Chapter 13
Pathogenesis of Graves' Orbitopathy

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Introduction

Graves' orbitopathy (GO), also known as Graves' ophthalmopathy or thyroid eye disease, is an inflammatory autoimmune disorder that primarily affects patients with a current or past history of Graves' hyperthyroidism [1]. While the onset of GO occasionally precedes or follows that of hyperthyroidism by several years, these conditions most commonly occur simultaneously or within 18 months of each other [2]. These clinical observations led clinicians early on to suspect that GO and Graves' hyperthyroidism share a common pathophysiology. Countering this concept appears to be the occasional finding of GO in euthyroid or hypothyroid individuals. However, with the advent of sensitive assays for circulating thyrotropin receptor antibodies (TRAb), the proximate cause of Graves' hyperthyroidism, it has been shown that at least minimally elevated levels of TRAb can be detected in essentially every patient with GO [3]. In addition, levels of TRAb correlate with the severity and clinical activity of the disease [4, 5] and higher titers of these antibodies in early disease portend a worse prognosis [6]. These clinical and laboratory observations point towards the orbital thyrotropin receptor (TSHR) as an autoimmune target that may play a central role in development of the ocular manifestations of Graves' disease. This chapter will discuss current concepts regarding the pathogenesis of GO.

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Mechanistic Explanation for Signs and Symptoms

Signs and symptoms of GO variously include proptosis (forward displacement of the globe), conjunctival and eyelid swelling and erythema, diplopia, and ocular pain. From a mechanistic standpoint, these features derive largely from enlargement of the orbital adipose tissues and extraocular muscles within the confines of the bony orbit. As the resulting orbital pressure increases, proptosis may develop and venous drainage may be impaired, facilitating the accumulation of inflammatory mediators within the orbit. This inflammation appears to be initiated by the migration of T-helper cells into the orbit [7]. This sets in motion the local production of cytokines, including interferon-γ (IFN-γ), interleukin-1 (IL-1), IL-6, as well as TRAb, although the latter may also reach the orbit via the circulation. In addition, chemoattractant cytokines, including interleukin-16 (IL-16), regulated upon activation, normal T cell expressed and secreted (RANTES), and CXCL10, may enhance mononuclear cell infiltration into the orbit [8]. Extraocular muscle dysfunction in early disease appears to result from active inflammation within the enlarged and edematous extraocular muscles. Diplopia encountered in late, inactive disease is likely due to fibrosis and may be impacted by local transforming growth factor-β production [1].

Histologic Underpinnings

Underlying the extraocular muscle and orbital adipose tissue remodeling characteristic of GO is an accumulation of hyaluronic acid (HA) with its attendant edema and the development of new fat cells, a process termed adipogenesis [9]. Additionally, within these orbital tissues can be found a perivascular and diffuse infiltration of CD4+ and CD8+ T cells, B cells, plasma cells, and macrophages. Several lines of evidence suggest that fibroblasts investing the extraocular muscle fibers and residing within the orbital connective tissues are the autoimmune target cells in GO [10–13]. These cells are heterogeneous and may be classified based on the presence or absence of the cell surface glycoprotein CD90, also known as thymocyte antigen-1 (Thy-1) [14]. Fibroblasts expressing this antigen are capable of copious HA production and are abundant in the extraocular muscles. In contrast, most fibroblasts found within the orbital connective tissues are Thy-1 negative and characteristically undergo adipogenesis under appropriate conditions.

The Role of TSH Receptor Activation

Insight into an important link between the thyroid and the orbit was gained by the demonstration that orbital fibroblasts, like thyrocytes, express the thyrotropin receptor (TSHR) [15, 16]. As such, this receptor could serve as a common autoantigen

allowing the pathogenic antibodies in hyperthyroidism to also impact the orbit. While orbital fibroblasts and tissues from both normal individuals and patients with GO express TSHR, significantly higher levels of the receptor are measured in GO tissues [17]. Further, ocular tissues from patients with active GO express higher levels of the receptor than do tissues from patients with inactive disease [4]. It has been shown in vitro that the expression of TSHR in these cells increases as they differentiate into mature adipocytes, perhaps leading to propagation of the autoimmune process within the GO orbit [18].

Sera from individual patients with Graves' disease contain a mixture of TRAb with the ultimate clinical expression of the disease influenced by the levels, varieties, and affinities of the TRAb present [19, 20]. Most TRAb-mediated TSHR signaling in thyrocytes is mediated through the Gαs protein subunit which activates the adenylyl cyclase/cAMP signaling cascade. Both TSH and some TRAb also activate a cAMP-independent cascade that increases phosphoinositide 3-kinase (PI3K) activity with subsequent phosphorylation of Akt and activation of the serine/threonine kinase mammalian target of rapamycin (mTOR). Recent evidence suggests that Forkhead box O-1 (FoxO-1) protein, a transcription factor and target of PI3K, may act as a downstream effector of both TSH and IGF-1 in thyrocytes [21]. In addition to these pathways, each Gα effector is impacted by growth factors that signal via MAPK pathways to regulate thyrocyte proliferation, differentiation, and survival [22].

TSHR signaling in orbital fibroblasts appears to be similar to that found in thyrocytes with TSH and TRAb-induced activation of both adenylyl cyclase/cAMP and PI3K/pAkt pathways [23]. In order to implicate TRAb as a proximate cause of the characteristic orbital tissue remodeling seen in GO, it would be necessary to show that activation of TSHR signaling in orbital fibroblasts enhances adipogenesis and/or HA production by GO orbital fibroblasts. The impact of TSHR activation on adipogenesis was studied by Zhang and colleagues using orbital fibroblasts into which an activating mutant TSHR was introduced [24]. This transfection led to an increase in adipocyte differentiation, as shown by two- to eightfold elevations in levels of early to intermediate adipocyte markers. Our laboratory similarly demonstrated pro-adipogenic effects of TSHR activation using a stimulatory monoclonal TRAb (termed M22) or bovine TSH as receptor ligands. Treatment with these agents resulted in increased expression of late adipocyte genes (adiponectin and leptin) and accumulation of lipid in GO orbital fibroblasts [25]. The adipogenesis promoted by M22 was inhibited by treatment with the PI3-kinase inhibitor LY294002, suggesting that this effect may be mediated via PI3K activation. While the cell systems used in the two studies differ, both suggest that TSHR activation may directly enhance adipogenesis in GO.

The Potential Role of IGF-1 Receptor Activation

The insulin-like growth factor-1 receptor (IGF-1R) has emerged as another receptor that might be activated within the GO orbit [26, 27]. While convincing evidence for the presence of elevated levels of autoantibodies against IGF-1R in GO is lacking, increased expression of IGF-1R and of IGF-1 itself has been shown in orbital cells from patients with GO [26]. The laboratory of Smith and colleagues studied the possible direct effects of human recombinant TSH (hrTSH) and purified IgG from patients with Graves' disease (GD-IgG) on HA synthesis in GO orbital fibroblasts. While they demonstrated IgG-induced HA synthesis, they found no increase in HA production following treatment of cultures with TSH [28]. Further, they showed that the stimulation of HA by GD-IgG was neutralized by a potent IGF-1R blocker (termed 1H-7) and that transfection of fibroblasts with a dominant-negative mutant IGF-1R inhibits GD-IgG-induced activation of T cell chemoattractant expression [29]. They concluded that these effects of GD-IgG on orbital fibroblasts were not due to TRAb, but rather to antibodies against IGF-1R that might be present in GD-IgG. The group of van Zeijl similarly found that GD-IgG, but not hrTSH, stimulates HA synthesis in GO fibroblasts using cells that had undergone adipocyte differentiation [30]. In contrast, Zhang and colleagues showed increased HA production in cultures treated with bovine TSH, or with two different monoclonal TRAb, in normal undifferentiated orbital fibroblasts, but not in GO fibroblasts [24]. We performed studies using undifferentiated GO orbital fibroblasts and found both bovine TSH and a potent stimulatory TRAb (termed M22) to increase cAMP production, phosphorylation of Akt, and HA production in these cells [31]. We also demonstrate that these effects can be abrogated by either 1-H7 or a small molecule inhibitor of TSHR activation [23], suggesting that HA synthesis resulting from TSHR activation in these cells may involve IGF-1R.

Direct interaction between TSHR and IGF-1R in orbital fibroblasts has been suggested in studies showing immunoprecipitation of both receptors using specific monoclonal antibodies directed against either receptor [26]. In these studies, the receptors appeared to be co-localized in the perinuclear and cytoplasmic compartments of the cells, suggesting physical and/or functional relationships between the receptors. In addition, both TSH and IGF-1 have been shown in thyrocytes to inactivate Forkhead box O-1 (FoxO-1) transcription factor by promoting its exclusion from the nucleus in an Akt-dependent manner [21]. Similarly, IGF-1 produced locally within the orbit may complement the effects of TSHR autoantibodies within the orbital tissues through modulation of downstream effectors, such as FoxO1. The combined effects of ligation of both receptors by their respective ligands may thus lead to full expression of the clinical features of GO [32]. A proposed model for the pathogenesis of GO is shown in Fig. 13.1.
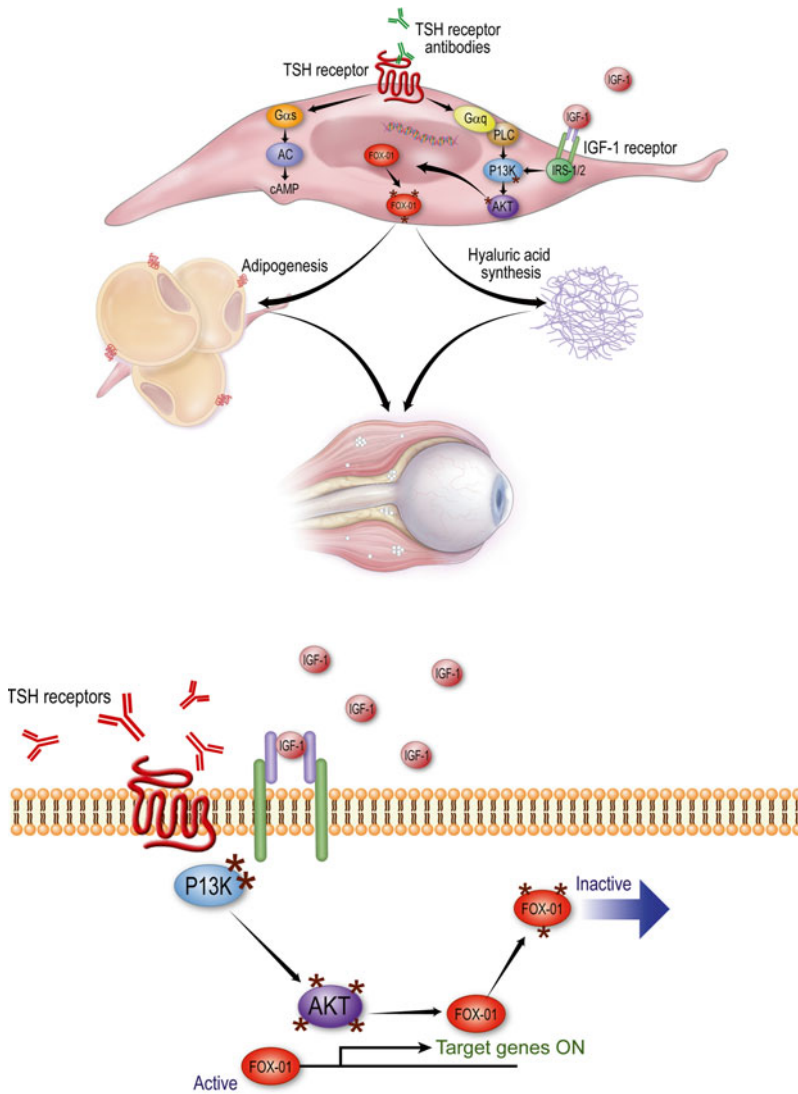
Fig. 13.1 Proposed model for pathogenesis of GO. (*Top*) Autoantibodies directed against the TSH receptor found in the sera of patients with GO engage this receptor on orbital fibroblasts while the IGF-1 receptor may be activated by locally produced IGF-1. Ligation of the TSH receptor activates the adenylyl cyclase/cAMP and the phosphoinositide 3-kinase (PI3K)/Akt signaling cascades. This may be augmented by IGF-1 receptor activation via physical and/or functional relationships between the receptors. This could involve the formation of receptor complexes and/or modulation of common downstream effectors, such as the transcription factor Forkhead box O-1 (Fox-01). This process results in increased production of hyaluronic acid, enhanced adipogenesis, and orbital infiltration of mononuclear cells with secretion of inflammatory cytokines. These cellular processes would lead to variable extraocular muscle enlargement, expansion of the orbital adipose tissues, and the inflammatory signs and symptoms of GO. (*Bottom*) Ligation of TSHR and IGF-1R in GO orbital fibroblasts by their respective ligands may lead to the inactivation of FoxO-1 by promoting its exclusion from the nucleus in an Akt-dependent manner. Inactivation of this known inhibitor of adipogenesis would be expected to enhance adipogenesis and may in addition augment hyaluronic acid production in these cells

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