THEMATIC REVIEW

40 YEARS OF IGF1

**IGF1 receptor and thyroid-associated
ophthalmopathy****Michelle Mohyi¹ and Terry J Smith^{1,2}**¹Department of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, Michigan, USA²Division of Metabolism, Endocrine, and Diabetes, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USACorrespondence should be addressed to T J Smith: terrysmi@med.umich.edu

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Abstract

Thyroid-associated ophthalmopathy (TAO) is a vexing and poorly understood autoimmune process involving the upper face and tissues surrounding the eyes. In TAO, the orbit can become inflamed and undergo substantial remodeling that is disfiguring and can lead to loss of vision. There are currently no approved medical therapies for TAO, the consequence of its uncertain pathogenic nature. It usually presents as a component of the syndrome known as Graves' disease where loss of immune tolerance to the thyrotropin receptor (TSHR) results in the generation of activating antibodies against that protein and hyperthyroidism. The role for TSHR and these antibodies in the development of TAO is considerably less well established. We have reported over the past 2 decades evidence that the insulin-like growth factor1 receptor (IGF1R) may also participate in the pathogenesis of TAO. Activating antibodies against IGF1R have been detected in patients with GD. The actions of these antibodies initiate signaling in orbital fibroblasts from patients with the disease. Further, we have identified a functional and physical interaction between TSHR and IGF1R. Importantly, it appears that signaling initiated from either receptor can be attenuated by inhibiting the activity of IGF1R. These findings underpin the rationale for therapeutically targeting IGF1R in active TAO. A recently completed therapeutic trial of teprotumumab, a human IGF1R inhibiting antibody, in patients with moderate to severe, active TAO, indicates the potential effectiveness and safety of the drug. It is possible that other autoimmune diseases might also benefit from this treatment strategy.

Key Words

- ▶ IGF receptor
- ▶ immune system
- ▶ thyroid
- ▶ signal transduction

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Introduction

Thyroid associated ophthalmopathy (TAO) encompasses the ocular manifestations of the autoimmune syndrome known as Graves' disease (GD) (Smith & Hegedus 2016). The thyroid dysfunction associated with GD is attributable to loss of immune tolerance to the thyrotropin receptor (TSHR) and the generation of activating antibodies against that protein. Further, hyperthyroidism, a central

manifestation of GD, can be easily treated with commonly used and effective anti-thyroid medications, radioactive iodine ablation of the thyroid gland, or surgical thyroidectomy. This pattern of treatment has been established in developed countries for several decades. In contrast, no US Food and Drug Administration (US FDA)-approved medical therapies are currently available for TAO,

a disfiguring and potentially sight-threatening disorder. Inadequate treatment of TAO represents a major unmet public health need. In TAO, the upper face and connective tissues surrounding the eye can become inflamed and undergo extensive remodeling (Wang & Smith 2014) (Fig. 1). This results in edema, fat redistribution, and fibrosis. These changes can have dramatically deleterious consequences on the function of tissues adjacent to the eye, such as the eyelids and extraocular muscles. The factors underlying TAO remain uncertain but appear to be very similar to those initiating the thyroid glandular processes responsible for hyperthyroidism in GD. Despite substantial barriers imposed by inexact animal models of human disease and difficulties in accessing human tissues for interrogation at the most informative disease stages, a number of recent advances have been made in understanding TAO. Some of these insights have resulted from studies, mostly conducted *in vitro*, prompted by the empirical testing of hypotheses challenging conventional wisdom. Prominent among them are concepts supporting the potential involvement of the insulin-like growth factor 1 (IGF1) pathway in TAO. This line of inquiry was triggered by observations made earlier that the IGF1 and TSH pathways intersect functionally and that IGF-1 might regulate immune function. Professional immune cells including T and B lymphocytes and monocytes express functional IGF1 receptors (IGF1R), respond to IGF1, and produce IGF1. This suggests that the pathway may serve as an autocrine/paracrine loop involved in regulating immune surveillance. Recent detection of anti-IGF1R antibodies in patients with GD and the demonstration of physical and functional interactions between IGF1R and TSHR have identified a novel potential therapeutic target in TAO. Results from these *in vitro* studies have elicited substantial criticism. Despite this skepticism, the great potential for interrupting the activities of IGF1R in

Figure 1

Facial portrait of a patient with relatively mild, asymmetric thyroid-associated ophthalmopathy. The patient manifests bilateral upper eyelid retraction, periorbital swelling and mild conjunctival inflammation.

effectively and safely treating active TAO has been revealed with the completion of a clinical trial involving the IGF1R inhibitory monoclonal antibody, teprotumumab (Smith *et al.* 2017). Its potential for application to other autoimmune diseases seems obvious and deserves serious consideration.

Historical perspectives of IGF1R as a therapeutic target in human disease

Shortly after its molecular cloning, IGF1R and its associated pathways were considered potential targets for disease therapy. Given the early recognition that IGF1 and IGF2 exerted important influence on cell survival and proliferation, this pathway was examined for its potential role in the pathogenesis of certain forms of cancer. Many studies have demonstrated effects *in vitro* of IGF1R-inhibitory agents on cell proliferation (Tracz *et al.* 2016). A role for IGF1 and components of its effector system in the pathogenesis of colorectal cancer is supported strongly by preclinical experimental results (Vigneri *et al.* 2015). The elevations in IGF binding proteins found in cancer are inconsistent and may be tumor-specific. For instance, ovarian cancer may be associated with elevations in IGFBP1 and IGFBP2 but not IGFBP3 or IGF1 (Gianuzzi *et al.* 2016). In old men, IGFBP3 is elevated in colon cancer and the abnormalities appear to be independent of IGF1 levels (Chan *et al.* 2018). The IGF1 pathway may play important roles in both prostate cancer initiation and progression (Cao *et al.* 2014). Moreover, IGF1R has been found to be overexpressed in neoplastic diseases. Several independent programs based in pharmaceutical companies began developing molecules that could interrupt the IGF1 pathway as potential therapies for cancer. These included both monoclonal antibodies and small molecule inhibitors targeting IGF1R. Several tumor types, including prostate, lung, colon, and ovarian carcinomas and several sarcomas have been examined in clinical trials with anti-IGF1R antibodies administered alone and in combination with other chemotherapeutic agents. Despite the growing evidence that abnormalities of IGF1, IGFBPs, and IGF1R are involved in the pathogenesis of many forms of cancer, it appears that treatment with agents targeting the IGF1R pathway in unselected disease will remain unrewarding (Philippou *et al.* 2017). There is a widespread opinion that identification of predictive biomarkers allowing stratification of cases likely to respond to these agents will be necessary (You *et al.* 2014). Therefore the effort to develop molecules targeting IGF1R for cancer has

been essentially curtailed (Qu *et al.* 2017). Despite the disappointing results concerning efficacy in cancer, invaluable experience in administering these drugs to relatively large cohorts of patients, many of whom were physiologically fragile, and the accumulation of substantial safety data has facilitated opportunities for repurposing these molecules. This was the case surrounding the emergence of teprotumumab as a potential candidate for evaluation in patients with active, moderate to severe TAO.

Clinical characteristics of TAO

TAO remains a vexing and poorly managed component of GD, an autoimmune syndrome exhibiting a distinct female bias (Smith & Hegedus 2016). Approximately 40% of patients with GD develop clinically impactful TAO at some point during their lifetime. The interval separating onset of thyroid dysfunction and ocular disease is extremely variable, ranging from coincidental to divergent by several decades. TAO can present initially with vague signs and symptoms, including eyelid retraction and periorbital edema, ocular dryness, and excessive tearing. These manifestations can linger, improve spontaneously, or worsen. More than 50% of those developing TAO have ocular disease limited to these relatively minor manifestations. This group of patients rarely requires systemic therapy and can be managed with topical treatments. The typical course of active TAO lasts 2–3 years (Rundle & Wilson 1945) during which time anti-inflammatory medications may improve the discomfort. This disease activity culminates in the stable (inactive) phase where progression ceases and many of the signs of inflammation resolve. The majority of cases involve both eyes but frequently the severity of TAO is asymmetric. Development of diplopia and proptosis can diminish the quality of life substantially. Proptosis can result in substantial anterior eye surface exposure which if severe can lead to sight-loss. It results from increased volume of the orbital contents, which is the consequence of enlargement of the extraocular muscles and expansion of orbital fat. Both can lead to compression of the optic nerve, a process known as optic neuropathy which, if uncorrected, can also lead to irreversible vision loss.

Current therapy for active TAO

Therapy during the active phase, if the disease is sufficiently severe, most often includes systemic glucocorticoids, administered either as a daily oral dose

or intravenously as pulse therapy (Bartalena *et al.* 2012, 2016). The latter is considered to be less associated with side effects but concerns emerging from liver toxicity have complicated this route of drug administration (Sisti *et al.* 2015). Unfortunately, glucocorticoids are effective in providing symptomatic relief in only half of those patients with TAO who receive them and are not considered disease modifying. They do not usually improve proptosis or strabismus. Their major impact in responding patients is the reduction of inflammation. Despite the widely held view that glucocorticoids are effective in TAO, the absence of adequately powered, placebo-controlled trials examining therapeutic benefit continues to cast uncertainty on their importance in this disease. Among the most informative studies of glucocorticoids was the examination of three different cumulative doses of methylprednisolone (2.25, 4.98, and 7.47 g) in 159 patients with active, moderate to severe TAO (Bartalena *et al.* 2012). Improvement was greatest in those receiving the highest dosage of the drug where reduction in the clinical activity score and improved ocular motility were observed. Proptosis failed to improve at any dosage. More recently, B cell depletion with anti-CD20 monoclonal antibodies such as rituximab, has been examined in two small pilot therapeutic trials, each performed at a single institution (Salvi *et al.* 2015, Stan *et al.* 2015). These two reports came to very different conclusions about the effectiveness of B cell depletion in moderate to severe active TAO. The disparate findings underscore the need for larger, more definitive studies of rituximab. Many practitioners continue to use the drug off-label but barriers in obtaining third party payment for them continues to limit its general availability for this indication. Agents targeting cytokine pathways putatively involved in the pathogenesis of TAO, such as IL-6 and TNF- α , have also been considered for their potential therapeutic utility in TAO (Perez-Moreiras *et al.* 2014, Luo *et al.* 2017). None has materialized in adequately powered and properly controlled clinical trials that would allow meaningful assessment of the value of this general therapeutic approach. Thus, the clinical management of TAO during its active phase currently remains inadequate and in need of improvement. Once the stable phase has been reached, remedial surgery, including orbital decompression, strabismus surgery (to correct muscle misalignment and diplopia) and eyelid repair can be undertaken. These surgical approaches have become more refined but their outcomes are somewhat unpredictable and they can reactivate stable disease (Baladeschi *et al.* 2007).

Pathogenesis of TAO

At the heart of GD is the loss of immune tolerance toward the thyrotropin receptor (TSHR) (Smith & Hegedus 2016). The underpinnings of susceptibility to GD reside in genetic, epigenetic and acquired factors (Tomer 2014). Among the candidate genes are CTLA4, CD40, TSHR, PTPN22 and HLA-DR β 1-Arg74. Some of these are shared with related forms of thyroid autoimmunity such as those occurring in Hashimoto's thyroiditis. Layered on to the genetic factors are those acquired from the environment, including experiential components, the details of which have yet to be identified. Epidemiological details such as geographical location, diet, previous drug exposure (including vaccinations), and antecedent illness are essentially left unexplored. Until very recently, little was known about the root mechanisms underlying TAO or how they might be related to the hyperthyroidism of GD. Many investigators in the field, including ourselves, strongly suspect that the antigens shared by the orbital

connective tissues and thyroid gland in some manner instigate both humoral and cell-mediated autoimmune responses, both systemically and within the orbit. Critical to the current thinking about disease pathogenesis inside the orbit was the discovery that CD34⁺ fibrocytes, coming from the bone marrow and belonging to the monocyte lineage, uniquely infiltrate the orbit in TAO (Douglas *et al.* 2010) (Fig. 2). These cells transition to CD34⁺ orbital fibroblasts and cohabit the orbit with CD34⁻ orbital fibroblasts, the normal residents of healthy orbits. CD34⁺ orbital fibroblasts are notably absent in tissues from healthy donors. Fibrocytes are prodigious antigen presenting cells which express high constitutive levels of MHC Class II expression (Chesney *et al.* 1997). The differentiation of fibrocytes from CD11b⁺CD115⁺Gr1⁺ monocytes is regulated by CD4⁺ T cells through their release of several molecular factors (Niedermeier *et al.* 2009). On the one hand, IL-4, interferon γ , TNF- α and IL-2 substantially retard fibrocyte differentiation from monocytes through as yet poorly understood mechanisms.

Figure 2

Theoretical representation: the pathogenesis of thyroid-associated ophthalmopathy. The orbit becomes infiltrated by B and T cells and CD34⁺ fibrocytes uniquely in thyroid-associated ophthalmopathy. Bone marrow-derived fibrocytes express several proteins traditionally considered 'thyroid specific'. They can differentiate into CD34⁺ fibroblasts, which in turn can further develop into myofibroblasts or adipocytes depending upon the molecular cues they receive from the tissue microenvironment. CD34⁺ fibroblasts cohabit the orbit with residential CD34⁻ fibroblasts. These heterogeneous populations of orbital fibroblasts can produce cytokines under basal and activated states. These include interleukins 1 β , 6, 8, 10, 16, IL-1 receptor antagonists, tumor necrosis factor α , the chemokine known as 'regulated on activation, normal T expressed and secreted' or RANTES, CD40 ligand and several other cytokines and chemokines. These cytokines can act on infiltrating and residential cells. Like fibrocytes, CD34⁺ fibroblasts express thyrotropin receptor, thyroglobulin and other thyroid proteins but at substantially lower levels. Thyroid-stimulating immunoglobulins and potentially other autoantibodies directed specifically at the insulin-like growth factor-I receptor, activate the thyrotropin/insulin-like growth factor receptor-1 complex, resulting in the activation of several downstream signaling pathways and expression of target genes. Orbital fibroblasts synthesize hyaluronan leading to increased orbital tissue volume. This expanded tissue can result in proptosis and optic nerve compression. Orbital fat also expands from *de novo* adipogenesis. From N. Engl. J. Med, Smith T.J. and Hegedus L., Graves' Disease, 375; 1552–1565. Copyright © (2016) Massachusetts Medical Society. Reprinted with permission.

In contrast, calcineurin-inhibited T cells enhance fibrocyte development. Fibrocytes themselves can further differentiate into mature adipocytes which accumulate cytoplasmic triglycerides. Alternatively, they can become myofibroblasts, which are important participants in fibrosis and scar formation (Moore *et al.* 2005, Hong *et al.* 2007). Their differentiation fate is determined by the cues they receive from their microenvironment. If they are treated with TGF- β , they can proceed down the myofibroblast pathway. PPAR γ agonists promote their adipogenesis. They traffic to sites of tissue disruption and wound repair through their responses to chemokines such as CXCL-12 (Phillips *et al.* 2004). The vast majority of evidence that fibrocytes are physiologically important and involved in human disease derives either from studies performed in animal models or from those conducted using human tissues and cells *in vitro*. Thus the findings concerning the behavior of these cells in experimental models must be reconciled with future *in vivo* studies such as those emerging from human therapeutic trials.

A notable property of fibrocytes is their capacity to express autoantigens relevant to several diseases, such as type I diabetes mellitus (ICA69, IA2), neuro-inflammatory disorders (myelin basic protein), and GD (Fernando *et al.* 2012, 2014a,b). With specific relevance to TAO, fibrocytes express several 'thyroid-specific' proteins, including TSHR, thyroglobulin (Tg), sodium-iodide symporter, and thyroperoxidase (TPO) (Fernando *et al.* 2012, 2014a). The expression of these proteins appears to be dependent on the thymic transcription factor, autoimmune regulator protein (AIRE). Of potential importance are the relatively high levels of TSHR displayed by fibrocytes, in some cases comparable to those found on thyroid epithelial cells. The receptor is functional in that both TSH and TSIs induce the synthesis of several cytokines with putative importance in GD. These include IL-1 β , TNF- α , IL-6, IL-10, IL-12, and IL-23 (Raychaudhuri *et al.* 2013, Chen *et al.* 2014, Fernando *et al.* 2014a, Li & Smith 2014). Further, TSH induces pentraxin-3 in fibrocytes (Wang *et al.* 2015). The detection of extra-thymic AIRE in fibrocytes raises the as yet unanswered question of its role in those cells and whether this expression usually enhances peripheral immune tolerance. Extra-thymic AIRE has been reported previously by Anderson and colleagues in eTac cells as an important component of peripheral tolerance (Gardner *et al.* 2008).

As fibrocytes infiltrate the orbit in TAO, their characteristic phenotypic attributes (Pilling *et al.* 2009) transition to those that more closely align with residential fibroblasts; however, these derivative fibroblasts retain

several fibrocyte markers, including CD34, collagen I, and CXCR4, albeit at substantially reduced levels of expression. Levels of the thyroid proteins and AIRE and the constitutive display of MHC class II are dramatically lessened in these fibroblasts compared to those found in circulating fibrocytes. Moreover, responses mediated through TSHR are considerably less robust in orbital fibroblasts than those found in fibrocytes. Recent evidence strongly suggests that a factor(s) expressed by residential orbital fibroblasts not derived from fibrocytes (i.e. CD34⁻ orbital fibroblasts) represses expression of these proteins and diminishes the responses to TSH in the CD34⁺ orbital fibroblasts. However, once CD34⁺ orbital fibroblasts are purified by cytometric cell sorting and CD34⁻ fibroblasts are removed, levels of the thyroid proteins and responses to TSH are substantially restored (Fernando *et al.* 2012). Conditioned medium from CD34⁻ fibroblasts down regulates this expression, suggesting that the repression factor is soluble.

Search for an orbital autoantigen in TAO

An early and persistently attractive concept in the development of TAO has been the existence of a shared 'thyroid-specific' protein expressed within the orbit. Several antigens have been considered for their potential roles in the orbital disease. Among them, Tg was first detected in the orbit of patients with GD by Kriss and his colleagues in the early 1970s (Kriss 1970). Those workers postulated that the Tg was transported from the thyroid to the orbit, perhaps through lymphatic channels. Characterization of orbital Tg, which could only be found in tissue coming from patients with TAO, was left virtually untouched for several years. Considerably more recent reports from Marino and colleagues contained studies that characterized Tg binding sites in cultured orbital fibroblasts and identified them as harbored on glycosaminoglycans (Lisi *et al.* 2002, Marino *et al.* 2003). Circulating anti-Tg antibodies are frequently detected in GD and Hashimoto's thyroiditis. They are considered to be both non-specific and non-pathogenic. Similarly, anti-TPO antibodies in the circulation are found widely in thyroid autoimmunity but no role for them in disease development has thus far been identified.

One group failed to detect Tg in the extraocular musculature (Kodama *et al.* 1984). They have proposed that extraocular muscles rather than fatty connective tissue are the primary targets in TAO, concluding that muscle proteins harbor the important immunogenic determinants (de Haan *et al.* 2010). Their contention has

not been supported by convincing experimental evidence. Importantly, late-stage disease in many cases culminates in muscle fibrosis, potentially generating the anti-muscle protein antibodies this group has detected.

TSHR as a relevant orbital antigen in TAO

Since its molecular cloning nearly 30 years ago (Parmentier *et al.* 1989), TSHR has been characterized extensively, mainly as a regulatory protein displayed in thyroid tissues. The receptor is a member of the rhodopsin-like G protein coupled receptor family. These integral proteins possess 7 plasma membrane spanning regions (Cornelis *et al.* 2001). TSHR comprises a ligand-binding domain located in the extracellular portion of the molecule made up of A and B subunits linked by a disulfide bond (Furmaniak *et al.* 1987). A principal mechanism through which TSHR signals downstream pathways involves activation of adenylate cyclase and the generation of cAMP (Kleinau *et al.* 2013). Within the thyroid, TSHR mediates the trophic actions of TSH on thyroid function and growth. TSH is a glycoprotein synthesized and released by the thyrotrophs in the anterior pituitary under negative feedback control. There is currently little doubt that TSHR represents the pathogenic, GD-specific autoantigen in GD (Smith & Hegedus 2016). In that disease, TSHR becomes targeted by antibodies that can either activate the receptor, independent of TSH or block the binding and actions of TSH. Ligation of TSHR with activating autoantibodies (Trab, TSI) results in the hyperthyroidism frequently occurring in GD. More recently, the complexity of TSHR signaling in GD has emerged, including the important insight that TSH and TSIs elicit similar but non-identical activation of signaling pathways downstream from the receptor (Latif *et al.* 2009, Morshed *et al.* 2009).

Besides its display on thyrocytes, TSHR has been detected in several tissues outside the thyroid gland (Roselli-Rehfuß *et al.* 1992, Endo *et al.* 1995). Many of these are fatty-connective tissues distributed widely in the human body. Its presence in these tissues has prompted inquiry into potential physiological roles in fat regulation, including the influence on cell metabolism and adipocyte proliferation. The presence of TSHR in the orbit carries obvious implications concerning the pathogenesis of TAO (Felicelli *et al.* 1993, Bahn *et al.* 1998). However, levels of the receptor protein found in fatty connective tissue are considerably lower than those in the thyroid. The roles of either TSHR or its cognate activating antibodies in TAO are considerably less well-established than those occurring within the thyroid gland. Much of the evidence

for participation in orbital disease is circumstantial. For instance, a relationship has been identified between the clinical behavior of TAO and levels of these antibodies (Jang *et al.* 2013, Kampmann *et al.* 2015, Ponto *et al.* 2015). While levels seem to be higher in patients with more severe and active TAO, the utility of following antibody levels in a given patient as a guide for clinical management has not been established with properly controlled studies nor is there wide consensus regarding its prognostic value. It must be noted that occasional patients, some with severe TAO, present with undetectable TSIs (Tabasum *et al.* 2016). This finding raises the possibility that another pathogenic antigen(s) might also play a role in TAO.

IGF1R was proposed as a ubiquitous therapeutic target

Type I IGF1R comprises 1368 amino acids and belongs to a family of structurally related transmembrane tyrosine kinase receptors (Lawrence *et al.* 2007). Its extracellular domain represents the region of the protein undergoing constitutive receptor dimerization. Once it undergoes cleavage at the second of three fibronectin domains, two polypeptides are formed, designated IGF1R α and IGF1R β . These are linked by disulfide bonds. The IGF1R α subunit contains the ligand binding site (Whittaker *et al.* 2001). IGF1R is expressed widely in many tissue and cell types. As testament to its far-reaching importance, haploinsufficiency of the pleiotropic transcription factor, MYC, in mice is associated with increased longevity and decreased serum levels of IGF1 (Hofmann *et al.* 2015).

Fundamental to the question of whether interrupting the IGF1 pathway might provide clinical benefit to patients with autoimmune diseases is whether the pathway is integrally involved in the regulation of immune function. In fact, the pathway exhibits multiple intersections with the 'professional' immune system and with mechanisms involved in host defense. IGF1 can alter cytokine actions as a consequence of the intertwining of the relevant signaling pathways. The IGF1 pathway influences immune function at a variety of levels including processes occurring within the thymus where IGF1 promotes thymic epithelial cell proliferation and intrathymic T cell development and migration (Kooijman *et al.* 1995a, Savino *et al.* 2002). Professional immune cells, including T and B lymphocytes and cells belonging to the monocyte lineage express functional IGF1R and respond to physiological concentrations of the growth factor. IGF1 plays important roles in the development of both T and B cells. It enhances thymidine

incorporation in human T cells and is chemotactic. These actions are mediated through IGF1R which is expressed at a higher level in activated cells (Tapson *et al.* 1988). The abundance of IGF1R⁺ CD45RO memory phenotype T cells is substantially lower than that of IGF1R⁺CD45RA⁺ T cells (Kooijman *et al.* 1995a). In transgenic mice over-expressing IGF2, thymic cellularity was substantially increased as was the frequency of normal T cells was increased, exhibiting a skew toward CD4⁺ lymphocytes (Kooijman *et al.* 1995b). IGF1 treatment of peripheral blood mononuclear cells from patients with GD could enhance the frequency of CD25⁺Foxp3⁺ regulatory T cells (Pawlowski *et al.* 2017). IGF1 produced by bone marrow stromal cells stimulates the development of pro-B cells (Landreth *et al.* 1992). It increases the B cell population in lethally irradiated mice (Jardieu *et al.* 1994). In the spleen, IGF1 enhances the mature B cell population by provoking cell proliferation (Clark *et al.* 1993, Jardieu *et al.* 1994). The molecule increases DNA synthesis in plasma cells and enhances these effects of IL-6 on these cells (Jelinek *et al.* 1997).

The relationship between IGF1 and effector and regulatory immune functions is complex and appears to be tissue context dependent. In a mouse model of intestinal inflammation, IGF1-primed monocytes can suppress immune inflammation (Ge *et al.* 2015). IGF1 reduces klotho expression in bone marrow-derived dendritic cells and attenuates lipopolysaccharide-induced TNF- α expression (Xuan *et al.* 2017). In contrast, in experimental endotoxin-induced uveitis, an antagonist of growth hormone-releasing hormone receptor attenuated the surge of IGF1 and the generation of proinflammatory cytokines and thus reduced inflammation (Qin *et al.* 2014). Osterix-expressing mesenchymal cells generate IGF1 and in so doing, promote pro-B to pre-B cell transition (Yu *et al.* 2016). IGF1 activity is important for IL-4-driven macrophage transition to the M2 phenotype and participates in the activation by IL-4 of Akt signaling in those cells (Barrett *et al.* 2015). High-dose IGF1 alone and synergistically with dihydrotestosterone alters migration, survival, and adhesion of peripheral lymphocytes by influencing cytokines and paxillin-related signaling proteins (Imperlini *et al.* 2015). Thus, the IGF1 pathway appears to regulate, both directly and through interactions with numerous molecules involved in immune function, the magnitude and characteristics of the inflammatory response. Both molecular and cellular environments undoubtedly determine the impact of this pathway on healthy responses and those involved in the development of disease.

Evidence for pathogenic anti-IGF1R antibodies in GD

The concept that antibodies targeting IGF1R might be generated, in addition to those against TSHR, Tg and thyroperoxidase, as a natural consequence of the immunopathophysiology of GD originated with the report from the group of Kendall-Taylor (Weightman *et al.* 1993). In that study, IgGs from patients with GD were shown to displace binding of radiolabeled IGF1 from the surface of orbital fibroblasts. In contrast, immunoglobulins from healthy controls had no impact on IGF1 binding. The study was not designed to assess the potential of these IGF1-displacing antibodies to initiate signaling in target cells (Weightman *et al.* 1993). Several years later, Pritchard and colleagues (Pritchard *et al.* 2002, 2003) found similar IGF1 displacing activity in IgGs from patients with GD and identified the relevant binding site as IGF1R using highly-specific IGF1 analogues (Fig. 3). They then demonstrated that these IgGs from GD could initiate signaling mediated through the mTor/FRAP/Akt/p70^{s6k} pathway (Pritchard *et al.* 2002). Moreover, the signaling leads to the induction of two T cell chemoattractant molecules, namely IL-16 and RANTES (Pritchard *et al.* 2002). These effects were absent in fibroblasts from healthy individuals. The induction of IL-16 and RANTES can be blocked with the IGF1R inhibitory antibody, 1H7, and by the transfection of a dominant negative mutant IGF1R, 486/STOP (Fig. 4). This same group also found that IgGs apparently recognizing IGF1R could induce the generation of hyaluronan in TAO-derived orbital fibroblasts, actions again absent in cultures from healthy donors (Smith & Hoa 2004). These findings have proven to be controversial. Several investigators in the field have attempted to detect activating anti-IGF1R antibodies in patients with TAO but were unsuccessful (Wiersinga 2011, Minich *et al.* 2013, Krieger *et al.* 2016). It is notable that those attempts at replicating the original observations involved experimental systems differing substantially from the one used by Pritchard *et al.* (Pritchard *et al.* 2002, 2003, Smith & Hoa 2004). One study was able to identify a subset of patients with TAO in whom activating anti-IGF1R antibodies could be detected (Varewijck *et al.* 2013). These disparate findings have led some investigators to conclude that the only fibroblast-activating antibodies in GD are those directed at TSHR. Absent from some of those discussions dismissing the existence of anti-IGF1R antibodies in GD has been the detection of these same IGF1R-activating antibodies in patients with rheumatoid arthritis (Pritchard *et al.* 2004) and the consistent

Figure 3

(Panel A) Cell surface IGF1R expression by normal and GD orbital fibroblasts. (Panel B) Western analysis of IGF1R β in normal and GD orbital fibroblasts. (Panel C) Displacement of [125 I]IGF1 binding with increasing concentrations of unlabeled IGF1, the IGF1R-specific IGF1 analogue, Des(1-3), IgG from patients with GD (GD-IgG) and that from healthy controls (N-IgG). (Panel D) Displacement of FITC-conjugated anti-IGF1R binding by N-IgG (left panels) and GD-IgG (right panels) in GD orbital fibroblasts (upper panels) and those from healthy controls (normal; lower panels). From J. Immunol, Pritchard J, et al., Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway, 170:6348-6354, 2003.

generation of anti-IGF1R immunoglobulins in an animal model of GD (Moshkelgosha *et al.* 2013). There are a number of potential explanations for the divergent results thus far obtained by different laboratory groups, not the least important of which is the lack of experimental standardization. Among the differences in these studies have been the use of different target cells, use of animal sera in which levels of IGF1- and IGF1-binding proteins were not determined and use of assays potentially possessing insufficient sensitivity. Clearly, additional studies are required, which would properly control for these and other likely factors.

Evidence for IGF1R/TSHR interplay

Initial clues that IGF1 and TSH pathways might functionally interact were provided by the studies of Ingbar and colleagues (Tramontano *et al.* 1986). They were later followed by the work of other investigators demonstrating that IGF1 and insulin could enhance the actions of TSH on thyroid epithelial cells in culture, including cell proliferation and tyrosine kinase activities (Tramontano *et al.* 1986, Takahashi *et al.* 1991). Importantly, conditional knock-out of the IGF1R gene in thyroid substantially lessens its responses to TSH (Ock *et al.* 2013). In contrast, the thyroid of transgenic mice over-expressing IGF1 and IGF1R selectively in that tissue appears to be more sensitive to the actions of TSH

(Clement *et al.* 2001). Critical aspects of these interactions have remained uncharacterized; however, key insights have begun to emerge. Signal transduction pathways used by the two receptors overlap (Dupont & LeRoith 2001, Latif *et al.* 2009, Morshed *et al.* 2009, 2010), suggesting the potential for functional interplay between the receptor proteins. Exploration of whether the two proteins actually interact physically yielded direct evidence that IGF1R and TSHR form a protein complex (Tsui *et al.* 2008). The two receptor proteins co-localize (Fig. 5) and can be co-immunoprecipitated from thyroid and orbital cells using highly specific monoclonal antibodies. Crucially, signaling initiated at either TSHR (with recombinant human TSH) or IGF1R (with IGF1) was attenuated with the monoclonal anti-IGF1R-inhibitory antibody, 1H7 (Fig. 6) (Tsui *et al.* 2008). Those initial observations were made using primary human thyroid epithelial cells and the end-response was measured as changes from baseline in Erk $\frac{1}{2}$ phosphorylation. Subsequent studies conducted in human fibrocytes and orbital fibroblasts demonstrated that the fully human IGF1R-inhibiting antibody, teprotumumab, could also attenuate the actions of both IGF-1 and TSH (Chen *et al.* 2014). Among the responses quantified was the induction of IL-6 and IL-8, two cytokines thought to be involved in the pathogenesis of TAO. These findings in aggregate formed the rationale for undertaking a study of clinical effectiveness and safety of teprotumumab in patients with active TAO.

Figure 4

A dominant negative mutant (DN, 486/STOP) IGF1R or empty vector was transiently transfected into GD orbital fibroblasts. The mutant protein attenuated chemoattractant activity and expression. (Panel A) T cell chemoattractant activity was assessed by treating cultures with GD-IgG (100 ng/mL) or nothing for 24 h. Media were analyzed for T cell migratory activity without (solid columns) or with either anti-IL-16 (empty columns) or anti-RANTES (stripped columns) neutralizing Abs (5 µg/mL), (panel B) IL-16 (solid columns) and RANTES (empty columns) protein expression. From J. Immunol, Pritchard J, et al., Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated through the insulin-like growth factor I receptor pathway, 170:6348–6354, 2003.

Does interrupting IGF1R result in clinical benefit of active TAO?

Initial testing of the central hypothesis, that IGF1R represents a viable therapeutic target in active TAO, was recently completed with the unmasking of a clinical

Figure 5

Immunofluorescence staining for IGF-1R β (red) and TSHR (green). The images demonstrate co-localization of the two receptor (yellow) by confocal microscopy. (Panels A, B and C) GD orbital fibroblasts and (panels D, E and F) thyrocytes. (Panels C and F) Merged images demonstrate co-localization appearing as yellow or orange. (Panels G, H and I) Images using a different pair of antibodies demonstrate IGF-1R β (green) and TSHR (red) and colocalization (yellow) in orbital fibroblasts. (Panels J, K and L) TSHR (green) and IGF-1R α (red) in orbital fibroblasts demonstrates different pattern than that for IGF-1R β . (Panel L) Merged image (yellow to orange). (Panels M, N and O) TAO orbital connective tissue stains for TSHR (M, green) and IGF-1R (N, red). (Panel O) Merged images (orange). From J. Immunol, Tsui et al., Evidence for an association between thyroid-stimulating hormone and insulin-like growth factor I receptors: a tale of two antigens implicated in Graves' disease, 181:4397–4405, 2008.

trial where 88 patients with relatively recent-onset (within 9 months of study enrolment) moderate-to-severe TAO were randomized into one of two treatment arms (Smith *et al.* 2017) (Fig. 7). Patients received either teprotumumab (20 mg/kg) or saline as 8 infusions over

Figure 6

(Panels A and B) Western blot analysis of proteins from orbital fibroblasts, human thyrocytes, and thyroid tissue that were immunoprecipitated (IP) with either anti-IGF-1R or anti-TSHR antibodies and immunoblotted (IB) with the antibodies indicated. (Panel C) Knocking down IGF-1R expression with siRNA disrupts TSHR/IGF-1R complexes. (Panel D) ERK activation in thyrocytes treated with IGF-1, rhTSH, or immunoglobulins from patients with GD (GD-IgG) without/with anti-IGF-1R monoclonal antibody 1H7 for 15 min. From J. Immunol, Tsui et al., Evidence for an association between thyroid-stimulating hormone and insulin-like growth factor I receptors: a tale of two antigens implicated in Graves' disease, 181:4397–4405, 2008.

a 24-week treatment period. Enrollees were required to be clinically euthyroid at the time of their participation, thus eliminating the potential for variations in thyroid function to confound interpretation of study results. The primary response endpoint was assessed at 24 weeks and comprised the aggregate of both an improvement ≥ 2 points in clinical activity score (CAS) (on a 7-point scale) and ≥ 2 mm proptosis reduction in the more severely affected eye. Those considered responders were required to not have similar worsening in the fellow (less affected) eye. Secondary responses included improved CAS ≥ 2 points, reduction in proptosis ≥ 2 mm, both measured as continuous independent variables over time, improvement in quality of life as assessed by a fully validated questionnaire and subjective improvement in diplopia. At 24 weeks, those receiving teprotumumab exhibited a greater clinical response than did those receiving placebo ($P < 0.001$). In fact, highly statistically significant differences in the two treatment groups was observed at week 6 of the treatment phase ($P < 0.001$). Many cases showed return to premonitory proptosis and CAS scores of 0 (inactive disease). Depending on the durability of these effects, teprotumumab could therefore represent a disease-modifying therapy. The results from the trial were unprecedented in that the improvements were equivalent to the best surgical outcomes thus far reported. In theory, therefore, teprotumumab possesses the potential for sparing patients from undergoing major, multi-phased rehabilitative surgery. The safety profile was considered encouraging in that the only adverse event that could be clearly attributed to drug treatment was worsening of glycemic control in a few patients who were diabetic prior to their participation

in the study. These cases were managed with adjustment in diabetes medicine. In every case, the medication requirements returned to baseline following the end of the treatment phase. Because no orbital imaging was performed before and immediately following treatment in the study, it remains uncertain whether the effects of teprotumumab were mediated primarily by improvement in the extraocular muscles, the orbital fat or both. A phase III confirmatory trial is now underway. On the basis of the recently completed trial, the US FDA designated teprotumumab a breakthrough therapy for active TAO.

Mechanism of teprotumumab action

The drug, a fully human IgG1, binds to the cysteine-rich region of IGF1R with high affinity and specificity. This in turn provokes receptor/antibody complex internalization and its entrance into degradation pathways. Its effects on IGF1 action have been characterized *in vitro* using a variety of established cell lines and primary cultures. In Ewing's sarcoma lines TC-32 and TC-71, teprotumumab decreased colony formation in a dose-dependent manner (Huang *et al.* 2011). These cells express relatively high levels of IGF2 while the relatively unresponsive cells, A4573 and RD-ES, express extremely low levels of the protein. In primary human fibroblasts and fibrocytes, teprotumumab attenuates the effects of not only IGF1 but also TSH and M22, a monoclonal TSHR-activating antibody (Chen *et al.* 2014). It is currently uncertain whether teprotumumab possesses physical/biological properties that would render its apparent clinical effectiveness and safety any different from that of other anti-IGF1R antibodies or small molecule inhibitors of the receptor.

Figure 7

Design of the trial and results of primary response. (Panel A) Patients underwent screening and were then randomized to receive either teprotumumab or placebo administered as 8 IV infusions at 3 week intervals. (Panels B, C and D) Primary endpoint was assessed at week 24. In panel B, the time to first response was determined. Panel C demonstrates the time course for patients who met responder status. In panel D, responders were graded according to the magnitude of their response. A full colour version of this figure is available at <https://doi.org/10.1530/JME-17-0276>. From *New England Journal of Medicine*, Smith T.J. et al. Teprotumumab for thyroid-associated ophthalmopathy, 376; 1748-1761. Copyright © (2017) Massachusetts Medical Society. Reprinted with permission.

The case for inhibition of IGF1R in other autoimmune diseases

Among current insights into pathogenesis of several autoimmune diseases are bodies of evidence that suggest blocking the IGF1 pathway might provide therapeutic benefit (Smith 2010). Similar to TAO, this pathway might play a significant role in rheumatoid arthritis (RA) (Pritchard *et al.* 2004). Polymorphism of a 192bp allele (cytosine-adenosine repeat) located 1 kb upstream from the transcription start site of the IGF1 gene has been identified (Dhaunsi *et al.* 2012). A majority of male non-carriers of the allele had RA. A recent study identified abnormalities in the IGF1 pathway in RA (Szeremeta *et al.* 2017). Low circulating IGF1 levels are associated with the severity and activity of the disease (Baker *et al.* 2015). T cells express higher levels of miR-223 in RA and lead to lower expression of IGF1-dependent IL-10 (Lu *et al.* 2014). Resistin actions in RA synovium involve the IGF-1R pathway (Bostrom *et al.*

2011). Reducing resistin synthesis lowers levels of IGF-1R expression and Akt phosphorylation in human RA synovium implanted in SCID mice. Immunoglobulins that activate IGF1R on RA synovial fibroblasts have been detected in patients with that disease (Pritchard *et al.* 2004). Those studies revealed that these IgGs can induce the expression of T cell chemoattractants such as IL-16 and RANTES. The effects are identical to those found when orbital fibroblasts from patients with GD are treated with their own IgGs (Pritchard *et al.* 2003). Moreover, RA-derived IgG could induce cytokine expression in GD orbital fibroblasts. Other lines of evidence suggest that IGF1R and other components of the IGF1 pathway might be therapeutically targeted for RA. These include the report from Suzuki and coworkers (Suzuki *et al.* 2015) who found elevations in serum IGF1 and IGF1R levels in patients with active disease. These authors also found that RA synovial fibroblasts synthesize IGF1, which provoked osteoclastic activation and angiogenesis. Those effects could be attenuated with anti-IGF1R inhibitory antibodies. Similar results were observed with the small-molecule IGF1R inhibitor, NVP-AEW541 (Tsushima *et al.* 2017).

Conclusions

The encouraging results recently obtained from the initial trial examining teprotumumab in TAO should provide impetus for moving that drug down the pathway to registration as the first in class therapy for severe disease. But that success might usher in a broader opportunity for developing similar treatments for other autoimmune diseases based on insights into the fundamental pathogenic mechanisms. Clearly, the IGF1 pathway may be therapeutically targeted either with agents inhibiting IGF1R or some other components of that cascade. Repurposing already-developed drugs seems an ideal solution for orphan diseases such as TAO since in principle it should help overcome economic barriers.

Declaration of interest

The author has been issued patents while a faculty member at the UCLA School of Medicine covering the diagnostic methods for monitoring anti-IGF1R antibodies and the therapeutic targeting of IGF1R in Graves' disease and other autoimmune diseases. I have requested that my current employer, the University of Michigan Medical School, adjudicate any conflicts of interest.

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