Published in final edited form as:

Expert Rev Ophthalmol. 2014 June ; 9(3): 227–234. doi:10.1586/17469899.2014.917960.

Cytokines are both villains and potential therapeutic targets in thyroid-associated ophthalmopathy: From bench to bedside

Fatemeh Rajaii^{#1}, Allison N. McCoy^{#1}, and Terry J. Smith^{1,2}

¹Department of Ophthalmology and Visual Sciences, University of Michigan Medical School, Ann Arbor, MI 48105

²Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48105

[#] These authors contributed equally to this work.

Summary

The pathophysiology underlying Graves' disease and its ocular manifestation, thyroid associated ophthalmopathy (TAO) is incompletely understood. Characterization of the mononuclear cells driving the disease and the cytokines they produce has led to significant advances in our understanding of TAO. This in turn has resulted in the identification of potentially attractive drug targets. For instance, development of inhibitors of specific cytokine pathways for use in other autoimmune diseases now presents an opportunity for their application in TAO. In this paper, we review the rationale for considering anti-cytokine therapy in TAO, evidence linking specific cytokines such as interleukin-6, tumor necrosis factor- α , and interleukin-17 pathways to TAO, and explore the potential for targeting of these pathways as therapy.

Keywords

Cytokines; Graves' disease; Graves' orbitopathy; interleukins; cytokines; inflammation; autoimmunity; thyroid-associated ophthalmopathy

1. Introduction

Absence of effective and safe medical therapies has resulted in important unmet needs of patients with thyroid-associated ophthalmopathy (TAO), the ocular manifestation of Graves' disease (GD). GD and TAO are currently believed to result from the autoimmune reaction to at least one self-antigen. Hyperthyroidism associated with GD results from the actions of thyroid stimulating immunoglobulin (TSI) on the thyrotropin receptor (TSHR) [1]. The promiscuity with which TSHR is expressed in tissues other than thyroid, including orbital fat, has raised the possibility that TSI might participate in the extra-thyroidal manifestations of GD [2,3]. While this is an attractive concept, the connectivity overarching the thyroid and orbit has yet to be demonstrated directly. Any insight into the downstream effects of TSHR signaling initiated by TSI or TSH within the orbit has resulted from inferences derived from treating cultured orbital fibroblasts with numerous agents and measuring cellular responses.

Several reports have demonstrated TSH-dependent up-regulation of cytokines such as interleukin 6 (IL-6) and members of the IL-1 family in fibroblasts and mononuclear cells [4-10]. These data provide evidence linking TSHR signaling to cytokine-driven inflammatory pathways. Their relevance to TAO remains speculative.

Cytokines play critical roles in normal processes and have been implicated in the pathogenesis of autoimmune diseases. As such, they have been therapeutically targeted. Monoclonal antibodies that block the actions of cytokines have emerged as effective treatment for rheumatoid arthritis (RA), psoriasis, seronegative spondyloarthropathies, inflammatory bowel disease, and multiple sclerosis [11, 12]. Although cytokines undoubtedly participate in some aspect of the tissue remodeling occurring in TAO, well-controlled and adequately powered prospective therapeutic trials involving agents that interrupt cytokine pathways have not yet been conducted. Many cytokines with broad roles in normal and pathologic immune function have been implicated in the pathogenesis of TAO, including IL-1 α , IL-1 β , IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1). In this article, we review evidence linking certain cytokines, including IL-6, tumor necrosis factor- α (TNF- α), and IL-17 to TAO. We have chosen to focus on these cytokines and not others, as exemplifying molecules the specificity of which has been tied previously to their roles in other autoimmune diseases. Therapeutic disruption of the associated pathways has been evaluated in allied autoimmune diseases and these may have acceptable side-effect profiles. We discuss the downstream targets of these cytokines as well as their disruption by small molecules and monoclonal antibodies as potential therapies in TAO.

2. Cell populations of importance in TAO

The histopathology of GD is characterized by infiltration of the thyroid and orbit by T and B lymphocytes, monocytes, and mast cells, but T cells predominate [13]. The precise functions of these cells in TAO are not well established. Both B and T cells play key roles in autoimmune disease, producing cytokines that are important in driving inflammation. In addition, B lymphocytes produce autoantibodies and present antigens while T cells provide co-stimulatory factors and serve cytotoxic functions. Th1 and Th2 cells accumulating in orbital tissue affected by TAO can be identified in a 1:2 ratio [14]. Circulating, activated T and B cells are more numerous in TAO compared to healthy individuals [15]. Activated T cells isolated from the orbit and peripheral blood of patients with GD express IGF-1 receptors (IGF-1R) at higher levels [16]. Further, IGF-1 promotes proliferation and inhibits apoptosis in these cells. Aberrant IGF-1R expression has also been demonstrated in B cells isolated from the orbit, blood, and bone marrow of these patients [17]. Moreover, IGF-1 enhances antibody production in these cells. Autologous T cells in co-culture induce proliferation of orbital fibroblasts derived from individuals with TAO, actions that are absent in cultures derived from healthy controls [18]. This effect is mediated by MHC class II, CD40, and CD40 ligand (CD40L) interactions.

Fibrocytes are CD34⁺ bone marrow-derived cells belonging to the monocyte lineage and circulate until recruited to sites of injury [19]. They are considerably more abundant in patients with GD than in healthy individuals and a greater fraction of these are TSHR⁺ [6, 20]. In fact, fibrocytes express TSHR at extremely high levels, comparable to those found on

thyroid epithelial cells [6]. The importance of fibroblasts apparently derived from bone marrow in mediating the pathology associated with GD is suggested by finding CD34⁺ fibroblasts in both thyroid and orbit [21]. Orbital fibroblasts can be divided into two distinct populations based on their surface display of CD34. CD34⁺ orbital fibroblasts may represent circulating CD34⁺ fibrocytes that have infiltrated affected tissue while CD34⁻ cells appear to be locally derived [22]. Evidence has emerged that CD34⁺ orbital fibroblasts are found uniquely in tissues from individuals with TAO [21]. CD34⁺ fibroblasts exhibit distinct phenotypic attributes from those of their CD34⁻ counterparts. Resident orbital fibroblasts differ from their systemic counterparts in that they derive from neural crest and are particularly responsive to inflammatory signals such as those provided by cytokines [23-25]. However, both CD34⁺ fibrocytes and orbital fibroblasts share certain attributes such as responses to cytokines and TSH by producing IL-1 receptor antagonist despite differences in the patterns of isoform expression [10].

3. Cytokine pathways potentially relevant to TAO

IL-6

IL-6 has been implicated in several autoimmune diseases [26, 27]. Initially identified as a product of T cells, IL-6 promotes B cell differentiation and immunoglobulin synthesis [26]. Further, it regulates immune responses, inflammation, acute phase responses, and hematopoiesis [26]. IL-6 plays a critical role in the interplay between innate and adaptive immunity. This cytokine has been shown to influence the development of both cytotoxic and regulatory T (Treg) cells. Together with TGF- β , it promotes T cell differentiation. In the presence of TGF- β , IL-6 enhances Th17 polarization whereas, in its absence, IL-6 promotes Treg differentiation [28]. The balance between Th17 and Treg cells, in turn, affects the inflammatory milieu. Furthermore, IL-6 promotes inflammation with multiple direct tissue target effects. Thus, the blockade of IL-6 may produce both anti-inflammatory and pro-regulatory effects in a tissue undergoing an autoimmune response, such as the orbit in TAO [29].

Orbital fibroblasts express CD40 and respond to its cognate, CD40 ligand (CD40L, aka CD154) by expressing IL-6, IL-8, and macrophage chemoattractant protein-1 (MCP-1) [30, 31]. In addition, fibrocytes also express CD40, and CD40L induces several pro-inflammatory cytokines, including IL-6, IL-8, IL-10, TNF- α , and regulated on activation, normal T cell expressed and secreted (RANTES) [8]. Prostaglandin E₂ is a powerful inducer of IL-6 in orbital fibroblasts [32]. TSHR stimulation also induces expression of IL-6, IL-8, and TNF- α in orbital fibroblasts and fibrocytes [6, 7, 32]. Serum IL-6 and IL-6 receptor levels are elevated in patients with GD and TAO [33-37]. They normalize when hyperthyroid patients are rendered euthyroid with methimazole or propylthiouracil [33-37]. Taken together, these findings suggest a potentially important mechanistic link between thyroid hormones and the IL-6 pathway.

TNF- α

TNF- α was initially isolated as a substance that could induce hemorrhagic necrosis of transplanted tumor cells in murine models [38]. It is produced by T cells, natural killer cells,

mast cells, and fibroblasts. Activation of TNF- α receptors by TNF- α stimulates apoptotic pathways and cell proliferation, and leads to activation of NF- κ B, Mitogen activated protein kinase, Erk and Jun kinase [39]. Activation of these kinase pathways, in turn, leads to expression of IL-6, IL-8, IL-18, and cyclooxygenase 2, and can result in inflammation and tissue remodeling such as that occurring in TAO [39].

Expression of TNF- α has been demonstrated in the orbits of patients with GD *in vivo* [14]. More recently, fibrocytes have been shown to express TNF- α in response to either TSH or CD40L [6, 8]. One study demonstrated that serum TNF- α levels are elevated in patients with TAO, but not in those with GD without TAO [37]. Serum TNF- α and soluble TNF receptor levels are elevated in patients with newly diagnosed GD, and decrease following correction of hyperthyroidism [40]. Gene polymorphism analyses have demonstrated that single nucleotide polymorphisms (SNPs) of the TNF- α gene are associated with TAO in Japanese and Iranian populations [41, 42]. These associations make the interrogation of the multiple currently available anti-TNF- α agents as potential therapies in TAO a very attractive line of investigation.

IL-23/IL-17

The IL-23/IL-17 pathway has been implicated in multiple autoimmune diseases, but only recently has it been mentioned in the context of GD [43]. IL-23 is secreted by dendritic cells and macrophages, and polarizes naive T helper cells toward the Th17 phenotype. Th17 cells, in turn, secrete IL-17, which acts as a potent pro-inflammatory cytokine [43]. IL-17 is also expressed by CD8⁺ T cells and natural killer cells. The importance of this pathway in autoimmune disease has been highlighted by the finding that IL-23 deficient mice are protected against the development of experimental autoimmune encephalitis (EAE), joint inflammation, and bowel disease [44-46]. Elevated serum levels of IL-17 have been detected in RA, systemic lupus erythematosus (SLE), and psoriasis. [47] In conjunction with IL-6 and TGF β , IL-23 induces the differentiation of Th 17 cells, while IL-17 induces IL-6 signaling via NF- κ B in a positive feedback loop, leading to dysregulated cytokine production characteristic of many autoimmune diseases [48].

Several case series implicate the IL-23/IL-17 pathways in GD. One study detected increased serum levels of IL-23 and IL-17 in Korean patients with TAO [49]. Another from China demonstrated that IL-17 mRNA expression induced by IL-23 was exaggerated in peripheral blood mononuclear cells (PBMCs) from donors with GD compared to those from healthy subjects. Whether fibrocytes contribute to IL-17 secretion in PBMCs remains uncertain [43]. A cohort study in the Han Chinese population revealed a specific polymorphism of the *IL-17F* gene that appeared to be associated with GD and TAO [50]. In addition to increased serum IL-17 levels, Th17 cells were more abundant in patients with autoimmune thyroid disease compared to healthy controls. Th17 cells were also more numerous in patients with intractable GD compared to those with GD in remission [51-53]. Both the frequency of Th17 cells and serum levels of IL-17 in Chinese patients with GD correlate with anti-TSHR antibody titers [52].

The specific downstream effects of IL-17 and IL-23 expression in orbital tissues have yet to be explored. It is not known whether orbital fibroblasts or fibrocytes express IL-17, IL-23,

or their receptors. It is also uncertain whether Th17 cells infiltrate the orbit in TAO. IL-17 receptors have been detected on cultured thyrocytes in GD [53]. Future studies evaluating the role of the IL-17/IL-23 pathway in GD could inform whether IL-17 and IL-23 are secreted by orbital cells, including residential fibroblasts, those derived from circulating fibrocytes, and lymphocytes trafficked to the orbit. These insights might then lead to the initiation of clinical trials with agents currently being evaluated for other autoimmune diseases.

4. A roadmap to future therapy for TAO: repurposing of agents designed for allied diseases

Progress in solving several autoimmune diseases has been considerably more rapid than the acquisition of insight into TAO. A number of possible factors have proven challenging to the development of therapies for TAO, including the absence of complete and robust animal models. A potentially productive avenue to pursue as a consequence of the relatively slow progress in this disease is to “borrow” wisdom from what has been learned recently in allied autoimmune diseases. This section will review the evidence for inhibitors of IL-6, TNF- α , and IL-17/23 pathways in related diseases and consider their potential usefulness as repurposed agents in TAO.

IL-6 receptor (IL-6R) antagonists

Given the putative role for IL-6 in TAO, a list of potential therapies for that disease should include small molecule antagonists and monoclonal blocking antibodies targeting IL-6R. Tocilizumab and atilizumab are both fully human monoclonal antibodies directed against IL-6R. Tocilizumab has demonstrated safety and effectiveness in treating and juvenile idiopathic arthritis (JIA) [54-57]. It is administered by intravenous infusion. Reported side effects include upper respiratory infections, nasopharyngitis, headache, elevated cholesterol, and hypertension [58]. The incidence of serious infections has been reported to be 4.7/100 patient-years, with only 0.13/100 patient-years of fatal infections [56]. Singh and colleagues (2011) found that the combination of tocilizumab and methotrexate significantly reduced RA disease activity but increased the ratio of total cholesterol to high-density lipoprotein in 12% of patients compared to 7% in patients treated with methotrexate alone [59]. The drug has been approved in the USA and Europe for use in patients with RA refractory to treatment with at least one anti-TNF- α agent, as well as in children 2 years or older with systemic JIA. Additionally, the drug has been approved in Japan for the treatment of Castleman’s disease, a rare B-cell tumor [12].

Inhibitors of TNF- α

The potential role for TNF- α inhibitors in TAO has been explored with greater detail than for any other cytokine-targeting therapy. TNF- α inhibitors first came to prominence for their efficacy in the treatment of RA over a decade ago [60]. Although effective, the excitement over this class of drugs has been tempered by its serious side effects, including tuberculosis reactivation and other life-threatening infections [60, 61]. One meta-analysis has reported that the odds ratio for serious infections was 2.0 in those treated with anti-TNF- α compared to controls and the odds ratio for developing malignancy was 3.3 [61].

TNF- α inhibitors may also benefit patients with TAO. In one pilot study, treatment with etanercept in 10 patients with newly diagnosed, mild-to-moderate TAO resulted in improved clinical activity scores and ophthalmopathy indices [63]. The drug had the greatest effect on soft tissue swelling and redness, with minimal improvement in strabismus and proptosis. Similarly, Durrani *et al.* reported the effects of infliximab in a single patient with recalcitrant, vision-threatening TAO [64]. The patient had failed to respond to corticosteroids, but had an impressive reduction in CAS from 10 to 2 one week after infliximab was administered. This agent has also been shown to benefit patients with intraocular inflammation [65]. Unfortunately, level III evidence from prospective, randomized controlled trials is lacking.

A more recent case study revealed onset of TAO in a patient with RA who was being treated with the TNF- α inhibitor, adalimumab [66]. Although the authors speculated that TNF- α therapy was responsible for provoking GD, it is unclear why the disease would present after 8 years of treatment with that agent. Moreover, it is well known that thyroid dysfunction occurs more frequently in patients with other autoimmune diseases [67]. No evidence has emerged that TNF- α blockade is responsible for thyroid disease. The evidence for a beneficial effect of TNF- α inhibitors in TAO remains preliminary. However, enthusiasm for this class of drugs has yielded to the pursuit of other targeted therapies perceived to have fewer side effects. Among them is B cell depletion with rituximab or inhibitors of the IGF-1R or TSHR. An ongoing trial targeting enrollment of 84 patients with severe, active TAO is currently being randomized to treatment with Teprotumumab, a fully humanized anti-IGF-1R monoclonal blocking antibody or to placebo [<http://clinicaltrials.gov/show/NCT01868997>].

Inhibitors of IL-17/IL-23

A more recently identified pathway that appears involved in chronic inflammation involves IL-17 as its terminal effector. Drugs that modulate the IL-17 pathway have shown promise in preclinical experimental models and clinical trials for psoriasis and ankylosing spondylitis [68, 69]. Secukinumab and ixekizumab neutralize IL-17, while brodalumab is an IL-17R antagonist [12]. Phase II trials for all three drugs demonstrated improvement of psoriasis activity and severity, and phase III trials are ongoing [68]. Secukinumab has also undergone an industry-sponsored phase III randomized, double-blinded, placebo-controlled trial for ankylosing spondylitis [69]. Patients with moderate-to-severe disease were screened and randomly assigned to the active drug (n=24) or placebo (n=6). Efficacy was assessed as the fraction of patients exhibiting a 20% response according to standardized criteria for clinical improvement (ASAS20 response). The safety of the drug was assessed for up to 28 weeks. Six weeks following treatment, the ASAS20 response estimates were 59% for drug versus 24% for placebo. Only one out of 24 secukinumab-treated patients experienced a serious adverse event, the development of a *S. aureus* subcutaneous abscess during the 28-week observation period. The authors concluded that secukinumab was well tolerated and may provide an alternative to anti-TNF therapy. This class of agents may be suitable for trial in TAO.

In addition to molecules that directly target IL-17 or IL-17R, other small molecules influence IL-17 levels indirectly. One such agent is developmental endothelial locus 1 (Del-1), an endogenous inhibitor of neutrophil adhesion mediated by the integrin, LFA-1. In a recent report, Del-1 was demonstrated to suppress IL-17-associated inflammation in a mouse model of periodontitis [70, 71]. Del-1 inhibited IL-17 expression and suppressed neutrophil recruitment. Conversely, mice deficient in Del-1 developed spontaneous periodontitis with excessive neutrophil infiltration and IL-17 expression. Manifestation of the disease was prevented when the animals were also deficient in LFA-1 or the IL-17 receptor. Locally delivered Del-1 inhibited IL-17 production, neutrophil infiltration, and periodontal bone loss in the mouse model [71]. These findings implicate Del-1 as a naturally occurring suppressor of neutrophil recruitment and IL-17-driven inflammation. It is not yet known if Del-1 is present in orbital tissues and if it acts as a universal regulator of IL-17. Future studies could determine whether locally or systemically delivered Del-1 holds promise as an anti-inflammatory therapeutic strategy in TAO.

p53 is a transcription factor best known for its regulation of the cell cycle and function as a tumor suppressor gene. Less well acknowledged is its putative role in autoimmune diseases such as RA where its mutation results in the proliferation of pathogenic cells [72]. In a mouse model of RA, the ratio of pro-inflammatory Th17 cells to anti-inflammatory Treg cells and IL-17 levels were assessed. CD4⁺ T cells from p53 knock-out mice showed reduced Treg cell differentiation. Under inflammatory conditions, p53 suppressed Th17 cell differentiation and skewed T cells toward the Treg phenotype. Conversely, p53 overexpression appeared to control development of experimental RA, providing evidence both *in vivo* and *in vitro* for p53-mediated regulation of IL-17 [73]. Thus p53 appears to control Th17 and Treg cell differentiation and in so doing influence the genesis or prevention of autoimmunity. Targeting the p53 pathway may therefore provide an additional strategy to silence IL-17-related inflammation underlying TAO.

IL-12, also known as the T cell-stimulating factor, is involved in the differentiation of naive T cells to those exhibiting a Th1 phenotype [74]. In addition, IL-12 modulates interferon-gamma (IFN- γ) production by T and natural killer cells [75]. Ustekinumab is a human IgG1 kappa monoclonal antibody that binds to the p40 subunit common to both IL-12 and IL-23. This in turn blocks cytokine/receptor interactions [76]. Ustekinumab has demonstrated efficacy in several autoimmune diseases, including psoriasis and Crohn's disease [77, 78]. Skin and bowel lesions associated with these diseases contain infiltrates of Th1 and Th17 cells, both of which are reduced following ustekinumab treatment [29]. Phase III clinical trials involving ustekinumab in psoriasis and Crohn's disease have thus far demonstrated both safety and efficacy, with serious adverse events occurring in 1.2% of patients; one patient developed basal cell carcinoma, but there were no other malignancies [77-79].

Antibodies targeting the p19 subunit of IL-23 have also been explored in animal models of autoimmune disease. In a model of psoriasis, monoclonal anti-mouse IL23p19 antibodies were effective in decreasing transcription of genes encoding IL-17 and IL-22, as well as decreasing epidermal hyperplasia [80]. They were as effective as anti-p40 antibodies in preventing acute and relapsing EAE [81]. The strategy of targeting IL-23p19 in disease is

currently being studied using a humanized IgG1/k, anti-IL-23p19 monoclonal antibody in patients with moderate to severe chronic plaque psoriasis [82].

Toll-like receptors (TLR) play enormously important roles in immunity [83]. TLR antagonists may be useful in inhibiting IL-17/IL-23 signaling in GD. In a mouse model of IL-23-induced psoriasis, an oligonucleotide-based antagonist of TLR7, 8, and 9 attenuated the induction of Th1 and Th17 cytokines in skin and serum. This antagonist also reduced the dermal expression of multiple inflammasome components [84]. It is tempting to speculate that TLR antagonists will show similar promise for the treatment of TAO.

Lymphocyte depletion

The insight that distinct lymphocyte populations play significant roles in TAO has driven B cell depletion with rituximab as treatment for the disease. Two prospective, randomized studies have recently concluded [85-86]. Although no results have yet been published, data from small case series of 6-13 patients appears promising and documented significant decreases in the clinical activity scores [87-91]. These preliminary findings demonstrate the potential for therapeutically targeting B cells as immunotherapy in TAO.

5. Conclusions and Expert Commentary

Despite the substantial challenges confronting our attempts to better understand the pathogenesis of TAO, recent observations have provided new insights into the cell populations and cytokine signaling pathways which may hold proximate relevance to the disease process. Ample evidence demonstrates the putative importance of IL-6, TNF- α , and IL-17/IL-23 in TAO. Additional studies will be needed to gain better clarity into the specific roles for each cytokine in immune cell trafficking, orbital tissue activation, and immune modulation as active TAO transitions to the stable, chronic phase. In addition, the determinants that switch cytokine function from physiological actions to those associated with disease are poorly understood. Growing understanding of the complex cytokine networks involved in TAO theoretically will offer expanded opportunities to specifically target disease-related pathways. The availability of agents targeting these cytokines, and the promising results associated with their use in allied autoimmune diseases, suggests exciting possibilities for effectively treating TAO.

6. Five Year View

As in other autoimmune diseases, cytokine pathways play a role in TAO. Development of specific cytokine-based therapies offers promising avenues for therapeutic intervention. In particular, IL-6, TNF- α , and IL-17/IL-23 appear to be involved in the pathogenesis of TAO. Further elucidation of their role in GD and TAO, perhaps in preclinical models, will allow translation of these insights into specific therapies. Given the existing evidence for efficacy and relative safety of agents that disrupt IL-6, TNF- α , and IL-17/IL-23 in allied diseases, we anticipate the conduct of adequately powered clinical trials of repurposed anti-cytokine agents in patients with TAO. The ultimate goal within the five year horizon will be to transition TAO from a surgically corrected disease to one that is effectively treated with agents that alter the natural disease course and obviate the need for invasive rehabilitation.

Acknowledgments

Grants: This work was supported in part by National Institutes of Health grants EY008976, EY011708, DK063121, Center for Vision grant EY007003 from the NEI, an unrestricted grant from Research to Prevent Blindness, and the Bell Charitable Foundation.

References

* Of interest

** Of Considerable Interest

1. Ginsberg J, Shewring G, Smith BR. TSH receptor binding and thyroid stimulation by sera from patients with Graves' disease. *Clin Endocrinol (Oxf)*. 1983; 19:305–11. [PubMed: 6138173]
- 2*. Feliciello A, Porcellini A, Ciullo I, Bonavolontà G, Avvedimento EV, Fenzi G. Expression of thyrotropin-receptor mRNA in healthy and Graves' disease retro-orbital tissue. *Lancet*. 1993; 342:337–8. [PubMed: 8101586] Initial demonstration of the expression of TSHR mRNA in orbital tissue of GD and control patients
3. Stadlmayr W, Spitzweg C, Bichlmair AM, Heufelder AE. TSH receptor transcripts and TSH receptor-like immunoreactivity in orbital and pretibial fibroblasts of patients with Graves' ophthalmopathy and pretibial myxedema. *Thyroid*. 1997; 7:3–12. [PubMed: 9086563]
4. Antunes TT, Gagnon A, Bell A, Sorisky A. Thyroid-stimulating hormone stimulates interleukin-6 release from 3T3-L1 adipocytes through a cAMP-protein kinase A pathway. *Obes Res*. 2005; 13:2066–71. [PubMed: 16421339]
5. Bell A, Gagnon A, Sorisky A. TSH stimulates IL-6 secretion from adipocytes in culture. *Arterioscler Thromb Vasc Biol*. 2003; 23:e65–6. [PubMed: 14672881]
- 6**. Douglas RS, Afifiyan NF, Hwang CJ, et al. Increased generation of fibrocytes in thyroid-associated ophthalmopathy. *J Clin Endocrinol Metab*. 2010; 95:430–8. [PubMed: 19897675] Demonstrated the abundance of CD34+ fibrocytes in orbital tissue of GD patients, which express levels of TSHR comparable to thyroid tissue and respond to TSH by producing inflammatory cytokines.
7. Kumar S, Schiefer R, Coenen MJ, Bahn RS. A stimulatory thyrotropin receptor antibody (M22) and thyrotropin increase interleukin-6 expression and secretion in Graves' orbital preadipocyte fibroblasts. *Thyroid*. 2010; 20:59–65. [PubMed: 20017620]
8. Gillespie EF, Raychaudhuri N, Papageorgiou KI, et al. Interleukin-6 production in CD40-engaged fibrocytes in thyroid-associated ophthalmopathy: involvement of Akt and NF- κ B. *Invest Ophthalmol Vis Sci*. 2012; 53:7746–53. [PubMed: 23092922]
9. Raychaudhuri N, Fernando R, Smith TJ. Thyrotropin Regulates IL-6 Expression in CD34(+) Fibrocytes: Clear Delineation of Its cAMP-Independent Actions. *PLoS One*. 2013; 8:e75100. [PubMed: 24086448]
10. Li B, Smith TJ. Regulation of IL-1 Receptor Antagonist by TSH in Fibrocytes and Orbital Fibroblasts. *J Clin Endocrinol Metab*. 2014; 21 jc20133977.
11. Baeten DL, Kuchroo VK. How Cytokine networks fuel inflammation: Interleukin-17 and a tale of two autoimmune diseases. *Nat Med*. 2013; 19:824–5. [PubMed: 23836225]
12. Kotsovilis, S.; Andreakos, E. Therapeutic Human Monoclonal Antibodies in inflammatory diseases. In: Steinitz, Michael, editor. *Human Monoclonal Antibodies: Methods and Protocols, Methods in Molecular Biology*. Vol. 1060. Springer Science+Business Media, LLC; 2014.
13. Weetman AP, Cohen S, Gatter KC, Fells P, Shine B. Immunohistochemical analysis of the retrobulbar tissues in Graves' ophthalmopathy. *Clin Exp Immunol*. 1989; 75:222–7. [PubMed: 2784734]
- 14*. Heufelder AE, Bahn RS. Detection and localization of cytokine immunoreactivity in retro-ocular connective tissue in Graves' ophthalmopathy. *Eur J Clin Invest*. 1993; 23:10–7. [PubMed: 8444271] Used immunohistochemistry to show that TNF- α , interferon γ , and IL-1 α are expressed in mononuclear aggregates in orbital tissue in TAO patients, but not controls.

15. Douglas RS, Gianoukakis AG, Goldberg RA, Kamat S, Smith TJ. Circulating mononuclear cells from euthyroid patients with thyroid-associated ophthalmopathy exhibit characteristic phenotypes. *Clin Exp Immunol.* 2007; 148:64–71. [PubMed: 17349012]
16. Douglas RS, Gianoukakis AG, Kamat S, Smith TJ. Aberrant expression of the insulin-like growth factor-1 receptor by T cells from patients with Graves' disease may carry functional consequences for disease pathogenesis. *J Immunol.* 2007; 178:3281–7. [PubMed: 17312178]
17. Douglas RS, Naik V, Hwang CJ, et al. B cells from patients with Graves' disease aberrantly express the IGF-1 receptor: implications for disease pathogenesis. *J Immunol.* 2008; 181:5768–74. [PubMed: 18832736]
- 18*. Feldon SE, Park DJ, O'Loughlin CW, et al. Autologous T-lymphocytes stimulate proliferation of orbital fibroblasts derived from patients with Graves' ophthalmopathy. *Invest Ophthalmol Vis Sci.* 2005; 46:3913–21. [PubMed: 16249464] Showed that autologous T cells can stimulate proliferation of orbital fibroblasts derived from patients with GD through a mechanism dependent on MHC class II and CD40-CD40L interactions.
- 19*. Bucala R, Spiegel LA, Chesney J, Hogan M, Cerami A. Circulating fibrocytes define a new leukocyte subpopulation that mediates tissue repair. *Mol Med.* 1994; 1:71–81. [PubMed: 8790603] Initial description of fibrocytes as circulating cells that are recruited to sites of tissue injury.
20. Gillespie EF, Papageorgiou KI, Fernando R, et al. Increased expression of TSH receptor by fibrocytes in thyroid-associated ophthalmopathy leads to chemokine production. *J Clin Endocrinol Metab.* 2012; 97:E740–6. [PubMed: 22399514]
21. Smith TJ, Padovani-Claudio DA, Lu Y, et al. Fibroblasts expressing the thyrotropin receptor overarch thyroid and orbit in Graves' disease. *J Clin Endocrinol Metab.* 2011; 96:3827–37. [PubMed: 21956421]
22. Fernando R, Atkins S, Raychaudhuri N, et al. Human fibrocytes coexpress thyroglobulin and thyrotropin receptor. *Proc Natl Acad Sci U S A.* 2012; 109:7427–32. [PubMed: 22517745]
23. Noden, DM. Periocular mesenchyme: neural crest and mesodermal interactions. In: Jakobiec, FA., editor. *Ocular anatomy, embryology and teratology.* Vol. 97. Harper and Row; Philadelphia: 1982. p. 119
24. Smith TJ, Ahmed A, Hogg MG, Higgins PJ. Interferon-gamma is an inducer of plasminogen activator inhibitor type 1 in human orbital fibroblasts. *Am J Physiol.* 1992; 263:C24–9. [PubMed: 1636680]
25. Cao HJ, Hogg MG, Martino LJ, Smith TJ. Transforming growth factor-beta induces plasminogen activator inhibitor type-1 in cultured human orbital fibroblasts. *Invest Ophthalmol Vis Sci.* 1995; 36:1411–9. [PubMed: 7775119]
26. Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev.* 2002; 13(4-5):357–68. [PubMed: 12220549]
27. Leech MD, Barr TA, Turner DG, et al. Cutting edge: IL-6-dependent autoimmune disease: dendritic cells as a sufficient, but transient, source. *J Immunol.* 2013; 190(3):881–5. [PubMed: 23267024]
28. Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. *Eur J Immunol.* 2010; 40(7):1830–5. [PubMed: 20583029]
29. Nepom GT, Ehlers M, Mandrup-Poulsen T. Anti-cytokine therapies in T1D: Concepts and strategies. *Clin Immunol.* 2013; 149(3):279–85. [PubMed: 23510726]
30. Sempowski GD, Rozenblit J, Smith TJ, Phipps RP. Human orbital fibroblasts are activated through CD40 to induce proinflammatory cytokine production. *Am J Physiol.* 1998; 274:C707–14. [PubMed: 9530102]
31. Hwang CJ, Afifiyan N, Sand D, et al. Orbital fibroblasts from patients with thyroid-associated ophthalmopathy overexpress CD40: CD154 hyperinduces IL-6, IL-8, and MCP-1. *Invest Ophthalmol Vis Sci.* 2009; 50:2262–8. [PubMed: 19117935]
32. Raychaudhuri N, Douglas RS, Smith TJ. PGE2 induces IL-6 in orbital fibroblasts through EP2 receptors and increased gene promoter activity: implications to thyroid-associated ophthalmopathy. *PLoS One.* 2010; 5:e15296. [PubMed: 21209948]

33. Celik I, Akalin S, Erba T. Serum levels of interleukin 6 and tumor necrosis factor- α in hyperthyroid patients before and after propylthiouracil treatment. *Eur J Endocrinol*. 1995; 132:668–72. [PubMed: 7788003]
34. Molnár I, Balázs C. High circulating IL-6 level in Graves' ophthalmopathy. *Autoimmunity*. 1997; 25:91–6. [PubMed: 9189010]
35. Salvi M, Girasole G, Pedrazzoni M, et al. Increased serum concentrations of interleukin-6 (IL-6) and soluble IL-6 receptor in patients with Graves' disease. *J Clin Endocrinol Metab*. 1996; 81:2976–9. [PubMed: 8768861]
36. Salvi M, Pedrazzoni M, Girasole G, et al. Serum concentrations of proinflammatory cytokines in Graves' disease: effect of treatment, thyroid function, ophthalmopathy and cigarette smoking. *Eur J Endocrinol*. 2000; 143:197–202. [PubMed: 10913938]
37. Pedro AB, Romaldini JH, Takei K. Changes of serum cytokines in hyperthyroid Graves' disease patients at diagnosis and during methimazole treatment. *Neuroimmunomodulation*. 2011; 18:45–51. [PubMed: 20628263]
38. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci U S A*. 1975; 72:3666–70. [PubMed: 1103152]
39. Vendramini-Costa DB, Carvalho JE. Molecular link mechanisms between inflammation and cancer. *Curr Pharm Des*. 2012; 18(26):3831–52. [PubMed: 22632748]
40. Pichler R, Maschek W, Hatzl-Griesenhofer M, Huber H, Crespillo-Gómez C, Berg J. Soluble tumour necrosis factor- α receptor I and interleukin-6 as markers of activity in thyrotoxic Graves' disease. *Horm Metab Res*. 2003; 35:427–33. [PubMed: 12931274]
41. Anvari M, Khalilzadeh O, Esteghamati A, et al. Graves' disease and gene polymorphism of TNF- α , IL-2, IL-6, IL-12, and IFN- γ . *Endocrine*. 2010; 37:344–8. [PubMed: 20960273]
42. Kamizono S, Hiromatsu Y, Seki N, et al. A polymorphism of the 5' flanking region of tumour necrosis factor alpha gene is associated with thyroid-associated ophthalmopathy in Japanese. *Clin Endocrinol (Oxf)*. 2000; 52:759–64. [PubMed: 10848881]
43. Zheng L, Ye P, Liu C. The role of the IL-23/IL-17 axis in the pathogenesis of Graves' disease. *Endocr J*. 2013; 60:591–7. [PubMed: 23327801]
- 44*. Cua DJ, Sherlock J, Chen Y, et al. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature*. 2003; 421:744–8. [PubMed: 12610626]
Using an IL-23 knockout mouse, demonstrated critical role of IL-23 in EAE.
45. Murphy CA, Langrish CL, Chen Y, et al. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. *J Exp Med*. 2003; 198:1951–7. [PubMed: 14662908]
46. Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med*. 2005; 201:233–40. [PubMed: 15657292]
47. Bedoya SK, Lam B, Lau K, Larkin J 3rd. Th17 cells in immunity and autoimmunity. *Clin Dev Immunol*. 2013; 2013:986789. [PubMed: 24454481]
48. Ogura H1, Murakami M, Okuyama Y, et al. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity*. 2008; 29(4):628–36. [PubMed: 18848474]
49. Kim SE, Yoon JS, Kim KH, Lee SY. Increased serum interleukin-17 in Graves' ophthalmopathy. *Graefes Arch Clin Exp Ophthalmol*. 2012; 250:1521–6. [PubMed: 22752189]
50. Guo T, Huo Y, Zhu W, et al. Genetic association between IL-17F gene polymorphisms and the pathogenesis of Graves' Disease in the Han Chinese population. *Gene*. 2013; 512:300–4. [PubMed: 23111159]
51. Nanba T, Watanabe M, Inoue N, Iwatani Y. Increases of the Th1/Th2 cell ratio in severe Hashimoto's disease and in the proportion of Th17 cells in intractable Graves' disease. *Thyroid*. 2009; 19:495–501. [PubMed: 19415997]
52. Peng D, Xu B, Wang Y, Guo H, Jiang Y. A high frequency of circulating th22 and th17 cells in patients with new onset Graves' disease. *PLoS One*. 2013; 8:e68446. [PubMed: 23874630]
53. Li JR, Hong FY, Zeng JY, Huang GL. Functional interleukin-17 receptor A are present in the thyroid gland in intractable Graves' disease. *Cell Immunol*. 2013; 281:85–90. [PubMed: 23501056]

54. Wendling D, Racadot E, Wijdenes J. Treatment of severe rheumatoid arthritis by anti-interleukin 6 monoclonal antibody. *J Rheumatol*. 1993; 20:259–262. [PubMed: 8474061]
55. Yoshizaki K, Nishimoto N, Mihara M, Kishimoto T. Therapy of rheumatoid arthritis by blocking IL-6 signal transduction with a humanized anti-IL-6 receptor antibody. *Springer Semin Immunopathol*. 1998; 20:247–259. [PubMed: 9836380]
56. Navarro-Millán I, Singh JA, Curtis JR. Systematic review of tocilizumab for rheumatoid arthritis: a new biologic agent targeting the interleukin-6 receptor. *Clin Ther*. 2012; 34:788–802. [PubMed: 22444783]
57. Chaitow J, De Benedetti F, Brunner H, et al. Tocilizumab in patients with systemic juvenile idiopathic arthritis: efficacy data from the placebo-controlled 12-week part of the phase 3 TENDER Trial. *Ann Rheum Dis*. 2010; 69(suppl 3):146.
58. Campbell L, Chen C, Bhagat SS, Parker RA, Östör AJ. Risk of adverse events including serious infections in rheumatoid arthritis patients treated with tocilizumab: systematic literature review and meta-analysis of randomized controlled trials. *Rheumatology (Oxford)*. 2011; 50:552–562. [PubMed: 21078627]
59. Singh JA, Beg S, Lopez-Olivo MA. Tocilizumab for rheumatoid arthritis: a Cochrane systematic review. *J Rheumatol*. 2011; 38:10–20. [PubMed: 20952462]
60. Maini R, St. Clair EW, Breedveld F, et al. ATTRACT Study Group. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. *Lancet*. 1999; 354:1932–9. [PubMed: 10622295]
61. Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA*. 2006; 295:2275–85. [PubMed: 16705109]
62. Grijalva CG, Chen L, Delzell E, et al. Initiation of tumor necrosis factor- α antagonists and the risk of hospitalization for infection in patients with autoimmune diseases. *JAMA*. 2011; 306:2331–9. [PubMed: 22056398]
63. Paridaens D, van den Bosch WA, van der Loos TL, Krenning EP, van Hagen PM. The effect of etanercept on Graves' ophthalmopathy: a pilot study. *Eye*. 2005; 19:1286–9. [PubMed: 15550932]
64. Durrani OM, Reuser TQ, Murray PI. Infliximab: a novel treatment for sight-threatening thyroid associated ophthalmopathy. *Orbit*. 2005; 24:117–9. [PubMed: 16191800]
65. Theodossiadi PG1, Markomichelakis NN, Sfrikakis PP. Tumor necrosis factor antagonists: preliminary evidence for an emerging approach in the treatment of ocular inflammation. *Retina*. 2007; 27:399–413. [PubMed: 17420690]
66. Van Lieshout AW, Creemers MC, Radstake TR, Elving LD, van Riel PL. Graves' disease in a patient with rheumatoid arthritis during treatment with anti-tumor necrosis factor-alpha. *J Rheumatol*. 2008; 35:938–9. [PubMed: 18464319]
67. Tektonidou MG, Anapliotou M, Vlachoyiannopoulos P, Moutsopoulos HM. Presence of systemic autoimmune disorders in patients with autoimmune thyroid diseases. *Ann Rheum Dis*. 2004; 63:1159–61. [PubMed: 15308528]
68. Chiricozzi A, Krueger JG. IL-17 targeted therapies for psoriasis. *Expert Opin Investig Drugs*. 2013; 22:993–1005.
69. Baeten D, Baraliakos X, Braun J, et al. Anti-interleukin-17A monoclonal antibody secukinumab in treatment of ankylosing spondylitis: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2013; 382:1705–13. [PubMed: 24035250]
70. Eskandari MA, Jotwan R, Abe T, et al. The leukocyte integrin antagonist Del-1 inhibits IL-17-mediated inflammatory bone loss. *Nature Immunology*. 2012; 13:465–74. [PubMed: 22447028]
71. Khader SA. Restraining IL-17: Del-1 deals the blow. *Nat Immunol*. 2012; 13:433–5. [PubMed: 22513326]
72. Firestein GS, Echeverri F, Yeo M, Zvaifler NJ, Green DR. Somatic mutations in the p53 tumor suppressor gene in rheumatoid arthritis synovium. *Proc Natl Acad Sci U S A*. 1997; 94:10895–900. [PubMed: 9380731]

73. Park JS, Lim MA, Cho ML, et al. p53 controls autoimmune arthritis via STAT-mediated regulation of the Th17 cell/Treg cell balance in mice. *Arthritis Rheum.* 2013; 65:949–59. [PubMed: 23280308]
74. Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. *Science.* 1993; 260:547–9. [PubMed: 8097338]
75. Gately MK, Wolitzky AG, Quinn PM, Chizzonite R. Regulation of human cytolytic lymphocyte responses by interleukin-12. *Cell Immunol.* 1992; 143:127–42. [PubMed: 1352483]
76. Benson JM1, Peritt D, Scallon BJ, et al. Discovery and mechanism of ustekinumab: a human monoclonal antibody targeting interleukin-12 and interleukin-23 for treatment of immune-mediated disorders. *MAbs.* 2011; 3(6):535–45. [PubMed: 22123062]
77. Leonardi CL, Kimball AB, Papp KA, et al. Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1). *Lancet.* 2008; 371:1665–74. [PubMed: 18486739]
78. Sandborn WJ, Gasink C, Gao LL, et al. Ustekinumab induction and maintenance therapy in refractory Crohn's disease. *N Engl J Med.* 2012; 367:1519–28. [PubMed: 23075178]
79. Yeilding N, Szapary P, Brodmerkel C, et al. Development of the IL-12/23 antagonist ustekinumab in psoriasis: past, present, and future perspectives--an update. *Ann N Y Acad Sci.* 2012; 1263:1–12. [PubMed: 22823582]
80. Nakajima K, Kanda T, Takaishi M, et al. Distinct roles of IL-23 and IL-17 in the development of psoriasis-like lesions in a mouse model. *J Immunol.* 2011; 186:4481–9. [PubMed: 21346238]
81. Chen Y, Langrish CL, McKenzie B, et al. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J Clin Invest.* 2006; 116:1317–26. [PubMed: 16670771]
82. Levin AA, Gottlieb AB. Specific targeting of interleukin-23p19 as effective treatment for psoriasis. *J Am Acad Dermatol.* 2014; 70:555–561. [PubMed: 24373779]
83. Fischer M1, Ehlers M. Toll-like receptors in autoimmunity. *Ann N Y Acad Sci.* 2008; 1143:21–34. [PubMed: 19076342]
84. Jiang W, Zhu FG, Bhagat L, et al. A Toll-like receptor 7, 8, and 9 antagonist inhibits Th1 and Th17 responses and inflammasome activation in a model of IL-23-induced psoriasis. *J Invest Dermatol.* 2013; 133(7):1777–84. [PubMed: 23370539]
85. Salvi, M. Rituximab in Graves' ophthalmopathy; Presented at: American Thyroid Association; San Juan, Puerto Rico. 2013;
86. Stan, MN.; Garrity, JA.; Thapa, P.; Bradley, EA.; Bahn, RS. Randomized double-blind placebo-controlled trial of rituximab for treatment of Graves' ophthalmopathy; Presented at: American Thyroid Association; San Juan, Puerto Rico. 2013;
87. El Fassi D, Nielsen CH, Bonnema SJ, Hasselbalch HC, Hegedüs L. B lymphocyte depletion with the monoclonal antibody rituximab in Graves' disease: a controlled pilot study. *J Clin Endocrinol Metab.* 2007; 92:1769–72. [PubMed: 17284622]
88. Salvi M, Vannucchi G, Campi I, et al. Treatment of Graves' disease and associated ophthalmopathy with the anti-CD20 monoclonal antibody rituximab: an open study. *Eur J Endocrinol.* 2007; 156:33–40. [PubMed: 17218723]
89. Heemstra KA, Toes RE, Sepers J, et al. Rituximab in relapsing Graves' disease, a phase II study. *Eur J Endocrinol.* 2008; 159:609–15. [PubMed: 18628345]
90. Khanna D, Chong KK, Afifiyan NF, et al. Rituximab treatment of patients with severe, corticosteroid-resistant thyroid-associated ophthalmopathy. *Ophthalmology.* 2010; 117:133–139. [PubMed: 19818507]
91. Silkiss RZ, Reier A, Coleman M, Lauer SA. Rituximab for thyroid eye disease. *Ophthal Plast Reconstr Surg.* 2010; 26:310–4.

Key Issues

- Cytokine signaling pathways are intimately involved in the pathogenesis of autoimmune diseases.
- Current evidence links several cytokine pathways to GD.
- Cytokine-based therapy has shown promise in allied diseases such as RA, psoriasis, and Crohn's disease.
- IL-6, TNF- α , and IL-17 are of particular interest as therapeutic targets in TAO.