

## The autoimmunity in Graves's disease

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## 1. ABSTRACT

Graves' disease (GD) is a systemic autoimmune syndrome manifesting complications in thyroid and orbital connective tissues. The thyroid gland plays a major role in the human body by producing the hormones necessary for appropriate energy levels and an active life. At the same time, the thyroid is highly vulnerable to autoimmune thyroid diseases. GD arises due to the complex interplay of genetic, environmental and endogenous factors, and the specific combination is required to initiate thyroid autoimmunity. Earlier studies have demonstrated the autoimmune response plays a dominant role in the development of GD. This review summarizes the inflammatory events which occur during the development of GD, such as Th17/Treg cell infiltration, Th1/Th2 cytokine and chemokine production, and the subtypes of immunoglobulins (IgGs) generated.

## 2. INTRODUCTION

Graves' disease (GD) is a systemic autoimmune syndrome manifesting complications in thyroid and orbital connective tissues. The clinical manifestations of GD include thyrotoxicosis and several extrathyroidal signs, such as ophthalmopathies, dermopathies, and acropathies (1).

Between 25% and 50% of patients with GD exhibit ocular complications, which are referred to as Graves' ophthalmopathy (GO) or thyroid eye disease. The clinical manifestations of GO include lid retraction, proptosis, soft tissue swelling, strabismus, and compressive optic neuropathy. The swollen soft tissues within the bony orbit displace the globe forward and impede venous outflow from the orbit. The swollen soft tissues result from adipogenesis and glycosaminoglycan accumulation, which

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follows activation of local fibroblasts secondary to inflammation (2).

Although it is widely accepted that GD is an autoimmune process and the primary antigen in the thyroid is the TSH receptor, the mechanism leading to autoimmunity in patients with GD remains poorly understood. The orbital manifestations of GD also involve lymphocytic infiltration and tissue remodeling, and can culminate in fibrosis(3, 4). Earlier studies have demonstrated inflammatory cell infiltration, cytokine and chemokine production, and negative regulation in patients with GD; this review summarizes the inflammatory events during the development of GD, such as Th17/Treg cell infiltration, Th1/Th2 cytokine and chemokine production, and the subtypes of immunoglobulins (IgGs) generated.

### 3. TH17 AND TREG CELLS

Treg cells function via negative co-stimulatory molecules, induction of anti-inflammatory signal transduction pathways in T-cells and antigen-presenting cells, direct or indirect destruction of effector cells or antigen-presenting cells, and secretion of suppressive cytokines (5). Treg cells are thought to be an essential element in suppression of autoimmune disorders, and therefore defects in Treg cell function may result in chronic autoimmunity. Treg cells can be divided into two subpopulations (naturally occurring and inducible)(6). CD4+CD25+ T cells are the most extensively studied naturally occurring Tregs, constitute 5–10% of peripheral CD4+ T cells, and are involved in the pathogenesis of a number of autoimmune diseases in humans and mice(7). CD8+CD122+ T cells are another type of naturally occurring Treg recently identified by Suzuki *et al.* (8-10). Mice genetically deficient for the CD122 gene spontaneously develop severe hyperimmunity (8) by expansion of abnormally activated T cells (9). This abnormal phenotype can be reverted by transfer of purified CD8+CD122+ T cells (10). Of interest, a more recent study showed that there is a difference in the mechanism(s) involved in the suppressive function between these two populations of Tregs. The suppressive function of CD4+CD25+ T cells is mediated by cell-cell contact (7), whereas the suppressive function of CD8+CD122+ T cells involves secretion of a regulatory cytokine IL-10, but not cell-cell contact (11). Several investigations report decreased frequency and/or function of Treg cells in human autoimmune diseases. In autoimmune thyroiditis, Treg cell frequency and/or function is enhanced when compared with normal donors (5), suggesting a compensatory attempt to overcome or reduce the autoimmunity by accelerating Treg cell activity (12).

There is no definitive evidence showing that autoimmune thyroid disease results from numerical and/or functional abnormalities of Tregs in humans. A recent study by Marazuela *et al.* (13) demonstrated that the number of CD4+CD25+T cells increases in thyroid glands from GD patients with impaired suppressor function. It remains to be investigated whether or not the suppressor function of Tregs is intrinsically impaired in GD patients or

that the thyroid microenvironments induce this abnormality. In human Hashimoto thyroiditis, one study showed a reduced suppressive capacity of CD4+CD25+Tregs (14), but another study has not confirmed this finding (15). Further investigation will be required to determine the significance of Tregs in human autoimmune thyroid diseases.

Recent studies have also reported different types of naturally occurring Tregs in the CD8+T-cell lineage (16-18). The similarities and dissimilarities of these CD8+ Tregs remain unclear, but it is likely that numerous distinct Tregs of different lineages (CD4 vs. CD8) intertwine intricately in the immune network system. Although there are still numerous issues of Tregs to be resolved in mice and humans, it is feasible that manipulation of Tregs may provide novel ways to treat immunologic disorders.

In mouse models of Hashimoto thyroiditis, the anti-CD25 antibody has recently been reported to overcome the resistance of Tg-tolerant CBA/J mice and BALB/c mice to Tg-induced thyroiditis (19, 20). It has also been shown in a mouse model, with adenovirus expressing the TSHR, that antibody-mediated depletion of CD4+CD25+ Tregs enhances the incidence and severity of hyperthyroidism in resistant and susceptible mouse strains, respectively, which suggests that a balance between effector T cells and Tregs is critical for disease development.

Another study was designed to evaluate the role played by another recently identified type of Treg, CD8+CD122+ T cells, in a mouse model; specifically, the significance of different types of Tregs in GD was delineated. With respect to CD4+CD25+ Tregs, CD8+CD122+ T cell depletion increases the incidence of hyperthyroidism in resistant and susceptible mice (21). Of interest, intrathyroidal lymphocytic infiltration was noted in some CD8+CD122+ T cell-depleted, hyperthyroid-resistant mice. These results indicate that in addition to CD4+CD25+ T cells, CD8+CD122+ T cells also play a crucial role in disease susceptibility in a murine model of GD.

Th17 cells were shown to belong to a subset of T helper cells producing IL17 in 2007. Th17 cells are considered to be developmentally distinct from Th1 and Th2 cells; an abundance of Th17 cells is thought to play a key role in autoimmune disease. Zhou *et al.* (22) reported that an imbalance between Th17 and Treg cells in a mouse model might play an important role in GD. They (22) showed that Graves' hyperthyroidism is associated with a decreased Treg population and an increased ratio of Th17 cells: Treg cells, but Th17 cells are not associated with the disease. Weaver *et al.* (23) also showed in 2009 that the Th17 cell: Treg cell ratio may be a useful marker for assessing the severity of disease in animal models and human disease, and important mechanisms were postulated to explain the skewed Th17 cell: Treg cell ratio. Additional studies are warranted to fully elucidate the complex immunologic mechanisms underlying Graves' hyperthyroidism.

### 4. TH1/TH2 CYTOKINES

Th lymphocytes consist of two subpopulations (Th1 and Th2) based on distinctive patterns of cytokine production (22). Th1 lymphocytes, which secrete interferon (IFN)- $\gamma$  and interleukin (IL)-2, are associated strongly with cell-mediated immune responses, while Th2 lymphocytes secrete IL-4 and IL-5 and are involved in humoral immunity (23). Th2 immune responses also down-regulate Th1 immune responses. Because these subpopulations tend to function antagonistically toward one another, the balance between Th1 and Th2 lymphocytes may determine the outcome of autoimmune diseases (24).

It is well-known that pro-inflammatory Th1-type cytokines, including tumor necrosis factor (TNF)- $\alpha$ , IFN- $\gamma$ , and IL-2 play pivotal roles in the pathogenesis of GD in the active state (25-27). While in the non-active state, a Th2 response, rather than a Th1 response, is dominant.

Nielsen *et al.* (24) assessed the production of Th1/Th2 cytokines elicited by a thyroid self-antigen, thyroglobulin (Tg), in cultures of peripheral blood mononuclear cells (PBMC) from healthy individuals and patients with GD. Initially, TNF- $\alpha$  and IL-2 are produced in both groups, accompanied by IL-10. Release of IFN- $\gamma$ , IL-4, and IL-5 then ensued. The GD patient group exhibited increased TNF- $\alpha$ , IL-2, IFN- $\gamma$ , and IL-10 responses. Conversely, a higher production of TNF- $\alpha$  and IL-5 occurred in the presence of autologous sera than in the presence of pooled normal sera in the GD patient group, indicating a dependency on serum constituents. Complement appeared to promote the production of IL-2, and particularly IL-5, the levels of which were reduced by neutralization of complement by heat or zymosan treatment. The production of IFN- $\gamma$  and IL-2 in both groups correlated directly with the serum anti-Tg activity. Moreover, TNF- $\alpha$ , IFN- $\gamma$ , IL-5, and IL-10 responses were markedly inhibited by the partial denaturation of Tg after boiling. Thus, it is hypothesized that autoantibodies and complement may promote mixed Th1/Th2 cell cytokine responses by enhancing the uptake of autoantigens by antigen-presenting cells (28).

It has been shown that C-X-C motif (CXC) - chemokines (Th1), especially chemokine CXC ligand (CXCL)9, CXCL10, and CXCL11, are important in the initial phases of autoimmune thyroid disorders (29-33). It has also been shown that the secretion of CXCL9, CXCL10, and CXCL11 in primary cultures of thymocytes from patients with GD can be stimulated by IFN- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  (34). Furthermore the treatment of thyroid follicular cells with peroxisome proliferator activated receptor (PPAR)- $\alpha$  activators at near-therapeutic doses significantly inhibit IFN- $\gamma$ -stimulated CXC chemokine secretion (35).

Another study demonstrated that a shift in the balance of IL-12/IL-5 cytokines was applied in judging the immunologic events in 74 patients with GD (50 patients had ophthalmopathies) during methimazole therapy and in 15 healthy controls. In patients with GD, only those

without ophthalmopathy had higher levels of IL-12 when compared to healthy controls. After 2 months of methimazole therapy in GD patients without ophthalmopathy, an increase in the ratio of IL-12:IL-5 was also observed as compared to those with ocular symptoms. The results demonstrated a difference in the balance shift of IL-12:IL-5 between GD patients with and without ophthalmopathies. The increased ratio of IL-12:IL-5 after methimazole therapy could be explained by the elevation of serum IL-12 due to methimazole therapy and the age-related decrease in serum IL-5 (36).

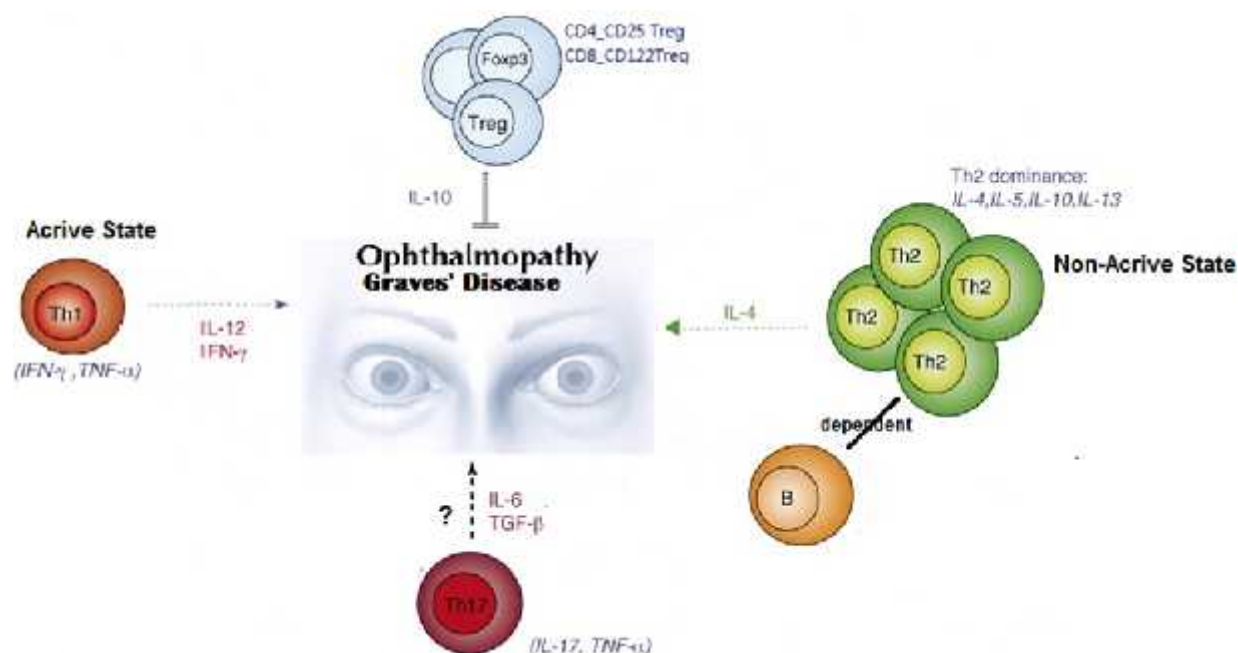
The Th2 dominance is achieved through "abnormal" T cell-dependent B-cell activation, which leads to the release of cytokines specific for a Th2 response, particularly IL-10 (37). Suppression of CD8 cells by this Th2 response may accentuate the symptoms of GD. CD8-expressing cells can work to counteract Th2 dominance. In fact, studies have shown that secretion of sCD8 is particularly marked during the active phase of GD (38). However, in hyperthyroid individuals, the levels of CD8-expressing cells are lower than in euthyroid individuals, indicating that the prevalence of the Th2 response is associated with more pronounced symptoms (39). Aust *et al.* (40) suggested that NK cells, as well as CD8 cells, may help prevent B cell infiltration of the thyroid. Some patients with a high number of NK cells within the thyroid had lower levels of B cells than patients with fewer NK cells (42).

Many studies have shown that IL-6, IL-10, and TNF- $\alpha$  are inflammation-involved cytokines; the enhanced expression of IL-6, IL-10, and TNF- $\alpha$  in GO has been reported earlier (12, 41, 42). IL-6 is especially important because it activates IL-17 production by Th17 T cells, which is implicated in autoimmunity and microbial immunity (43). Th17 cells and Treg cells display a reciprocal function, thus when Th17 cells are up-regulated, Treg cell activity is reduced, resulting in inflammation enhancement and vice versa. Despite the high level of IL-6 in GD, which normally shifts the Th17 cell-Treg cell balance in favor of the Th17 (43), similar levels of Treg cells are detected in GO patients and normal donors. Because IL-10 and TNF- $\alpha$  (44) are pro-Treg cells, the anti-Treg effect of IL-6 may be counteracted (45) (Figure 1). Nevertheless, additional experiments are required to resolve the IL-6-Treg cell co-existence enigma (12).

### 5. IMMUNOGLOBULINS (IGGS)

GD is a classic autoantibody-mediated disease, but T-cell help is presumably required for the differentiation of autoantibody-producing B cells into plasma cells. GO is a serious manifestation of GD, the pathogenesis of which suggests a role for T cells and autoantibodies.

Increased levels of particular IgG subclasses may act as a surrogate for cytokine profiles, typifying Th pathways. Autoantibodies are more stable in serum in long-term storage than cytokines. The best defined relationship in humans involves IgG4 and the Th2 pathway driven by



**Figure 1.** Treg cells are considered to be an essential element in suppression of GD. The Th1 cytokine response dominates in the active state of GD, while the Th2 cytokine response dominates in the non-active state of GD; the role of Th17 cells needs further investigation.

IL-4 (46), but drivers of the Th1 pathway are controversial in that IgG1 and IgG3, and sometimes IgG2, have been attributed to this response. There is often a mixture of subclasses, implying a mixed response that is almost impossible to separate. One possible explanation for the subclass dichotomy between thyrotoxicosis (TT) and chronic lymphocytic thyroiditis (CLT) is that TT is an antibody-driven autoimmune disease driven by a Th2 response, while CLT is considered to be a cell-mediated disorder driven by a Th1 response (47).

Interestingly, Outschoorn *et al.* (47) reported that differences in the maternal and paternal contributions to the heritability of IgG subclasses in TT families, but not CLT families. Among the TT group, positive heritability was consistently observed in juvenile patients for IgG2, IgG3, and IgG4. Maternal contributions consisted of IgG2, IgG3, and IgG4, whereas paternal contributions consisted only of IgG2 and IgG3. There was a stronger maternal influence and essentially no paternal influence on the IgG4 subclass.

In summary, subclasses do reflect differences in contributions to the total anti-Tg response, as do maternal and paternal differences. In the CLT group in which patients were followed over time, the over-expression of IgG2 shows a dominant and heritable pattern in some families. Ethnic differences in the inheritance of antibodies exist among TT patients and their family members.

Another study by Santoh *et al.* (48), showed that the serum IgG3:IgG ratio and goiter size may represent different factors of GD intractability and that the combined analysis may be useful in the detection of patients with intractable GD

under treatment with anti-thyroid drugs. In GD patients, the serum IgG3:IgG ratio was higher in euthyroid patients than patients with thyrotoxicosis. The IgG3:IgG ratio was inversely correlated with the serum concentration of thyroid hormones. In conclusion, the serum IgG3:IgG ratio and goiter size may be used as independent indicators of GD intractability in euthyroid patients with GD under treatment with anti-thyroid drugs.

## 6. CONCLUSIONS

GD is the result of a complex autoimmune response; indeed, a number of immune cells, cytokines, and chemokines are involved. In understanding the process culminating in GD, manipulation of the predominant cells, cytokines and chemokines may provide novel ways to treat this immunologic disorder.

## 7. ACKNOWLEDGMENTS

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- Abbreviations:** GD: Graves' disease; IgGs: immunoglobulins; GO: Graves' ophthalmopathy; IFN- : interferon- ; IL-2: interleukin-2; TNF- : tumor necrosis factor; PBMC: peripheral blood mononuclear cells; Tg: thyroglobulin; CXC: C-X-C motif; PPAR- : peroxisome proliferator activated receptor- ; TT: thyrotoxicosis; CLT: chronic lymphocytic thyroiditis
- Key Words:** Graves' disease, IFN-gamma, IL-2, Review
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