Does autoimmunity against thyroglobulin play a role in the pathogenesis of Graves’ ophthalmopathy: a review

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Abstract: While most authors believe that autoimmunity against the TSH receptor expressed in the orbital connective tissue cells is the main reaction that leads to the development of ophthalmopathy in patients with Graves’ hyperthyroidism, an older hypothesis that deserves fresh consideration is based on the notion that thyroglobulin (Tg) in the thyroid gland passes in a retrograde fashion to the orbit where it is recognized by Tg autoantibodies, leading to inflammation. Here, we review new evidence that supports a role of Tg and propose a new hypothesis based on the notion that Tg is targeted in the orbit leading to a complex cascade of reactions that leads to Graves’ ophthalmopathy.

Keywords: ophthalmopathy, Graves’ disease, thyroglobulin, thyroid peroxidase, TSH receptor, lymphocytes, autoantibodies

Introduction
The pathogenesis of the ophthalmopathy that occurs in approximately 40% of patients with Graves’ hyperthyroidism¹ and, less often and usually in a less severe form, in 25% of patients with Hashimoto thyroiditis² remains controversial.³ While most authors favor the hypothesis that autoimmunity against the TSH receptor (TSHr) expressed in the orbital fibroblasts, preadipocytes, and fibrocytes is the dominant autoimmune reaction that leads to orbital inflammation⁴ not all the evidence supports this, namely, Graves’ disease and worsening ophthalmopathy may be associated with decreasing levels of TSHr antibodies in individual patients (Wall et al, 2013, unpublished observations) and ophthalmopathy may develop many years after the onset of hyperthyroidism. Most importantly, patients with so-called euthyroid Graves’ disease⁵ have a similar ophthalmopathy but no evidence for thyroid autoimmunity including negative TSHr antibodies as measured in the TRAb assay and as thyroid-stimulating immunoglobulin in a Thyreporter bio-assay,⁶ suggesting that other possibilities should be considered. Moreover on a recent case report we showed that eye signs and other soft tissue manifestations of Graves’ disease worsened in the context of psychological stress, with reduced levels of TSHr antibody, occurred following stress.⁷

Regardless of the nature of the inflammatory process in the orbital connective tissue and fat and extraocular muscles (EOM), the eye disorder is presumed to begin in the thyroid since the great majority of patients with Graves’ ophthalmopathy (GO) have active thyroid inflammation, ie, thyroiditis, at the time they develop eye signs.⁸,⁹ Thus, a logical hypothesis for the orbital reaction is that thyroid antigens are released in the context of a thyroiditis and travel (“home”) to the orbital tissues where they bind to various cell types and are targeted by autoantibodies and/or sensitized...
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patients with GO by Western Blotting and ELISA.
identified intact Tg in the retro-orbital tissue from three
which they used monoclonal antibodies against Tg that
absorbed out by Tg in the orbital tissues. Feldt-Rasmussen et al
demonstrated the presence of Tg in the orbit but the presence of
Tg/anti-Tg immune complexes has not been reported. Serum
anti-Tg antibodies did not correlate closely with severity of
the eye disease in one study.14
Subsequently, using a panel of well-characterized
monoclonal antibodies against Tg and patients sera, we
demonstrated reactivity against a human orbital connective
tissue membrane protein which correlated with serum
anti-Tg antibody levels but not ophthalmopathy.15 We
concluded that the orbital protein was not native Tg but
another putative thyroid and orbital tissue shared antigen.
In retrospect, these early studies had partly confirmed the
hypothesis of McDougall and Kriss.13 Later, we carried out
further studies with anti-Tg monoclonal antibodies to test
for the presence of Tg in orbital tissues from patients with
GO and, as controls, from patients undergoing orbital sur-
gery for non-immunological disorders. However, we could
not identify intact Tg in the orbit.16 Goh et al17 found that
ophthalmopathy tended to be associated with lower titers
of Tg and TPO antibodies in patients with Graves’ disease
compared to those without eye involvement. Although they
postulated that Tg antibodies somehow protected against
ophthalmopathy, an alternative explanation is that serum
antibodies are decreased in the circulation because they are
absorbed out by Tg in the orbital tissues.
The “Tg hypothesis” lay dormant for a further 15 years
until an Italian group reported a series of experiments in
which they used monoclonal antibodies against Tg that
identified intact Tg in the retro-orbital tissue from three
patients with GO by Western Blotting and ELISA.18 They
did not find Tg and anti-Tg immune complexes in the orbit
and concluded that autoimmunity against Tg was probably a
factor in the development of ophthalmopathy but likely cell
mediated. A year later the same group carried out additional
studies in more patients with GO, again identifying intact
Tg in fibroadipose tissue from three of seven patients by
immunoprecipitation and Western Blotting with Tg mono-
clonal antibodies. They did not detect Tg in EOM extracts,
similar to our own earlier findings in which eye muscle mem-
branes did not react with our Tg monoclonal antibodies.19
They also demonstrated intact Tg in sub-palpebral skin
extracts from two of two patients with GO and they sug-
gested that we should re-visit the Kriss hypothesis and the
possibility that Tg is an important target for the autoimmune
reactions in ophthalmopathy.19
On the other hand, Kendall-Taylor et al20 identified an IgG
which they called “ophthalmopathic immunoglobulin” that
bound to retro-orbital but not to thyroid membranes, thyroid
microsomes, or Tg. They concluded that GO was associated
with what they called a “specific ophthalmopathic immuno-
globulin” that reacted with retro-orbital antigen, as distinct
from thyroid antigens, suggesting that the ophthalmopathy
was an entity distinct from autoimmune thyroid disease.

Serum Tg levels and ophthalmopathy
Very recently, we demonstrated a significant positive correla-
tion between serum Tg levels and the presence and severity of
ophthalmopathy in patients with Graves’ disease.21 In patients
with Graves’ disease, but not Hashimoto thyroiditis, Tg levels
also correlated with serum titers of TSHR antibodies, favoring
the notion that not only TSHR but also Tg, may be released
from the thyroid gland in the course of a thyroiditis and home
to the orbit where they become targets of autoantibodies and
cytotoxic T lymphocytes.

Special nature of the orbital
fibroblasts
Smith,22 Smith et al,23 Fernando et al,24,25 and Smith et al26
have convincingly demonstrated that orbital fibroblasts from
patients with GO respond differently to skin fibroblasts and
orbital fibroblasts from normal subjects in vitro. However,
the fibroblasts may actually be the same in all situations but
different in GO only because they have been primed in the
context of the orbital reaction and have new surface markers
which makes them seem different, ie, we need to differen-
tiate between cause and effect before promoting the GO
orbital fibroblasts, preadipocytes, and fibrocytes as “unique”.
However, if considered in this light, the results described
by Smith et al23 are compatible with, and supportive of, the
“throid antigen hypothesis”.
While an effect of cytotoxic antibodies targeting Tg
bound to EOM cells or orbital fibroblasts is one possible
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mechanism for the initialization of orbital inflammation, there are other potential mechanisms for reactivity against Tg (and other thyroid antigens) in situ in the orbit. While it is known that orbital fibroblasts can be stimulated by TSHr antibodies in vitro, suggesting that the prominent orbital scarring and glycosaminoglycans overproduction of GO is due to a reaction with the TSHr, this could also result from a reaction between Tg antibodies and Tg attached to the orbital fibroblasts; more likely, both mechanisms contribute to the orbital pathology and clinical features (Table 1).

Thyroiditis and ophthalmopathy

Subacute thyroiditis and radioiodine treatment of Graves’ hyperthyroidism may be good models for what may happen to the orbit in patients with thyroiditis. What happens to the thyroid gland following radioactive iodine treatment of patients with Graves’ hyperthyroidism. Following $^{131}$I, there is a flare up in the thyroiditis with release of thyroid proteins and increased production of the corresponding antibodies, against Tg, TPO, and the TSHr.\(^{31,32}\) In addition, we have recently shown an increase in serum Tg concentrations following radioiodine which correlated with an increase in TSHr antibody titer, initial worsening of the eye signs, but later improvement (Wall et al, 2013, unpublished observations). It is also likely, but not yet shown, that the numbers of thyroid antigen-sensitized T cells increase in the thyroid following $^{131}$I treatment; antibodies and sensitized T cells could pass to the orbital tissues, leading to a flare up in the severity of existing eye disease. Indeed, studies confirming that radioactive iodine treatment leads to worsening of eye signs – or even the new development of ophthalmopathy, although this is more controversial – would be good evidence for a role of autoimmunity against thyroid antigens in the development of ophthalmopathy in patients with thyroid autoimmunity.\(^{33,34}\)

While there is no mechanistic data that radioactive iodine worsens GO by increasing thyroid destruction, release of Tg, and production of Tg/anti-Tg immune complex, this is our working hypothesis for present in vitro and clinical studies (Table 2). In the longer term, one would expect a decrease in the severity of the ophthalmopathy as the “thyroid antigen mass” is destroyed by $^{131}$I, and this has been shown in earlier observations that total thyroidectomy is associated with the improvement of existing eye disease and prevention of new ophthalmopathy.\(^{35-37}\)

While this review focuses on the role of Tg, we believe that there are other thyroid antigens that are involved in the pathogenic process of GO. This process can be characterized as a complex series of reactions involving multiple thyroid antigens, the corresponding serum autoantibodies, cellular immunity, cytokines, and a large number of other chemical mediators and co-factors. Our proposed hypothesis for the role of Tg in the development of ophthalmopathy in patients with thyroid autoimmunity is shown schematically in Figure 1.

*Figure 1* Hypothetical role of thyroglobulin in Graves’ ophthalmopathy.
Proposed pathogenic mechanisms of Tg for Graves’ ophthalmopathy

**Table 1 Evidence supporting a role of thyroglobulin in the pathogenesis of Graves’ ophthalmopathy**

1. Thyroglobulin is a large and very antigenic protein and the corresponding autoantibodies react with it in many clinical and experimental situations, so why not in the orbit?
2. Intact thyroglobulin can be detected in the orbits of patients with Graves’ ophthalmopathy so would be seen by circulating thyroglobulin antibodies
3. In patients with Graves’ hyperthyroidism, serum thyroglobulin levels correlate with the prevalence and severity of any associated ophthalmopathy
4. In patients with Graves’ ophthalmopathy, serum thyroglobulin levels correlate with serum titers of TSHr antibodies
5. In patients with Graves’ ophthalmopathy, serum thyroglobulin levels correlate with TSHr antibody titers, suggesting that both thyroid antigens are released at the same time
6. Thyroglobulin antibodies in the circulation and from the thyroid would bind to Tg in the orbit, although this has not been demonstrated

**Abbreviation:** TSHr, TSH receptor.

Role of chemokines in GO

The importance of chemokines such as CXCL10 and CCL2 on GO has been investigated by the Italian team. In their in vivo and in vitro studies, they have demonstrated that EOM involves in the self-perpetuation of inflammation by secreting CXCL10 and CCL2 chemokines under the influence of cytokines especially IFNγ. In addition to that, CXCL9 and CXCL10 have been assessed as surrogate markers of GO while patients undergoing treatment and were proposed to be useful guidelines in therapeutic decision-making.

**Future studies**

The evidence for a role of autoimmunity against Tg is, as is the case for the TSHr, mainly circumstantial. In order to prove the “thyroid antigen hypothesis”, we must demonstrate that normal orbital fibroblasts and EOM cells, to which are attached Tg, become targets for antibodies and/or sensitized T lymphocytes from patients with ophthalmopathy, but not from those without eye disease. We can compare GO patient sera vs normal sera, fibroblasts to which is bound Tg vs control antigen and peripheral blood mononuclear cells, or purified T-cell subsets from patients with GO vs those from patients with Graves’ disease without eye signs. We can also isolate mononuclear cells from the thyroid of patients with GO and test for reactivity against the same target cells vs mononuclear cells from patients with Graves’ disease without ophthalmopathy. Specific IgG autoantibodies obtained by affinity chromatography, and purified subpopulations of T cells targeting Tg can be incubated with Tg-coated EOM cells or orbital fibroblasts to confirm findings using serum and unfractionated mononuclear cells. As end points, one can measure changes in gene expression for inflammatory markers and cytokine levels. It is also possible to study the putative benefits of selenium supplementation in GO in this model. We can carry out serial studies of patients with Graves’ hyperthyroidism, measuring serum Tg antibodies and correlating with eye signs. The notion that Tg antibodies tend to be low in patients with ophthalmopathy and those without eye disease due to their absorption by the orbital tissues can be studied by correlating orbital Tg levels in immunohistochemistry with parameters of the orbital autoimmune reactions, scores for eye signs, and serum Tg and anti-Tg titers in serial studies of patients with and without ophthalmopathy, including those treated with radioactive iodine.

**Conclusion**

The orbital reactions in patients with Graves’ disease are complex, involving antibodies and T lymphocytes, as well as other mononuclear cells, homing receptors on orbital cells, and cytokines. Moreover, we must determine why it is that only approximately 50% of patients with Graves’ hyperthyroidism develop ophthalmopathy. Environmental and genetic factors will play a role, an example being the presence of a recently shown informative single nucleotide polymorphism in the CASQ1 gene which is linked to ophthalmopathy. Smoking is well recognized as a major risk factor for ophthalmopathy and stress is likely to play an important role in its initiation. In conclusion, it does seem appropriate to give serious attention to the possibility that the pathogenesis of GO is not just about the TSHr but the contribution from Tg and other thyroid antigens as well.

**Disclosure**

The authors report no conflicts of interest in this work.

**References**


