical role in the autoimmune process** [38-43].

In C57BL6 transgenic mice (that aberrantly express IFNγ under the control of the thyroglobulin promoter) it has been shown an increased expression of **CXCL10** [44].

CXCL10 is not produced in normal human thyrocytes; however, the CXCL10 dose-dependent secretion is induced by IFNγ in thyroid follicular cells, and the co-

stimulation with IFNγ+tumor necrosis factor (TNF)α induces a synergistic CXCL10 release, in comparison with IFNγ alone [45].

Indeed, increased levels of **CXCL10** were found (by immunohystochemistry) in thyroid tissues from AITD [38]. Thyroid follicular cells in HT expressed **CXCL10**, suggesting the local production of Th1 chemokines is important in the recruitment of inflammatory cells into the thyroid gland [38]. In patients with newly diagnosed AT, circulating CXCL10 were significantly higher, than in controls; overall in those patients with hypothyroidism, or a hypoechoic ultrasonographic pattern [4], suggesting **CXCL10** is a marker of a more aggressive thyroid autoimmune process, leading to the destruction of the thyroid gland [4].

The above mentioned results underline the importance of the Th1 immune attack in the early phase of AITD, while other studies exclude the involvement of the Th2 chemokines [such as (C-C motif) ligand 2 (CCL2) chemokine] [4].

Furthermore, CXCL9, -10, -11, co-stimulate the IFNγ production by (CD)4+ T-cells, producing a positive cytokine/chemokine feed-back loop, that reiterates the Th1-immune response and enhances the inflammatory process linked to IFNγ [46].

To sum up, thyroid cells under the stimulation by cytokines (IFNγ, TNFα) produce **CXCL10**, that induces the Th1 lymphocytes migration into the thyroid, which secrete more cytokines, stimulating a further chemokine release by the follicular cells, initiating and perpetuating the autoimmune response [47].

IFNγ dependent chemokines in GD

TSH-R stimulating autoantibodies are pathogenetic of GD [48], and GD is an autoantibody-mediated, Th2-dominant disease. However, a Th1-immune predominance is present at the beginning of GD [4].

IFNγ dependent chemokines, in GD, are expressed by thyrocytes and in infiltrating inflammatory cells and endothelial cells [4], and the C-X-C chemokine receptor (CXCR)3 has been demonstrated in endothelial and inflammatory cells.

In the early GD phase the secretion of CXCL10 by follicular cells induces the recruitment of Th1 lymphocytes into the thyroid [49]. IFNγ dependent chemokines secreted into the thyroid gland enter the circulation, and serum levels of these chemokines are high in GD patients [4]. Circulating IFNγ dependent chemokines are higher in hyperthyroid GD, than euthyroid or hypothyroid patients, and methimazole (MMI) therapy determines a decrease of them. Newly diagnosed GD patients had similar circulating CXCL10, than relapsed GD patients, demonstrating that high serum levels of these chemokines are associated with the active phase of GD (both at the beginning, such as in relapses) [50].

Also in peripheral blood lymphocytes the Th1 profile predominates (on the Th2) in GD patients in the early active phase, while a Th1 to Th2 switch is observed after the beginning of MMI therapy [51].

Human GD thyrocytes (in primary cultures) do not secrete CXCL10; however, IFNγ is able to induce a dose-dependent chemokine secretion; also in this case, the co-stimulation with IFNγ plus TNFα of thyrocytes induces a potent synergistic CXCL10 release (with respect to IFNγ alone) [45].

Other studies have shown the severity of GD can be predicted by CXCL10 polymorphism [52], and that CXCL10 could not decline during the remission phase [53].

The findings that, in GD patients, circulating CXCL10 levels decline after radioiodine ablation, or near-total thyroidectomy, suggest the chemokine is secreted mainly in the thyroid gland in AITD patients [54-56].

IFNγ dependent chemokines, or CXCR3, as therapeutic targets in HT and GD

Small antagonists, neutralizing monoclonal antibodies, modified chemokines, and binding proteins can be used to interfere with chemokines or CXCR3 function [4].

The pharmacological use of such molecules in animal models have demonstrated some problems, and primary human cell cultures are used to evaluate the modulating ability of these drugs on the cytokine-induced chemokine production [4].

Peroxisome proliferator-activated receptor (PPAR)γ are implicated in the modulation of IFNγ-induced chemokine expression in human autoimmune disorders, and in the down-regulation of inflammatory responses. Many papers have critically reviewed the evidences of the anti-inflammatory action of PPAR-agonists [57]. PPARγ agonists reduce the recruitment of activated T lymphocytes at sites of Th1-mediated inflammation [58].

PPARγ activator rosiglitazone (0.1–10 μM) reduces dose-dependently the secretion of IFNγ dependent chemokines (induced by IFNγ+TNFα) in thyrocytes, suggesting PPARγ-agonists modulate CXCR3 chemokines [45, 47].

PPARα ligands have shown therapeutic action in rodent models of autoimmune and inflammatory diseases [59], and it has been recently hypothesized that they could exert a similar action in human disorders. The PPARα agonist fenofibrate reduces the expression of interleukin (IL)-17 and IFNγ and improves colitis in IL-10 deficient mice, and inhibits the CXCL10 gene expression, repressing the activity of its

promoter, in TNFα-treated HT (HT)-29 cells [58]. Furthermore, PPARα agonists inhibit the secretion of CXCL10 and CCL2 in thyrocytes, suggesting that PPARα is implicated in the immune response modulation in AITD [60, 61].

PPAR ligands act repressing transcriptional activation, *via* nuclear factor-kB (NF-kB) through ligand-dependent transrepression [62]; so, NF-kB is considered a possible target to repress the transcription of CXCL10 by PPARα agonists.

MMI has an immunomodulatory effect in GD. Hyperthyroid patients with GD had high levels of serum CXCL10, and circulating CXCL10 decreased significantly when euthyroidism was reached after MMI therapy. Instead, patients with toxic nodular goiter showed a slight, not significant, decrease in the chemokines levels when rendered euthyroid by MMI itself. On this base it has been hypothesized that the strong reduction in circulating CXCL10 in GD patients treated with MMI therapy is related to the immune-modulatory activity of the drug [4]. Furthermore, MMI has been shown to be able to reduce CXCL10 production by thyrocytes, and promotes the Th1 to Th2 switch in GD patients [63].

In recent studies the role of drugs targeting IFNγ dependent chemokines or CXCR3 has been investigated in several autoimmune disorders.

CXCL10 has been shown to be involved in the immunopathogenesis of RA. MDX-1100, "a fully human, anti-IP-10 monoclonal antibody", has been studied in a phase II study [64], in 35 RA patients who did not respond to methotrexate (MTX). The response rate was significantly higher in MDX-1100 patients than in the placebo group (54% *vs* 17%). These results suggest that MDX-1100 is well tolerated and clinically effective in RA patients.

BMS-936557, "a fully human, anti-CXCL10 monoclonal antibody", has been investigated in a phase II study [65] in 55 patients with moderately-to-severely active

ulcerative colitis (UC). The results suggested that BMS-936557 is an effective therapy for active UC [64, 65].

These studies underline the important efforts that are ongoing in the attempt to find new drugs able to block IFNγ dependent chemokines or CXCR3 in autoimmune diseases.

New therapies of GO

For many decades corticosteroids (CS) have been used in the therapy of active GO. Many studies have shown that steroids decrease orbital congestion and inflammation [66]. Pulsed intravenous methylprednisolone (ivMP) has been shown to be more effective and is safer than oral prednisone [66]. In a recent large multicenter trial ivMP (a cumulative dose of 7.5 g) ameliorated inflammation in about 70% of patients, while eye muscle function in 50% of patients [66]. About 20% of GO patients were not responsive to the steroids and up to 4% of GO patients experienced disease progression or developed compression of the optic nerve within the orbit.

For these reasons, recently alternative immunosuppressive therapy has been evaluated in GO, and more recently, with novel agents that target different antigens involved in GO pathophysiology.

The main targets of these new drugs are the TSH-R and the IGF-1R on the fibroblasts, or cytokines and chemokines implicated in various stages of disease progression, or immune effector-cells such as B or T cells.

Rituximab (RTX)

RTX is a chimeric monoclonal antibody directed against the CD20 protein, present in the surface of B cells. RTX destroys B cells and it is used for the therapy of diseases characterized by excessive numbers of B-lymphocytes, overactive B-cells, or dysfunctional B-lymphocytes. RTX does not destroy antibody-producing plasma cells [67], for this reason, antibody production is maintained [67]. RTX has been approved by Food and Drug Administration for the therapy of RA, non-Hodgkin's lymphoma, and Wegener's granulomatosis. RTX is used off-label in different autoimmune disorders.

In humans, clinical trials in RA with RTX have shown it is effective in improving symptoms (usually within 8 – 16 weeks); this amelioration persists for the duration of B-lymphocytes depletion (typically 16 – 24 weeks) [68].

The most frequently reported RTX side effects are infusion-related reactions, usually occurring at the first infusion [69]. However a recent review of more than 3000 RA patients showed that RTX is well tolerated over time. Progressive multifocal leukoencephalopathy (PML) has been reported in RTX-treated patients, but mainly in patients with SLE, suggesting that SLE may predispose to PML development [70, 71].

The rationale for using RTX in GO is the depletion of B cells to block the production of inflammatory cytokines, and of pathogenic autoantibody generation [72].

Few reports have suggested that RTX may be effective in GO patients [72, 73].

More recently 2 randomized studies have reported contradictory results for the use of RTX in GO. Stan *et al.* [74], to evaluate the efficacy of RTX in GO, designed a prospective, randomized, double-masked, placebo-controlled trial. Twenty-five patients with GO were enrolled. Two RTX infusions (1000 mg each) (or two saline infusions) were given 2 weeks apart. No differences were found in the rate of patients

showing improvement of clinical activity score (CAS) at 24 weeks (25% placebo; 31% RTX) or in decrease of CAS at 24 or 52 weeks. There were four adverse events (AE) in 3 out of 12 placebo patients and 11 AE in 8 out of 13 RTX-treated patients. The Authors suggested that RTX is not effective in GO patients with active and moderate to severe GO.

In another study Salvi *et al.* [75] conducted a double-blind, randomized trial to compare RTX with ivMP in GO patients. Thirty-two patients were randomized to be treated with either RTX (2000 or 500 mg) or ivMP (7.5 g). CAS decreased with both treatments but more after RTX, whether 1000 mg RTX twice or 500 mg RTX once was used. At 24 weeks 100% of RTX patients improved in comparison with 69% after ivMP. Disease reactivation was not observed in RTX patients but was present in 5 after ivMP. Patients treated with RTX showed better motility. The results of this trial demonstrate a better therapeutic outcome with RTX in patients with GO, in comparison with ivMP.

Other studies are needed to evaluate RTX in GO patients with different phenotype and clinical history.

TSH-R as target

Small TSH-R molecules have been recently developed as TSH-R agonists (that activate the receptor), neutral antagonists (that inhibit the receptor activation by agonists), and inverse agonists (that inhibit the activation of receptor by agonists and the basal activity) [76]. The effects of a small TSH-R antagonist (NCGC00229600) [77] in GO orbital fibroblasts or adipocytes, on adenylate cyclase or phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B signaling, has been tested, demonstrating that inhibits the ligation of TSH-R on orbital fibroblasts and the

accumulation of hyaluronic acid in the orbit, and suggesting that similar molecules with increased potency may be effective in the future for GO [78].

IGF-1 receptor as target

Orbital fibroblasts coexpress IGF-1R with the TSH-R in patients with GO [79]. Teprotumumab (RV 001, R1507) is a human monoclonal antibody that binds to the extracellular IGF-R1 domain designed for the treatment of hematologic and solid hematologic tumors [80]. Teprotumumab has been very recently demonstrated to reduce cell proliferation in GO fibrocytes [80] and it is currently used in a phase II randomized clinical trial in patients with active GO, to be completed in 2016 [81].

TNFα as target

TNFα has been shown to be important in many autoimmune disorders and in GO [8]. Etanercept is a fusion protein (produced by recombinant DNA) that fuses the TNF receptor to the constant end of IgG1. It is a large molecule (150 kDa) that binds to TNFα and inhibits its role in inflammatory diseases, including autoimmune diseases such as ankylosing spondylitis, RA, psoriasis, psoriatic arthritis, sarcoidosis, and in others [82].

Paridaens *et al*. [83] have used etanercept for the therapy of 10 patients with active GO showing inactivation of the disease in 6. Other studies are needed to evaluate it in comparison with ivMP.

IL-6 as target

IL-6 acts as a pro-inflammatory cytokine, and it is secreted by T cells and macrophages to stimulate immune response, e.g. during inflammatory diseases and infections.

The IL-6 and the soluble IL-6 receptor have been demonstrated to be activated in GO, and elevated circulating soluble IL-6 receptor concentrations have been shown in patients with active GO [84].

Many studies have been devoted to develop anti-IL-6 agents as therapy against many of inflammatory diseases. Tocilizumab is a humanized monoclonal antibody against the IL-6 receptor, which has been approved for RA [85], Castleman's disease and systemic juvenile idiopathic arthritis [86].

Recently a prospective nonrandomized study in 18 GO patients (resistant to previous corticosteroids) with tocilizumab has been conducted. Thirteen patients reduced proptosis, fifteen patients had an amelioration in extraocular motility, and 7/13 patients resolved their diplopia. No relapse of GO were observed at the end of the follow up. This study suggests that tocilizumab may be effective in the therapy of patients with GO refractory to steroids [87].

Th1 Chemokines in GO and as a possible target

GO is clinically evident in about 50% of GD patients, and in 3-5% with severe forms. According to what reported above, a Th1-dominance could prevail in the initial active phase of GD, during which high levels of circulating CXCL10 are present. **In fact orbitopathy may be T cell mediated and all the antibodies identified secondary to a reaction involving cytotoxic T cells [88].**

The role of T cell mediated cytotoxicity in GO has been postulated [89].

Circulating CXCL10 are significantly higher in GO patients in the active phase of the disease than in the inactive one. Primary cell cultures of retrobulbar fibroblasts and preadipocytes obtained from GO patients did not secrete CXCL10 in basal conditions; however IFNγ alone induces a dose-dependent secretion of IFNγ dependent chemokines by orbital cells, and this secretion is enhanced by TNFα. These results suggest that GO retrobulbar cell types take part in the self-perpetuation of inflammation secreting chemokines, once stimulated by cytokines [45]. The chemokines secreted by orbital cells enter the circulation explaining why circulating Th1 chemokines are significantly higher in GO patients in the active phase of the disorder. Other studies confirmed that GD patients with ophthalmopathy had significantly higher circulating CXCL10 than GD patients without GO [4]. In a recent study to evaluate the importance of circulating CXCL9 and CXCL10 in GO activity, 42 subjects were subdivided into 4 groups [90]. The first group was composed by 15 euthyroid patients with clinical symptoms of orbitopathy in therapy with CS [infusions of ivMP and teleradiotherapy (TR)]. The second group comprised 10 hyperthyroid GD patients; the third 10 patients with GD in euthyroidism; and the fourth 7 controls age- and sex-matched to groups 1-3. Serum CXCL9 and CXCL10 levels significantly reduced during CS and TR treatment in GO patients, suggesting that the elevated levels of these chemokines are linked to the activity of orbital inflammation. CXCL9 and CXCL10 may serve as guide in the therapeutic decision-making in GO patients [90]. These findings were more recently confirmed in another study that demonstrated a reduction of CXCL10 in patients treated with intravenous CS [91].

PPARγ activator rosiglitazone (0.1–10 μM) reduces dose-dependently the secretion of CXCL9, -10, -11 in orbital fibroblasts, and preadipocytes, suggesting PPARγ-agonists

modulate CXCR3 chemokines [45, 47]. The possibility that PPARγ-agonists exert anti-inflammatory action, without expanding the retrobulbar fat, in GO needs further studies [92].

PPARα ligands have shown therapeutic action in animal models of autoimmune and inflammatory diseases. In humans the PPARα agonists fenofibrate, gemfibrozil, ciprofibrate inhibit the secretion of CXCL10 and CCL2 in orbital fibroblasts, and preadipocytes, suggesting that PPARα is implicated in the immune response modulation in GO [60, 61].

Other studies with drugs targeting IFNγ dependent chemokines or CXCR3 are needed in GO.

Conclusion

Th1 lymphocytes are very important in the autoimmune process, such as IFNγ and the IFNγ dependent chemokines. CXCR3 and its IFNγ dependent chemokines (CXCL10, CXCL9, CXCL11) are important in the immune-pathogenesis of autoimmune thyroiditis (AT), **Graves** Ophthalmopathy (GO), and **Graves** disease (GD).

Attempts have been made to modulate the autoimmune process in AITD. Peroxisome proliferator-activated receptor (PPAR)γ or -α agonists exert a modulatory role on CXCR3 chemokines in autoimmune thyroid disorders. Also methimazole has an immune-modulatory effect on CXCR3 and its chemokines in GD. Corticosteroids have been shown to inhibit the production of IFNγ and the IFNγ dependent chemokines in AITD, too. Other studies are ongoing to evaluate the use of new molecules antagonists of CXCR3, or that block CXCL10, in **Hashimoto** thyroiditis (HT), GD and GO.

In recent years, novel molecules which target the different agents involved in the immune-pathogenesis of GO have been proposed as an alternative to corticosteroids. A randomized trial with rituximab suggests good efficacy in patients with active GO. However discordant results have been reported, too. Promising results have been observed with small antagonists of TSH-R molecules (that interact with the receptor in thyrocytes and fibroblasts), with the anti-IGF-1 receptor monoclonal antibody teprotumumab, and with tocilizumab (an antibody anti-soluble IL-6 receptor) in GO. However, randomized and controlled studies are needed to generalize these interesting results.

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