Novel Therapies for Thyroid Autoimmune Diseases: an update

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Abstract

A Th1 immune-preponderance has been shown in the immunopathogenesis of autoimmune thyroiditis (AT), Graves' disease (GD) and Graves' Ophthalmopathy (GO), in which the Th1-chemokines (CXCL9, CXCL10, CXCL11), and their (C-X-C)R3 receptor, have a crucial role. Methimazole, and corticosteroids have been shown to modulate these chemokines; several efforts have been done to modulate the autoimmune reaction with other drugs, i.e. PPAR-γ or -α ligands, or antibodies, or small molecules directed against CXCL10, or CXCR3. Antigen-specific therapy for GD, by inducing T cell tolerance through an immunization with TSH-R peptides, has been published.

Drugs targeting cytokines [anti-TNFα (Etanercept), and anti-IL-6 (Tocilizumab)], and RTX (a chimeric monoclonal antibody *vs.* CD20) have been used in GO, with promising results. Teprotumumab (a human monoclonal anti-IGF-1R blocking antibody) has been investigated in a trial, showing it was very effective in GO patients. Still, more studies are needed for new therapies targeting autoimmune thyroid disorders.

Keywords: autoimmune thyroid disorders; corticosteroids; rituximab; teprotumumab; tocilizumab; antigen-specific immunotherapy.

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1. Introduction

Autoimmune thyroid disorders (AITD) are characterized by the break of tolerance of the immune system against thyroid antigens (1) and are the most frequent autoimmune diseases (2). A raised incidence of AITD has been reported, together with an advancing decrease in female/male ratio and age at the onset (3).

Chronic autoimmune thyroiditis (AT) and Graves' disease (GD) are the main AITD, characterized clinically by hypothyroidism and thyrotoxicosis, respectively, and by circulating antithyroid antibodies (ATA) and infiltration of autoreactive lymphocytes in the thyroid (1).

It has been shown that: a) women are at higher risk than men (about 4–8/1); b) the frequency of hypothyroidism in the presence of AT increases with age, with the peak at 45–55 years; c) the frequency of ATA increases with age; d) the prevalence of AITD changes geographically; e) a higher prevalence of AITD is present in areas with iodine sufficiency than in those with iodine deficiency (1).

AITD are usually indolent diseases, but sometimes they can impact negatively the quality-of-life (QOL), causing a notable medical cost (4).

GD is characterized by hyperthyroidism, that is associated with the presence of circulating thyrotropin receptor (TSH-R) stimulating autoantibodies.

Graves' Ophthalmopathy (GO) is a debilitating condition, that is present in 30-50% of patients with GD, leading to facial disfigurement and altered vision, that impacts negatively patients' employment, and psychosocial activity (5,6). The GO pathogenesis is led by an autoimmune reaction whose main autoantigen is TSH-R which is expressed mainly in the thyroid, but also in orbital adipocytes, fibroblasts and other sites. In orbital fibroblasts, also the expression of insulin growth factor-1 receptor (IGF-1R) has a crucial role, too (7). TSH-R antibodies were not detected in any euthyroid GD patient defined as ophthalmopathy,

emphasizing the idea that they are not the cause of ophthalmopathy in GD (8). Further investigations are proposing as autoantigens of GO also calsequestrin, flavoprotein sub-unit SDH and collagen XIII, even though antibodies directed versus these antigens are usually secondary in importance to the TSH-R in disease initiation (9).

Patients with polyglandular autoimmune syndrome (defined as organ specific autoimmune diseases distinguished by the failure of various endocrine/non-endocrine glands) present hypogonadism, alopecia, vitiligo, Addison's disease, AT, GD or type 1 diabetes (T1D) (with a prevalence of 5%, 6%, 20%, 19%, 33%, 33%, and 61%, respectively) (10).

Furthermore, an association between AITD and another autoimmune disease [systemic lupus erythematosus (SLE), Sjögren syndrome (SS), systemic sclerosis (SSc), sarcoidosis, rheumatoid arthritis (RA), type 1 diabetes, autoimmune gastritis, vitiligo, celiac disease, HCV-related cryoglobulinemia] has been demonstrated in about 19% of three thousand sixty-nine patients with AT, *vs*. to 2 age- and gender-matched control groups (1023 healthy subjects; 1023 non-toxic multinodular goiter patients). Also the association of 3 different autoimmune disorders was observed in AT patients, *vs*. controls (11). Similar results were shown in another study that estimated the prevalence of another autoimmune disease in 3209 GD patients (984 with GO), *vs*. 1069 controls, 1069 AT and 1069 multinodular goiter patients (12). Approximately 17-18% of GD patients showed also another autoimmune disease (12).

An association between AITD and papillary thyroid cancer (PTC) has been shown by epidemiological studies. A paper reported in 13738 AT patients that the raised TSH levels were significantly associated with PTC frequency (13). On the other hand, other papers reported that both an elevated TSH, and thyroid autoimmunity, are independent risk factors for PTC (14).

Increased TSH is a growth factor for TC cells in AT; however autoimmunity and inflammation, *per se*, are TC risk factors (3). In fact, in the tumor microenvironment,

inflammatory cells (such as macrophages and lymphocytes) are linked to endothelial cells, adipocytes, fibroblasts, and extracellular matrix *via* cytokines, chemokines and adipo-cytokines, and may exert a pro-tumoral effect on TC cells (13).

2. Genetic susceptibility and environmental factors

The mechanisms underlying the autoimmune reaction against the thyroid tissue depend on both genetic susceptibility and environmental factors, causing the failure of the immune-tolerance (1).

Since AITD are primarily a women disease, the immunological changes associated with pregnancy and postpartum, and estrogens play a key role in the onset of AITD. It has been supposed that during pregnancy, microchimerism could be a determinant endogenous factor at the basis of AITD (15).

2.1 Genetic susceptibility

Evidence for genetic susceptibility to AITD includes : A) the familial clustering of the disease (25% of AITD, in siblings of AITD patients); B) AITD sibling risk ratio of approximately 17; C) an elevated ATA prevalence in siblings of AITD patients. Moreover, twin studies have reported a heritability of GD of approximately 80%, and of ATA of about 70%, and a concordance rate for AITD of 0.5 for monozygotic (15).

Approximately 70% of the genes associated with the risk of AITD, whose effects are known, take part in T cell functions, emphasizing the role of T lymphocytes in AITD pathogenesis (16); indeed, chronic AT can be present even in the absence of circulating ATA (17).

Patients with systemic rheumatologic disorders (SLE, SS, SSc, RA, sarcoidosis, etc) show frequently the presence of ATA and thyroid dysfunctions (1), and have a common genetic susceptibility with AITD (18). In SLE, the 5q14.3-q15 has been proposed as the the major

locus of susceptibility at the basis of the association with AITD. Furthermore, DR3 and HLA-B8 are significantly more frequent in AITD and SLE patients, *vs.* control subjects (19).

2.2 Environmental factors

Several environmental risk factors (smoking, radiation, viruses, iodine, stress, drugs, and others) are considered triggers of AITD in susceptible individuals (1,20).

Smoking is a risk factor for Graves' hyperthyroidism (21) and an even stronger risk factor for Graves' orbitopathy, while it decreases the risk of overt hypothyroidism (22).

The thyroidal tissue express specific selenoproteins, and the lack of selenium is involved in the onset of thyroid autoimmunity, whereas its supplementation protects from AITD (23).

Moreover, the importance of viruses has been investigated in the occurrence of AITD, with contrasting results (24).

The association between AITD and HCV infection has been demonstrated in children, and in adults (25,26). Women with HCV infection carry a higher prevalence of AT and hypothyroidism, with high level of anti-thyroperoxidase (AbTPO) (1). Furthermore, elevated serum anti-thyroglobulin antibodies (AbTg), and/or AbTPO, and subclinical hypothyroidism are more prevalent in “mixed cryoglobulinemia and hepatitis C” (MC+HCV) patients (27). Furthermore, in MC+HCV patients a higher prevalence of GD has been shown (11). The presence of HCV has been reported in the thyroidal tissues, and HCV can infect human thyroid cells (ML1), emphasizing its role in thyrocytes for the association between HCV and AITD (28).

During IFN-α therapy, 40% of HCV patients show thyroid disorders (ATA, AT, hypothyroidism, GD, or destructive thyroiditis). Thyroiditis can be induced by IFN-α through direct toxic effects, or by the stimulation of the immune system(29).

Vitamin D deficiency is another environmental possible risk factor (30).

3. Th1 chemokines

Chemokines are small cytokines (of about 8–10 kDa in mass), or signaling proteins secreted by cells (31). Four main subfamilies (CXC, CC, CX3C and XC) exist, and they interact with G protein-linked transmembrane receptors on the surface of target cells (31). In particular, CXCR3 binds the Th1 dependent chemokines: chemokine ligand 10 (C-X-C motif) (CXCL)10, CXCL9 and CXCL11 (32).

Various types of cells express CXCR3, such as activated Natural Killer, T lymphocytes, certain epithelial and endothelial cells, and it is strongly expressed on Th1 cells, that are attracted in the tissues by the Th1 chemokines, released by the target cells during inflammation (31,33).

Elevated circulating and tissue levels of the Th1 chemokines have been shown in autoimmune diseases, such as AT (27,34,35), GD (36), T1D (37), GO (38), or systemic rheumatological disorders, such as sarcoidosis (39), SLE (40), RA (41), SSc (42), psoriasis or psoriatic arthritis (43), HCV-related cryoglobulinemia (44), other HCV-immune-mediated disorders (27,45,46), and also in cancers (3).

3.1 Th1 chemokines in AT

AITD is characterized by an inflammatory infiltrate of the thyroid, where the immune cells release autoantibodies against Tg and TPO, with subsequent damage of the gland parenchyma and onset of hypothyroidism. The thyroidal infiltrate is constituted by CD4+ and CD8+ T cells, CD19+ B cells, macrophages and plasma cells. B lymphocytes may also act as antigen presenting cells (APCs), activating naïve autoreactive CD4+ T cells, by presenting thyroid autoantigens to them (47). Th1 lymphocytes, and IFNγ and the IFNγ dependent chemokines (CXCL9, CXCL10, CXCL11), have a critical role in this process (33,34).

An elevated expression of CXCL10 has been reported in C57BL6 transgenic mice (expressing abnormally IFNγ under the control of the Tg promoter) (48). CXCL10 is not released in normal human thyrocytes, but its secretion is induced dose-dependently by IFNγ, and the treatment with IFNγ+tumor necrosis factor (TNF)α induces synergistically the CXCL10 secretion, *vs.* IFNγ alone (38).

Increased CXCL10 levels were detected by immunohystochemistry in thyroid samples obtained from AITD patients (33). In AT, thyrocytes expressed CXCL10, indicating that the local production of Th1 chemokines has a crucial role in recruiting inflammatory cells in the gland (33). In AITD patients with new diagnosis, serum CXCL10 was significantly higher, especially in the presence of hypothyroidism, or a hypoechoic pattern at neck ultrasound (1). This evidence candidates CXCL10 as a marker of a higher thyroid autoimmune burden, which might lead to the gland atrophy (1).

Furthermore, CXCL9, -10, -11 induce the IFNγ release by (CD)4+ T-cells, stimulating a chemokine/cytokine feed-back loop, that carries on the Th1 immune response and strengthens inflammation (49).

3.2 Th1 chemokines in GD

The Th1 immune response predominates at the initial/active phases of GD (1), that is induced by Th1 lymphocytes trough the main Th1 cytokines, IL-2, but overall IFNγ (50). *In vitro* experiments show that in human primary cultures of GD thyrocytes, CXCL10 is not secreted, but the treatment with IFNγ induces it dose-dependently, and the co-treatment with IFNγ and TNFα induces a strong synergistic CXCR3 chemokines secretion (*vs.* IFNγ alone) (38).

During the initial phase of GD, the release of CXCL10 by follicular cells recruits Th1 lymphocytes into the gland (51), and the locally produced IFNγ dependent chemokines reach the circulation, with subsequent high circulating levels of these chemokines (1). In GD,

hyperthyroidism is associated with more elevated levels of Th1 chemokines, and methimazole (MMI) lower them. New cases of GD show similar level of CXCL10 to those of relapsed GD patients, suggesting that these chemokines distinguish the active phase of GD (at the onset, and in relapse) (36). In the early GD active phase, the Th1 profile prevails even in peripheral blood lymphocytes, whereas a Th1-Th2 switch is reported once in MMI therapy (52). In GD patients after radioiodine ablation, or near-total thyroidectomy, serum CXCL10 declines, suggesting that it is released principally in the thyroid (53). GD severity can be predicted by CXCL10 polymorphism (54), and CXCL10 could not decline in remission (55).

Other studies showed that the frequency of Th17 cells and the level of interleukin (IL)-17A in the peripheral blood of GD patients are significantly increased, while the changes of single nucleotide polymorphism of the IL-17A gene were correlated with GD susceptibility (56).

3.3 Th1 chemokines in GO

About half cases of GD patients develop also GO. At the onset, GD is a Th1-dominant autoimmune process, and also in GO, T cells (especially cytotoxic T cells) and their related antibodies are predominat (57). The fibroblasts in the connective tissues of the orbit have been identified as target cells in GO (58), and they are involved in lymphocyte infiltration and B cell differentiation. Cytokines and growth factors are able to regulate the orbital fibroblasts in GO (56).

The active phase of Graves' orbitopathy is characterized by elevated serum CXCL10 levels (1). Retro-orbital fibroblasts and preadipocytes in primary cultures from GO patients did not secrete CXCL10 basally, but the treatment with IFNγ alone induces it dose-dependently, and this secretion is enhanced in the presence of IFNγ and TNFα. These data lead to hypothesize that GO retrobulbar cells participate in the self-perpetuation of inflammation through the secretion of chemokines, once stimulated by cytokines (38).

Recently in a paper, in order to investigate the role of serum CXCL9 and CXCL10, patients with GO were divided in 4 groups: 15 euthyroid patients with orbitopathy in treatment with corticosteroids (CS) [intravenous (iv) methylprednisolone (MP) and teleradiotherapy (TR)]; 10 hyperthyroid GD patients; 10 euthyroid GD patients; 7 control subjects sex- and age-matched *vs* groups 1-3 (59). During CS and TR in GO patients, serum CXCL9 and CXCL10 significantly decreased, indicating that their elevated levels were associated with the activity of orbital inflammation, and suggesting that they could be used in the therapeutic decision-making in GO (59). Another paper confirmed a decrease of CXCL10 during the treatment with ivCS (60).

4. Th1 chemokines as therapeutic targets in AITD

The inhibition of chemokines or CXCR3 action through small compounds, monoclonal antibodies (mAbs), or binding proteins may be considered a new therapeutic strategy (1). However, the first trials using these compounds on animal models resulted in some issues. The use of human primary cell cultures *in vitro* permits to investigate the modulating effects of these drugs on chemokines secretion (1).

Peroxisome proliferator-activated receptor (PPAR)γ modulates the IFNγ stimulated chemokines release in human autoimmune diseases, down-regulating the inflammatory process. The PPARγ ligand rosiglitazone (0.1–10 μM) lowers in a dose-dependent manner the release of CXCL9, CXCL10, CXCL11 in orbital fibroblasts, and preadipocytes, advocating for a modulating effect on CXCR3 chemokines of PPARγ agonists (38). The hypothesis that PPARγ agonists induce an anti-inflammatory action in GO, without inducing the expansion of the retro-orbital fat, needs more investigations (61), and the anti-inflammatory role of PPAR-agonists have been recently critically reviewed (62,63).

PPARα ligands have also demonstrated a therapeutic activity in rodent models of

autoimmune and inflammatory diseases (64), suggesting this effect also in human diseases. Fenofibrate (a PPARα agonist) decreases IL-17 and IFNγ expression, and it ameliorates colitis in IL-10 deficient mice (64). Furthermore, it represses CXCL10 promoter in HT-29 colon epithelial cells treated with TNFα (64). Moreover, PPARα seems to take part in the immune response modulation in AITD, since it inhibits CXCL10 and CCL2 secretion in orbital fibroblasts, and preadipocytes (65). PPARα agonists actions are exercised *via* the repression of transcriptional activation, through nuclear factor-kB and ligand dependent trans-repression, suggesting that it could be a target for PPARα ligands (66).

MMI has an immune-modulatory effect in GD. In hyperthyroid patients CXCR3 chemokines circulating levels are high, and they decline after MMI therapy, when euthyroidism is reached; while in patients with toxic nodular goiter CXCL10 levels are only slightly increased and do not decline significantly during MMI therapy. For this reason, the decrease in serum CXCL10 under MMI treatment in GD is supposed to be interconnected with the immune-modulatory action of the therapy (1), promoting the Th1-Th2 switch in GD (67).

5. New therapies in GO

High-dose intravenous immune-globulins (68), or CS have been used for the treatment of active GO, decreasing orbital congestion and inflammation (69). CS are the usual treatment of GO. A multicenter trial demonstrated that ivMP improved inflammation in approximately 80-70% of the cases, whereas eye muscle function in 50%. However, approximately 20% of GO subjects did not respond significantly to steroids, and compression of the optic nerve or disease progression were shown in up to 4% (69).

Currently, thanks to a more extensive knowledge of GO pathophysiology, alternative immunomodulant therapies, targeting different antigens, are considered. TSH-R and the IGF-1R present on fibroblasts, B and T lymphocytes, cytokines, and chemokines involved in the

autoimmune reaction, are all potential targets of the new drugs (70).

5.1 Targeting TSH-R

Small TSH-R molecules with either agonist, neutral or antagonist activities have been recently developed (71). Small TSH-R antagonist (NCGC00229600) showed promising results in GO retro-orbital fibroblasts/adipocytes reducing the production of hyaluronic acid (72).

5.2 Targeting TNFα

In several autoimmune disorders, including GO, TNFα has a crucial role (6). Etanercept is a large molecule, deriving from recombinant DNA, from the fusion of the TNF receptor to the constant end of IgG1protein. Binding to TNFα, it inhibits its role in autoimmune diseases (RA, ankylosing spondylitis, psoriasis, sarcoidosis, psoriatic arthritis, etc.) (73). Paridaens *et al*. have explored the use of etanercept as possible treatment of ten patients with active GO, achieving the remission in 6/10 (74). Further researches are necessary to investigate this therapy *vs*. ivMP.

5.3 Targeting IL-6

IL-6 is secreted by T cells and macrophages to stimulate immune response and it acts as a pro-inflammatory cytokine. In patients with active GO, IL-6 and its soluble receptor are activated, and high serum IL-6 receptor levels have been reported (75). Tocilizumab is a humanized mAb directed *vs*. the IL-6 receptor; it has reached the approvation for the treatment of RA (76), systemic juvenile idiopathic arthritis (77) and Castleman's disease.

A prospective nonrandomized study about tocilizumab has been done in 18 GO patients (resistant to previous CS) (78). Proptosis decreased in thirteen patients, extraocular motility improved in fifteen, and 7/13 resolved their diplopia. At the end of the follow up, there was no relapse of GO. These data suggested the effectiveness of tocilizumab in the treatment of GO patients refractory to steroids (78).

A paper recently discussed 3 case reports (79). Three patients with CS-resistant or advanced diplopia-associated GO were administered with monthly iv tocilizumab (8 mg/kg). A significant improvement in ocular symptoms was shown (79).

5.4 Rituximab (RTX)

RTX (a chimeric mAb directed *vs.* CD20) is on the surface of B cells. By this way, inducing B cells death, RTX is indicated for the treatment of disorders caused by elevated levels of B-lymphocytes or dysfunctional B-lymphocytes, and overactive B-cells. RTX has no effect on antibody-producing plasma cells, without interfering with the antibody synthesis (80). RTX has been approved by Food and Drug Administration for the treatment of chronic lymphocytic leukemia, RA, Wegener's granulomatosis, and non-Hodgkin's lymphoma, but it is also used off-label in various autoimmune disorders (81).

RTX has been proposed for the treatment of GO since it reduces the number of B lymphocytes, the burden of cytokines and the secreted autoantibodies; nevertheless only few studies support the use of RTX in GO patients (82).

Two randomized studies reported conflicting data about the use of RTX. A prospective, placebo-controlled, randomized trial evaluated the effectiveness of RTX in 25 GO patients (83). Two RTX (or 2 saline infusions) were administered 2 weeks apart. The improvement of CAS was not different, suggesting that RTX is not effective in GO (83).

In another double-blind, randomized trial, 32 patients were treated with RTX or ivMP. CAS

was reduced by both treatments, but in particular with RTX. At 24 weeks 100% of RTX patients improved compared to 69% after ivMP, demonstrating in GO patients that RTX is more effective than ivMP (84).

A systematic review and meta-analysis of 4 randomized controlled trials was conducted in 293 GO patients (who received RTX or glucocorticoids, or saline) to investigate the efficacy and safety of RTX in GO patients. Compared to controls, in the RTX group CAS was significantly reduced at 24 weeks, and a notable proptosis reduction was also observed, even if not significant (85).

In another paper (86), 219 GO patients were treated with pulse MP, and then oral steroids and/or orbital radiotherapy, 15 (6.8%) finally received 100 mg RTX doses (100–400 mg) for the presence of active disease. It was shown that low-dose RTX showed sustained anti-inflammatory action in the major part of patients with active GO resistant to conventional therapies (86). These data have been confirmed by another study conducted in 12 patients with active GO managed primarily with a 100 mg RTX infusion, showing that low-dose RTX is an effective, and safe therapy for active GO, able to reduce disease activity, permitting a decreased administration of systemic steroid (87).

In a further study, 14 patients with active and moderate-to-severe GO, 11 of whom refractory to CS, received ivRTX, 1000 mg twice at a 2-week interval (88). A limited improvement in CAS was shown (P=0.002, 35.7%). Disease inactivation occurred in 50% of the cases. At 12 weeks, CAS improved in 14.3% of patients and inactivation of GO in 28.6%. At 24 weeks, in 33% and in 28.6% of patients, respectively, proptosis and total eye score improved. The Authors concluded that RTX is well-tolerated and safe in these patients (88).

Further evaluations are needed to establish the real role of RTX therapy in GO patients.

5.5 Targeting IGF-1R

The IGF-1 receptor (IGF-1R) and TSH receptor (TSH-R) constitute a physical and functional complex in orbital fibroblasts. A subset of these fibroblasts is derived from infiltrating CD34(+) fibrocytes. Teprotumumab (RV 001, R1507) is a human monoclonal anti-IGF-1R blocking antibody. *In vitro*, teprotumumab reduced the fibrocyte display of IGF-1R and TSH-R, such as the IGF-1- and TSH-dependent phosphorylated Akt levels. TSH induction of IL-6 and IL-8 mRNA and protein was also reduced by the monoclonal antibody (89).

On the above mentioned bases, a multicenter, double-masked, randomized, placebo-controlled trial was conducted, to determine the efficacy and safety of teprotumumab, in patients with active, moderate-to-severe ophthalmopathy (90). Eighty-eight patients were randomly assigned to receive placebo, or active drug (for a total of 8 infusions). The primary end point was the response in the study eye, defined as a reduction of 2 points or more in the Clinical Activity Score (CAS), and a reduction of 2 mm or more in proptosis at week 24. Twenty-nine of 42 patients who received teprotumumab (69%), with respect to 9 of 45 patients who received placebo (20%), had a response at week 24 ($P<0.001$). Therapeutic effects were rapid; at week 6, 18 of 42 patients in the teprotumumab group (43%) and 2 of 45 patients in the placebo group (4%) had a response ($P<0.001$). The only drug-related adverse event was hyperglycemia in patients with diabetes; this event was controlled by adjusting medication for diabetes. This study showed that teprotumumab was more effective than placebo in reducing proptosis and CAS, in patients with active ophthalmopathy (90).

6. Antigen-Specific Immunotherapy for GD

None of the current treatment options are ideal for the treatment of hyperthyroidism in GD. Anti-thyroid drugs are effective in producing long-term remission only in about half of the patients, and thyroidectomy and radioiodine treatment lead to long-term medication

dependence (91). The discovery of ATA and the development of tests for their detection has permitted to study more deeply the pathogenesis of AITD (92).

Emerging antigen-specific immunotherapies are attracting for the treatment of autoimmune, inflammatory, and allergic diseases, because they aim only to restore immune tolerance to the immune-dominant epitopes taking part in the aberrant autoimmune response. They do not cause generalized immunosuppression with the associated risk of infection, and do not alter the immune response (91).

Recent approaches to antigen-specific therapy for AITD have been published. The treatment with broad-range immunosuppressive drugs can lead to important side effects, so that antigen-specific therapies have been designed to induce tolerance in various autoimmune conditions (93).

A first human study was conducted attempting immunotherapy in GD by inducing T cell tolerance through an immunization with TSH-R peptides (ATX-GD-59) (91). This study was based on the fact that in transgenic mice expressing the human HLA-DR3 molecule, the pre-treatment with these peptides blunted the humoral and cell-mediated immune responses to TSH-R A-subunit adenovirus immunization. On the other hand, injection of TSH-R A-subunit protein after the induction of hyperthyroidism had no effects. In this phase I open label trial, 12 patients with earlier untreated mild to moderate Graves' hyperthyroidism were administered with ATX-GD-59 for 18 weeks. Ten subjects received all 10 doses of ATX-GD-59, 50% of whom had free triiodothyronine within the reference interval by the 18-week visit. At the end of the study, 2 further subjects had improved free thyroid hormones, while 3 subjects showed worsening thyrotoxicosis. Serum TSH-R autoantibody concentrations decreased during the study and correlated with changes in free thyroid hormones. These data suggested that ATX-GD-59 is a safe and well-tolerated treatment (91).

7. Conclusion

The immunopathogenesis of AT, GO, and GD is characterized by a Th1-type response where IFNγ, CXCL10, CXCL9, CXCL11 (IFNγ dependent chemokines), and their receptor CXCR3 have a crucial role. The mainstay therapy of GD and GO already includes immune-modulating agents such as MMI, or CS, which are able to act on IFNγ and CXCR3 chemokines. Yet several studies have recently explored further potential drugs in AT, GD and GO, able to interfere with the autoimmune process of AITD, such as PPARγ or -α ligands and other molecules directed against CXCR3, or CXCL10.

Emerging antigen-specific immunotherapies are attracting for the treatment of autoimmune, inflammatory, and allergic diseases, because they aim only to restore immune tolerance to the immune-dominant epitopes taking part in the aberrant autoimmune response. A first human study was conducted attempting immunotherapy in GD by inducing T cell tolerance through an immunization with TSH-R peptides (ATX-GD-59) (91), showing a normalization of T3 in more than 50% of treated patients, associated with a decline of TSH-R autoantibody concentrations (91) (**Table 1**).

Innovative immune-modulant therapies, targeting different antigens, are considered for GO (**Table 1**). TSH-R and the IGF-1R present on fibroblasts, B and T lymphocytes, cytokines, and chemokines involved in the autoimmune reaction, are all potential targets of the new drugs. Among drugs targeting cytokines anti-TNFα (Etanercept), and anti-IL-6 (Tocilizumab) have shown promising results. Many studies have evaluated RTX (a chimeric mAb directed *vs.* CD20), on the surface of B cells, for the treatment of GO, suggesting it is safe and an effective in active GO (87). More recently teprotumumab (a human monoclonal anti-IGF-1R blocking antibody) has been investigated in a multicenter, double-masked, randomized, placebo-controlled trial. This study showed that in patients with active ophthalmopathy, teprotumumab was very effective in reducing proptosis and inflammation in GO patients (90).

Still, higher quality evidences from other large, randomized and controlled studies are necessary for new therapies targeting autoimmune thyroid diseases.

Conflict of Interest

The Authors have nothing to declare.

Role of the funding source

The Authors have nothing to declare.

Summary

Chronic autoimmune thyroiditis (AT) and Graves' disease (GD) are the main autoimmune thyroid disorders (AITD), characterized clinically by hypothyroidism and thyrotoxicosis, respectively, and by circulating antithyroid antibodies (ATA) and infiltration of autoreactive lymphocytes in the thyroid.

The mainstay therapy of GD and GO already includes immune-modulating agents such as MMI, or CS, which are able to act on IFNγ and CXCR3 chemokines. Further potential drugs have been explored in AT, GD and GO, able to interfere with the autoimmune process of AITD, such as PPAR-γ or -α ligands and other molecules directed against CXCR3, or CXCL10.

Emerging antigen-specific immunotherapies are attracting for the treatment of autoimmune, inflammatory, and allergic diseases, because they aim only to restore immune tolerance to the immune-dominant epitopes taking part in the aberrant autoimmune response. A first human study was conducted attempting immunotherapy in GD by inducing T cell tolerance through an immunization with TSH-R peptides (ATX-GD-59) (91), showing a normalization of T3 in more than 50% of treated patients, associated with a decline of TSH-R autoantibody concentrations (91).

Innovative immune-modulant therapies, targeting different antigens, are considered for GO. Among drugs targeting cytokines anti-TNFα (Etanercept), and anti-IL-6 (Tocilizumab) have shown promising results. Many studies have evaluated RTX (a chimeric mAb directed *vs.* CD20) for the treatment of GO, suggesting it is effective, and safe for active GO (87). More recently teprotumumab (a human monoclonal anti-IGF-1R blocking antibody) has been investigated in a multicenter, double-masked, randomized, placebo-controlled trial. This study showed that in patients with active ophthalmopathy, teprotumumab was very effective in reducing proptosis and inflammation in GO patients (90).

Still, higher quality evidences from other large, randomized and controlled studies are needed for new therapies targeting autoimmune thyroid diseases.

Practice Points

- New knowledge has been reached about the immunopathogenesis of AITD.
- IFNγ, IFNγ dependent chemokines (CXCL9, CXCL10, CXCL11) and the CXCR3 receptor have a crucial role in the pathogenesis of AITD.
- Elevated serum levels of Th1 chemokines have been reported in AT patients (in the presence of hypothyroidism), and in GD or GO (overall in the active phase).
- MMI, PPARγ or -α ligands and corticosteroids modulate CXCR3 chemokines secretion in AITD.
- Recent approaches to antigen-specific therapy for AITD have been published.
- A first human study was conducted attempting immunotherapy in GD by inducing T cell tolerance through an immunization with TSH-R peptides (ATX-GD-59).
- Drugs targeting cytokines anti-TNFα (Etanercept), and anti-IL-6 (Tocilizumab) have shown promising results in GO.
- RTX (a chimeric mAb directed *vs.* CD20) has been used in GO, suggesting it is an effective, and safe.
- Teprotumumab (a human monoclonal anti-IGF-1R blocking antibody) has been investigated in a multicenter, double-masked, randomized, placebo-controlled trial; showing that it was very effective in reducing proptosis and inflammation in GO patients.

Research Agenda

- Researches are going on in order to investigate new compounds able to antagonize CXCR3, or blocking CXCL10, in AITD.
- In AT, GD and GO, new selective PPARγ or PPARα ligands, able to exercize an anti-inflammatory effect, without inducing potentially dangerous secondary effects, will be explored.
- Trial are needed to evaluate new compounds targeting the agents taking part in GO pathogenesis [including rituximab, small antagonists of TSH-R molecules, teprotumumab (an anti-IGF-1R monoclonal antibody), tocilizumab (an antibody anti-soluble IL-6R)].
- Recent approaches to antigen-specific therapy for AITD have been published: treatment with broad-range immunosuppressive drugs can lead to important side effects, so that antigen-specific therapies have been designed to induce tolerance in a variety of autoimmune conditions.

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Table 1. New therapies in Graves' disease, or Graves' ophthalmopathy.

New therapy	Mechanism	Findings	References
NCGC00229600	TSH-R antagonist	Showed promising results in GO retro-orbital fibroblasts/adipocytes reducing the production of hyaluronic acid	(72)
Etanercept	Binding to TNFα	Inhibited TNFα in autoimmune diseases; remission in 6/10 patients with active GO	(73) (74)
Tocilizumab	mAb targeted to IL-6 receptor	A general significant improvement in ocular symptoms	(78) (79)
Rituximab	Induce B cells death	Able to reduce the disease activity; Conflicting data about the improvement of CAS	(85) (86) (87) (88)
Teprotumumab	Bind the extracellular IGF-1R domain	Lowered GO fibrocytes proliferation; in patients with active ophthalmopathy, reduced proptosis and CAS	(89) (90)
ATX-GD-59	TSH-R peptides that induce T cell tolerance through an immunization	Decreased serum TSH-R autoantibody concentrations in Graves' hyperthyroidism	(91)

CAS: clinical activity score; GO: Graves' ophthalmopathy; IGF-1R: insulin growth factor-1 receptor; IL-6: interleukin 6; TNFα: tumor necrosis factor α; TSH-R: thyroid-stimulating hormone receptor