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# Progress in the pathogenesis of thyroid-associated ophthalmopathy and new drug development

Yazhuo Huang<sup>1,2†</sup>, Sijie Fang<sup>1,2†</sup>, Shuo Zhang<sup>1,2</sup>, Huifang Zhou<sup>1,2\*</sup>

## Abstract:

Thyroid-associated ophthalmopathy (TAO) is the most common extrathyroidal manifestation of toxic diffuse goiter (Graves' disease), also known as Graves' ophthalmopathy/orbitopathy. As an organ-specific autoimmune disease, the pathogenesis of TAO is still unclear. In recent years, great progress has been made in revealing the mechanism of TAO. Various biological and immunosuppressive agents have emerged in an endless stream, showing encouraging results. Strengthening the basic research, establishing ideal animal models, deeply understanding the pathogenesis, and developing novel targeted drugs are of great significance to guide the clinical diagnosis and management of TAO and improve the prognosis of patients.

## Keywords:

Immunotherapy, new drug development, thyroid-associated ophthalmopathy

## Introduction

Thyroid-associated ophthalmopathy (TAO), also known as Graves' ophthalmopathy/orbitopathy, the most common extrathyroidal manifestation of Graves' disease (GD), is found in 25%–50% of patients with GD. TAO also occurs in 2% of patients with chronic thyroiditis and in a small number of people with normal thyroid function.<sup>[1]</sup> Previous studies showed that TAO is a tissue-specific autoimmune disorder, which is affected by many factors such as heredity, immunity, and environmental elements.<sup>[1,2]</sup> The clinical manifestations of TAO are complex, including eyelid retraction, exophthalmos, diplopia, restrictive strabismus, exposed keratitis, and dysthyroid optic neuropathy.<sup>[3,4]</sup> TAO is divided into an early active stage and a late chronic inactive stage and is classified as mild, moderate-to-severe, and very severe, namely sight-threatening, forms according to the impact of TAO on the quality of life and the risk of vision loss.<sup>[5]</sup>

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Recently, novel hypotheses in the pathogenesis of TAO such as fibrocyte, T-helper (Th) cell 17, and insulin-like growth factor-1 receptor (IGF-1R) are being confirmed. As a result, the development of therapeutic strategies for TAO based on immunologic perspective is rising. A variety of immunosuppressants and biological agents have shown great clinical potentials. In this review, we summarized the latest theories of pathogenesis and the emerging new drugs to provide insights into future management of TAO.

## Autoantigens

Thyroid-stimulating hormone receptor (TSHR) is the first recognized autoantigen of TAO, which is expressed on orbital fibroblasts (OFs).<sup>[6]</sup> In recent years, a number of studies have confirmed that IGF-1R might be another important autoantigen in disease progression. IGF-1R and TSHR overlap in the postreceptor signal transduction, and they synergistically regulate the function of OFs *in vitro*.<sup>[7]</sup>

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<sup>1</sup>Department of Ophthalmology, Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, <sup>2</sup>Shanghai Key Laboratory of Orbital Diseases and Ocular Oncology, Shanghai, China

<sup>†</sup>These authors contributed equally to this work.

## \*Address for correspondence:

Dr. Huifang Zhou,  
Department of Ophthalmology, Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, 639 Zhizaoju Road, Huangpu, Shanghai, China.  
E-mail: fangzzfang@163.com

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Compared with healthy individuals, GD patients have higher IGF-1R expression on the thyroid glands, OFs, and lymphocytes,<sup>[8,9]</sup> and IGF-1R is highly expressed on OFs obtained from patients with active TAO. It has been demonstrated that activation of IGF-1R by IGF-1 enables OFs to differentiate into adipocytes and secrete hyaluronic acid.<sup>[10-12]</sup> In addition, IGF-1 secreted by OFs plays a pathogenic role through autocrine and paracrine ways.<sup>[13]</sup>

In order to block TSHR-stimulating production of autoimmune antibodies, researchers developed a monoclonal antibody named K1-70 and carried out a clinical study on its efficacy in controlling hyperthyroidism of GD patients (NCT02904330), which is expected to be completed in 2020.<sup>[14]</sup> Since there is crosstalk between the pathogenesis of TAO and GD, K1-70 is also a hopeful drug for suppressing the ocular complications. Teprotumumab, a monoclonal blocking antibody of IGF-1R, is originally designed as an antitumor drug. Two randomized, double-blind, placebo-controlled clinical results with global multicenter participation published in 2017 and 2020, respectively, showed that 82.9% of TAO patients had lower exophthalmos (reduction of exophthalmos  $\geq 2$  mm) at the 24<sup>th</sup> week after teprotumumab treatment, whereas the placebo group had a value of 9.5%, while achieving secondary endpoints such as reduced diplopia, improved quality of life, and decreased clinical activity score (CAS). The adverse reactions of teprotumumab are mild, including potential hyperglycemia and hearing loss.<sup>[15,16]</sup> At present, teprotumumab has been approved by the US Food and Drug Administration as the first drug for TAO treatment.

## Lymphocytes

Both humoral and cellular immunities play important roles in orbital inflammation. Lymphocytes participate in the pathogenesis of TAO through the following pathways: (1) B-cells. B-cells migrate to the orbit and recognize TSHR and/or IGF-1R through B-cell receptors, the first signal for B-cell activation. T-cells bind to CD40 on the surface of B-cells through CD40L, which provides the second signal for B-cell activation. Meanwhile, interleukin (IL) 4 secreted by T-cells is essential for further activation of B-cells and their antibody-class switching.<sup>[17,18]</sup> Activated B-cell clones proliferate and differentiate into plasma cells that produce autoantibodies. Autoantibodies in TAO including stimulating, blocking, and neutralizing types recognize and attack OFs, leading to orbital inflammation. (2) T-cells. Antigen-presenting cells recognize TSHR and/or IGF-1R and then activate T-cells. The second signal for T-cell activation is provided by B7 on B-cells and CD28 on the surface of T-cells.<sup>[17]</sup> Activated T-cells, mainly CD4+ helper

T-cells, express adhesion molecules, secrete cytokines, and recruit more lymphocytes, which cause orbital inflammation, adipogenesis and fibrosis of orbital connective tissues, extracellular matrix deposition, and ultimately leading to orbital tissue remodeling.<sup>[17]</sup>

At present, there are two kinds of antimetabolic drugs that inhibit lymphocytes mycophenolate mofetil (MMF) and methotrexate (MTX). MMF can inhibit the *de novo* synthesis of guanosine and play an immunosuppressive role by inhibiting lymphocyte proliferation. MMF has been used to treat moderate-to-severe TAO in active stage. The results showed that MMF was superior to glucocorticoid therapy in many aspects with fewer side effects.<sup>[19]</sup> However, the high price limits wide use of the drug and its long-term efficacy and safety still need to be confirmed by a large number of follow-up clinical studies.<sup>[20]</sup> MTX is an antifolate antimetabolite that exerts immunosuppressive effect by interfering with the DNA/RNA synthesis of proliferating cells. It has been used in the treatment of glucocorticoid-insensitive TAO patients, showing good results but generally needs a long course of treatment.<sup>[21]</sup> Other drugs with more accurate targets are currently undergoing clinical trials for a variety of autoimmune diseases. For instance, orelizumab and teplizumab are used as CD3 antibodies to deplete T-cells, whereas CTLA-4 analogs such as abatacept limit the further activation of T-cells; these drugs have been approved for the treatment of type 1 diabetes and rheumatoid arthritis.<sup>[22,23]</sup> CFZ533 is a monoclonal antibody against CD40, which can inhibit the activation of B-cells and has been used in the treatment of myasthenia gravis and Sjogren's syndrome.<sup>[24,25]</sup> Furthermore, CFZ533 is currently approved for the treatment of GD (NCT02713256). We speculate that these drugs are expected to be used in TAO treatment in the near future.

As an important part of orbital inflammation, blocking the activation of B-cells is also expected to be used in the treatment of TAO. Rituximab (RTX) is a monoclonal antibody against CD20 on B-cell surface. RTX depletes B-cells and blocks antigen presenting processes, thus inhibiting T-cell activation.<sup>[26]</sup> As a second-line treatment recommended by the European Group on Graves' Orbitopathy (EUGOGO) guidelines,<sup>[5]</sup> many studies have focused on the application of RTX in the treatment of TAO, especially in patients who are insensitive or resistant to glucocorticoid therapy.<sup>[27]</sup> In 2015, Savino *et al.* reported that intraorbital injection of RTX was safe and effective.<sup>[28]</sup> Salvi *et al.* found that intravenous RTX was even better than methylprednisolone in improving eyeball movement, decreasing CAS scores, and reducing surgical rates.<sup>[29]</sup> However, another research by Stan *et al.* demonstrated that the effect of treating TAO with RTX was not different from that of the placebo group.<sup>[30]</sup> In

the latter study cohort, patients with longer duration, older age, higher male ratio, and higher levels of autoantibodies may all contribute to the reduction of RTX reactivity. Recently, a study by Chen *et al.* has shown that the removal of functionally defective B-cells by RTX can eliminate pro-inflammatory B-cells, which is beneficial for the treatment of TAO patients.<sup>[31]</sup> The EUGOGO guidelines recommend the use of RTX as one of several options for patients who are not sensitive or ineffective with intravenous glucocorticoid therapy.<sup>[5]</sup> Similar to RTX, belimumab is another monoclonal antibody targeting at the B-cell activating factor and has been used to treat systemic lupus erythematosus. Clinical trial of belimumab for TAO is currently undergoing (EUDRACT 2015-002127-26).<sup>[26]</sup>

## Cytokines

Previous studies have shown that interferon (IFN)- $\gamma$ -producing Th1 cells are dominant in the active phase of TAO, whereas IL-4-producing Th2 cells are dominant in the inactive phase.<sup>[32]</sup> In 2008, Huber *et al.* reported for the first time that the IL23R single-nucleotide polymorphism was related to the occurrence of TAO.<sup>[33]</sup> Several studies suggested a correlation between IL-17A and TAO development.<sup>[34-37]</sup> Our group first confirmed the inflammatory responses in TAO orbital connective tissue mediated by IL-17A-producing Th17 cells<sup>[38,39]</sup> and elucidated the regulatory mechanism of pathogenic Th17 cells on the adipogenesis and fibrosis of TAO orbital connective tissues.<sup>[40]</sup> Xin *et al.* reported that the methylation of IL-17RE was positively correlated with the CAS of TAO.<sup>[41]</sup> In addition, the expression of T-cell immunoglobulin and mucin domain-3 on Th17 cells in patients with TAO is reduced, which cannot inhibit the secretion of IFN- $\gamma$  and IL-17A by Th1 and Th17 cells, respectively.<sup>[42,43]</sup> Thus, the local orbital immune responses of TAO might be a complex regulatory process involving multiple T-cell subsets.

IFN- $\gamma$ , IL-4, IL-17A, and other cytokines form a precise network to coordinately promote the autoimmunity of TAO. IL-2 promotes the proliferation of T-cell clones; IFN- $\gamma$  upregulates CD40 expression on OFs and releasing of monocyte chemokines;<sup>[44]</sup> IL-4 activates B-cells; IL-17A and IL-1 $\beta$  stimulate OFs to secrete RANTES or IL-16 through different pathways;<sup>[3,39]</sup> TNF- $\alpha$  stimulates OFs to express adhesion molecules.<sup>[45]</sup> In addition, IL-1 $\beta$  is involved in maintaining the phenotype of Th17 cells and promoting prostaglandin E2 (PGE<sub>2</sub>) production by OFs.<sup>[38,46,47]</sup> On the one hand, PGE<sub>2</sub> stimulates OFs to produce IL-6 through the cyclic AMP pathway<sup>[48]</sup> and enhances the differentiation and pathogenic phenotype of Th17 cells;<sup>[40,46]</sup> meanwhile, PGE<sub>2</sub> assists B-cell maturation and antibody-type switching, activates mast cell degranulation, and induces Th2

cell immunity.<sup>[45]</sup> These are the molecular basis for the application of nonsteroidal anti-inflammatory drugs to inhibit the synthesis of prostaglandin as adjuvant therapy for TAO.

Based on the above cytokine network, many specific therapeutic targets can be applied. Cyclosporine inhibits the calcineurin pathway and reduces the secretion of IL-2 by Th2 cells. Clinical studies have shown that cyclosporine combined with glucocorticoid treatment for moderate-to-severe TAO has a good effect, but cyclosporine alone is not better than glucocorticoid alone, and the specific protocol has yet to be verified by more clinical trial data.<sup>[49]</sup> Studies have shown that IL-1R antagonists inhibit the production of glycosaminoglycan by OFs<sup>[50]</sup>, but this phenomenon has not been verified in patients with TAO. Tocilizumab (TCZ) is a monoclonal antibody against the human IL-6 receptor. A number of studies have shown that TCZ is safe and effective in treating TAO patients who were glucocorticoid insensitive. TCZ was also demonstrated to block the inflammatory cascade, improve clinical performance, and reduce CAS,<sup>[51-54]</sup> but it needs to be further verified in the clinical application. A variety of TNF- $\alpha$  monoclonal antibodies, such as etanercept, infliximab, and adalimumab, have shown good efficacy in TAO,<sup>[55-57]</sup> however most studies are case reports and large-scale population based studies have not been carried out. At present, many monoclonal antibodies have been successfully developed IL-17A-producing Th17 cells, including Cosentyx (secukinumab), Taltz (ixekizumab), and Siliq (brodalumab). The indications are psoriasis and mandatory spondylitis. The first Chinese monoclonal antibody of IL-17, SHR-1314, is currently under Phase II clinical trials. However, these anti-IL-17 monoclonal antibodies have not been used in the clinical treatment of TAO. In view of the vital role of Th17 cells in the pathogenesis of TAO, large-scale clinical research needs to be carried out, and it is expected to become an effective therapy.

## Orbital Fibroblasts

OFs are the target cells of TAO autoimmune responses. OFs differentiate into adipocytes and myofibroblasts under the stimulation of autoantibodies and cytokines, indicating that OFs have heterogeneous phenotypes and function. It has been revealed that OFs can be divided into two main subgroups: CD90+ OFs, which are prone to differentiate into myofibroblasts, and CD90- OFs, which are mostly transformed into adipocytes.<sup>[45,58]</sup> Both types of OFs can synthesize extracellular matrix such as hyaluronic acid and glycosaminoglycan in the inflammatory environment of TAO, leading to orbital connective tissue edema.<sup>[59]</sup> OFs express a variety of chemokines, such as ICAM-1, MIP-1, CXCL9/10/11,

and RANTES,<sup>[3,39,60,61]</sup> which can recruit T-cells infiltrating into the orbit. Meanwhile, OFs secrete various cytokines, such as IL-1 $\beta$ , IL-6, IL-8, and PGE<sub>2</sub>, which are involved in regulating the local immune responses in the orbit.<sup>[62]</sup>

It has been shown that platelet-derived growth factor (PDGF)-AA, AB, and BB are increased in orbital connective tissues of TAO patients, and OFs express PDGF receptor.<sup>[63,64]</sup> PDGFs induce the proliferation of OFs, stimulate their production of hyaluronic acid and IL-6,<sup>[65-67]</sup> and increase the expression levels of TSHR on OF surface.<sup>[68]</sup> Tyrosine kinase inhibitors, such as imatinib and nilotinib, can inhibit PDGF transduction signaling by blocking the phosphorylation of PDGF receptor on the surface of OFs. However, these inhibitors have adverse effects such as periorbital edema, peripheral arterial occlusive disease, and cerebrovascular event,<sup>[69]</sup> thus, the safe dose and efficacy of tyrosine kinase inhibitors need to be further explored.

### Fibrocytes

Smith *et al.* proposed that fibrocytes from peripheral blood are involved in the pathogenesis of TAO. Fibrocytes are derived from bone marrow and are found in very small amounts in peripheral blood. Surface markers of fibrocytes include CD45, CD34, CXCR4, and TSHR. In peripheral blood of GD patients, the proportion of CD34+ fibrocytes increases significantly.<sup>[70]</sup> When TAO occurs, CD34+ fibrocytes may infiltrate the orbit and differentiate into CD34+ OFs, coexisting with the original CD34- OFs in the orbit. Both CD34+ and CD34- OFs express TSHR and IGF-1R.<sup>[71]</sup> CD34- OFs in the orbit and CD34+ OFs originated from fibrocytes are mutually regulated. Some studies have pointed out that this regulatory effect may be controlled by Slit2 and AIRE.<sup>[72-75]</sup> Our study found that fibrocytes can recruit Th17 cells through the MIP-3/CCR-6 pathway.<sup>[76]</sup> In addition, TSH and CD40L can induce the secretion of IL-12 of fibrocytes,<sup>[77]</sup> which might be involved in the induction of Th1 cell immunity or transformation of Th17 cell to Th1 phenotype. Therefore, fibrocytes are also a potential target for precision treatment of TAO.

### Animal Models

The establishment of TAO animal model is not only an important means to study and verify the pathogenesis of this immune disorder but also a useful platform for new drug development. Over the years, researchers have carried out a lot of work in this field. Banga *et al.* constructed plasmids using TSHR subunits and induced TAO murine model successfully.<sup>[78-80]</sup> However, due to the large differences in the orbital structure of rodents and human beings, and the proneness of Th2-type immunity in BALB/c mice, the murine model cannot

accurately reflect the real inflammatory environment of the human orbit. Hence, it is still challenging to explore animal models of TAO with stable incidence and typical pathological and clinical manifestations.

### Conclusion

The onset of TAO involves autoantigens, lymphocytes, OFs, and many other parts. Glucocorticoid therapy is still preferred for patients with very severe and moderate-to-severe TAO, whereas orbital decompression surgery is considered for patients with inactive TAO, all of which are symptomatic approaches. In the last decades, progresses have been made in the field of basic research of TAO, and several potential drugs have been developed, bringing hope for the cure of the disease. However, the problems of how autoimmune tolerance is broken in TAO, the molecular mechanism that promotes fibrocyte differentiation into OFs, and the synergistic effect of different T-cell subsets need to be further studied. The clinical research of many new drugs for TAO is in different process, and their safety and efficacy also need to be clinically evaluated and verified. The establishment of a perfect TAO animal model is also an important part of new drug development. In the future, the basic research of TAO should be reinforced to uncover its pathogenesis, so as to accelerate the development of novel targeted drugs and benefit more patients.

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### Conflicts of interest

The authors declare that there are no conflicts of interest of this paper.

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