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Recent insights into the pathogenesis and management thyroid-associated ophthalmopathy

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Abstract

Purpose of review—To identify and critique the most recent experimental findings regarding the pathogenesis and therapy of thyroid-associated ophthalmopathy (TAO).

Recent findings—Much of the recent work in this field has focused on identifying genetic alterations associated with the phenotypes of Graves' disease (GD) and TAO and investigating their functional consequences. Identified candidate genes include CD40, cytotoxic T-lymphocyte antigen-4 (CTLA-4), protein tyrosine phosphatase-22 (PTPN22), HLA-MHC and those associated with the X-chromosome. Efforts to generate a complete rodent model of GD continue with little progress. These uniformly involve the immunization of animals with the thyrotropin receptor (TSHR). Studies conducted *in vitro* have focused on the actions of cytokines in orbital fibroblasts, the potential role of the insulin-like growth factor-1 receptor and activating antibodies directed against it as a fibroblast and T cell activation pathway. Reports continue to appear examining the potential relationship between the TSHR and orbital adipogenesis. Regarding therapy for TAO, small molecules and antibodies disrupting cytokine pathways and lymphocyte function are currently under examination and have yielded promising albeit preliminary results.

Summary—TAO remains a vexing medical problem, the pathogenesis of which remains uncertain. A number of obstacles continue to plague major advances, not the least of which is the absence of a robust animal model. A few new insights seem to represent departure from traditional thinking about this disease and may herald important innovation.

Keywords

Autoimmune; cytokines; Graves' disease; ophthalmopathy; thyroid

Introduction

Graves' disease (GD) represents a partially genetic autoimmune process affecting the thyroid gland as well as orbital and dermal connective tissue. Most patients present with an enlarged, hyperplastic thyroid and hyperthyroidism. Many also manifest thyroid-associated ophthalmopathy (TAO), a syndrome comprising proptosis, ocular edema and inflammation.

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Dermopathy, a localized infiltrative process of the lower leg occurs in very few patients. The pathogenesis of these components of GD is poorly understood as is the mechanistic connection between them. At the center of hyperthyroidism in GD lie activating antibodies directed against the thyrotropin receptor (TSHR). But why tissues of the thyroid, orbit and skin undergo remodeling remains uncertain and may be unrelated to the TSHR or its antibodies. These tissues are infiltrated by inflammatory cells, fat expansion, and hyaluronan accumulation. Our inability to develop a robust and complete animal model has hindered better understanding GD.

In this brief review we attempt to identify recent progress being made in identifying the mechanisms underlying GD and TAO. New insights are needed before we can approach therapy more rationally. TAO remains a vexing problem associated with substantial morbidity.

Emerging concepts in disease pathogenesis

Progress in identifying the genetic contribution to GD

Autoimmune thyroid disease (AITD) occurs with increased frequency in families harboring susceptibility genes (1). Twin studies have revealed a pattern of familial clustering, a 35% concordance of GD disease in monozygotic twins and have attributed 79% of disease susceptibility to genetic factors (2). In addition to as yet unidentified environmental factor(s), multiple genetic abnormalities appear to converge in GD and these may interact synergistically (3). Much of the recent investigation has focused on 5 genes; CD40, cytotoxic T lymphocyte antigen-4 (CTLA-4), protein tyrosine phosphatase-22 (PTPN22), HLA-MHC and the X-chromosome associated genes.

i) CD40, a member of the tumor necrosis factor receptor super-family, was originally identified as an important B-cell activation factor involved in the development of humoral immune responses (4). Subsequently, it has been detected on the surface of many cell-types including thyrocytes (5,6) and orbital fibroblasts (7). Activated $CD4^+$ T lymphocytes express CD40 ligand (CD40L, aka CD154) (4). Thus, cross-talk between thyrocytes or orbital fibroblasts and infiltrating lymphocytes might occur through the CD40-CD40L molecular bridge in the setting of autoimmune disease. Orbital fibroblasts from patients with GD proliferate when incubated with autologous T cells, an effect dependent on the CD40-CD154 pathway (8). CD40-mediated signaling results in cellular activation through the up-regulation of specific downstream genes including those encoding IL-6, IL-8, PGHS-2, and enhanced hyaluronan synthesis, all of which have been implicated in the pathogenesis of GD (7,9). A single nucleotide polymorphism (SNP) C/T in the un-translated region of the CD40 gene has been associated with susceptibility to GD in Caucasian (10) and Korean (11) populations. Recently, this SNP was also implicated in disease susceptibility in an older cohort of Japanese (12) and has been linked to production of anti-thyroid Abs (13). Interestingly, it was not detected in 12 mice strains known to be susceptible to GD (two animals) and Hashimoto's thyroiditis (ten animals) (14), suggesting that any contribution of CD40 to autoimmunity in mice and human beings may differ substantially. In man, this SNP may alter the translational efficiency of CD40 protein (14). Jacobson and colleagues, utilizing an *in vitro* transcription/translation system, demonstrated that approximately 15.5% more CD40 protein was produced by cells harboring this SNP (14). When surface expression of CD40 was examined by flow cytometry, rat embryonic kidney fibroblasts (Rat2) transfected with the C/T SNP expressed 32% more surface CD40 compared to controls. Furthermore, human B cells harboring this SNP expressed 39% and 27% higher levels of surface CD40 at rest and following activation, respectively, compared to controls. No difference was detected in steady state CD40 mRNA levels. It is possible that the elevated levels of surface CD40 might amplify cell activation during tissue injury,

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potentially predisposing the host to GD. The CD40-CD40L bridge may represent an important therapeutic target for GD and its interruption has been examined in other inflammatory diseases (15).

ii) CTLA-4- The CTLA-4 gene encodes a negative regulator of T lymphocyte activation and proliferation (16). In fact, CTLA-4 knock-out mice develop a lethal lymphoproliferative disorder (17). CTLA-4 inhibits T cell responses by opposing the metabolic consequences of CD28 activity, which ordinarily promotes several T cell functions. A SNP (CT60) in the 3′UTR of the CTLA-4 gene has been associated with increased incidence of GD in caucasians (18) a finding recently extended to Japanese (19), Italian (20), Chinese (21) and Taiwanese (22) populations, and confirmed in a metanalysis (23). Other alterations of the CTLA-4 gene A49G (20,24), aT-n (20) have also been identified. Saverino *et. al.* recently studied a soluble form of CTLA-4 (sCTLA-4), levels of which are elevated in serum of subjects with GD (25). sCTLA-4 was isolated from these individuals and found to be functional in *in vitro* studies. The role of sCTLA-4 and its full-length cell-bound analogue in autoimmunity remains uncertain.

iii) **PTPN22**- Lymphoid protein tyrosine phosphatase (LYP, aka PTPN22) represents another negative regulator of T cell activation. A SNP at 1858CT, 620AT of the PTPN22 gene encoding LYP has been detected with increased frequency in autoimmune diseases such as GD (26-28). LYP inhibits T cell activation by de-phosphorylating T cell receptor (TCR)-associated kinases (29-31). Interestingly, the 1858CT, 620AT SNP results in a gain of function mutant phosphatase that inhibits T cell signaling more completely than wild type LYP (31). Yu *et. al.* recently synthesized a highly specific salicylic acid-based inhibitor of LYP, I-C11 (32). I-C11 inactivates LYP by binding to its active catalytic site. Furthermore, the authors identified serine 25 as a potentially critical phosphorylation site on LYP. PKC-dependent phosphorylation of this serine residue leads to a conformational change of the LYP molecule and disrupts LYP's ability to recognize and bind adaptor molecules. When phosphorylated at serine-25, LYP cannot inactivate kinases critical to T cell activation. I-C11 as well as inhibitors of PKC dependent serine-25 phosphorylation may represent useful agents for studying T cell activation in chronic inflammatory and autoimmune disease (32)

iv) HLA class I- HLA class II genes are associated with susceptibility to GD. The HLA class II region codes for cell surface receptors involved in antigen presentation as well as thymic selection/deletion of T cells. Recently HLA class I molecules also have been implicated (33). Simmonds *et. al.* genotyped 871 patients with GD and 621 controls for DRB1, DQB1 and DQA1 loci. All three loci were associated with GD (33). Further analysis examining peptide binding domains of these molecules suggests that position B74 located on exon 2 of DRB1 exhibits the strongest disease-association, making this peptide a prime candidate for involvement in auto-antigen presentation.

v) Monosomy X- Female gender propensity for autoimmune disease was recognized many years ago and women are approximately 10 times more likely than men to develop GD. Some, but not all studies have shown linkage of AITD with regions of the X-chromosome. Genes residing there apparently play critical roles in the maintenance of immune tolerance. Thus, X-gene haplo-insufficiency has been scrutinized for its potential as a common mechanism for autoimmune disease. The rate of X- chromosome monosomy, particularly in cells of the immune system, such as T and B cells, is significantly higher in women with AITD (34). In addition, skewed X-chromosome inactivation (>80% of cells inactivating the same X-chromosome) occurs with a high frequency in female AITD patients compared to controls (35,36). Brix *et. al.* studied skewing of X-chromosome inactivation in twins with GD compared to healthy unrelated twin controls as well as to their unaffected twin sibling (37). 32 female twins [mono (MZ)- and dizygotic (DZ)] with AITD were compared for x-

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chromosome skewing to 96 healthy unrelated twin controls matched for age and zygosity. Skewed inactivation of the X-chromosome occurred in 37% of subjects with GD compared to 14% for matched controls (37). Furthermore, the rate of skewed x-chromosome inactivation was 32% in 26 female twins (6 MZ, 20 DZ) compared to 12% in their clinically discordant twin. Skewed inactivation of the X-chromosome may exhibit divergence with respect to tissue involvement. Preferential inactivation of one of the two X-chromosomes (in more than 80% of cells) instead of the random 50%/50% may result in inadequate thymic exposure to potential X-chromosome-encoded self-antigens. Should these antigens be expressed in sufficiently high frequency in peripheral tissues, an immune response to these insufficiently self tolerized X-linked antigens may lead to an organ specific autoimmune response (37).

Animal models of GD

Attempts to develop a complete and robust animal model of GD continue (38-41). For instance, Flynn *et. al.* showed that chronic stimulation of an autoimmune-prone HLA DR3 transgenic non-diabetic mouse with stimulating anti-TSHR Mab leads to sustained hyperthyroidism. Histologic examination or the thyroid gland after 65 days revealed enlarged, hyperplastic follicles but an absence of inflammatory infiltrates (38). Kaneda *et. al.,* using BALB/c mice in an electroporation (EP)-based gene transfer technique, greatly enhanced TSHR expression *in vivo* and achieved a high incidence of hyperthyroidism. Moreover, TSHR simulating Ab generation persisted for more than 8 months (40). No thyroid lymphocytic infiltration was noted. McLachlan *et. al.* have implicated $CD25^{+}$ regulatory T cells as important determinants of whether an induced AITD phenotype resembles that of GD or Hashimoto's disease in mice (42). Baker *et. al.* failed to reproduce a murine model of thyroiditis and TAO, developed by immunizing BALB/cbyJico mice with the human TSHR and transferring primed T cells to a syngeneic animal. The authors determined that the microbial environment may influence the immune responses in experimental animals (41). Our inability to develop an animal model of GD reflects our poor understanding of its underlying pathophysiology.

Cytokines

IL-6 plays critical roles in B cell development and differentiation. It drives Ig synthesis and is critical to normal plasma cell development. Chen *et. al.* found that IL-6 production was dramatically enhanced by IL-1β in orbital fibroblasts but not in those from other tissues (43). The induction resulted from coordinate enhancement of IL-6 gene promoter activity and stabilization of IL-6 mRNA. This same group reported that IL-4 induced 15-lipoxygenase-1 gene expression and in so doing promoted the production of 15-HETE (44). Notably this effect was shared by IL-13, another Th2 cytokine and was specific to orbital fibroblasts from patients with TAO. 15-HETE may promote fibrosis, thus these findings might tie together the transition from Th1 to Th2 dominated immunity found as TAO progresses to the irreversible fibrotic tissue changes associated with late-stage disease.

The proteolytic environment surrounding cells determines a host of molecular events, including extracellular matrix turnover and the processing of cell-surface molecules. This environment comprises both proteases and their physiological inhibitors such as tissue inhibitor of metalloproteinases (TIMPS). TIMP-1 is differentially regulated in TAO orbital fibroblasts by IL-1β, an induction which is mediated through enhanced gene promoter activity and is down-regulated by both Th1 and Th2 cytokines (45). Thus it is very likely that pericellular proteolysis occurring in the microenvironment surrounding these cells differs from that of other fibroblasts, perhaps accounting in part for the unique phenotypic attributes displayed by these cells.

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IL-16, RANTES and CXCL10 represent potent T lymphocyte chemoattractant molecules, and their production by thyrocytes (46,47) and orbital fibroblasts (48,49) represents an important basis for lymphocyte trafficking to diseased thyroid and orbit. Serum CXCL10 levels are elevated in patients with GD, particularly early in the disease (50) and during the active phase of TAO (51). CXCL10 production by thyrocytes is inhibited by methimazole (52). IL-16 and RANTES production is mediated through the IGF-1 receptor (IGF-1R) (53). Circulating activating autoantibodies against this receptor have been identified in GD and may drive lymphocyte infiltration (46,53). The T cell phenotype in GD appears skewed toward the IGF-1R$^+$ phenotype (54). Their activation through IGF-1R enhances survival and promotes proliferation, resulting in a disproportionate increase in CD45RO$^+$IGF-1R$^+$ memory T cells (54). Trafficking of these lymphocytes to affected tissues may be dependent on cell surface expression of the IGF-1R (54) and CXCR6 (55) or on the tissue expression of markers such as CD1 (56).

Adipogenesis

Orbital fat commonly expands in TAO. TSHR is expressed by orbital adipocytes in culture (57) and the transcript can be detected in orbital fat (58). Therefore, several studies have examined a potential connection between orbital fat and TSHR. Recently, Zhang *et. al.* using retroviral vectors, introduced activating mutant TSHR (L629F and M453T) into orbital fibroblasts (59). This receptor over-expression led to inhibition of proliferation and rendered the cells refractory to PPARγ-induced adipogenesis (59). In another study, orbital fibroblasts exhibited enhanced TSHR expression after adipogenesis (60). The authors concluded that the receptor was functional because treatment with TSH and stimulating anti-TSHR Abs resulted in small increases in cAMP generation (60). Differentiation of orbital fibroblasts into adipocytes may be driven by infiltrating activated T cells through their elaboration of PPARγ ligands (61). Another report examined frizzled related protein-1 (sFRP-1) and the wnt pathway. sFRP-1 inhibits signaling through this pathway and usually attenuates adipogenesis. sFRP-1 treatment of orbital fibroblasts increased leptin, adiponectin, and TSHR mRNA expression and was associated with enhanced Oil-Red-O staining (62). sFRP-1 and the wnt pathway may therefore represent targets for interrupting potentially aberrant adipogenesis in TAO.

Therapy

Treatment of hyperthyroid GD is easily accomplished with antithyroid drugs, surgical thyroidectomy or radioactive iodine. Despite this, El Fassi *et. al.* administered Rituximab (RTX), an anti CD20 mAb, to patients with uncomplicated GD (63). RTX depletes peripheral B cells depletion through its action on CD20. In this uncontrolled study, patients with GD were treated with antithyroid medication without or in combination with RTX. They were then withdrawn from the anti-thyroid medication and followed longitudinally for signs of disease relapse and elevated TSI levels. While treatment with RTX may have sustained remission in a subset of patients with low TSI titers, the high cost, low efficacy and potential side effects may not justify its use in uncomplicated GD. Salvi *et. al.,* compared RTX to intravenous glucocorticoid therapy in a small, open label non-randomized pilot study involving patients with mild to moderate TAO (64). The investigators concluded that RTX positively affected the clinical course of TAO. Unfortunately, that study too was uncontrolled and inadequately powered. Prospective trials to further investigate the efficacy of RTX in TAO are currently underway. Somatostatin analogues were initially considered promising therapeutic agents for TAO, based on non-randomized open trials (65). The drug has been abandoned as therapy for TAO based on results from four recent randomized, prospective trials (66-69) showing no efficacy.

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Emergence of anti-cytokine and small molecule therapies for other autoimmune diseases has led to questions about their potential efficacy in TAO. TNF-α was found to be over-expressed in orbital connective tissue in TAO (70,71), and serum levels are elevated in hyperthyroid GD. Furthermore, a SNP in the TNFα gene promoter has been associated with increased incidence of GD (72,73). In addition, TNF-α may activate circulating monocytes and promote their differentiation into dendritic cells and macrophages in affected tissues (74). Thus, TNF-α may represent a therapeutic target particularly for patients with TAO. Agents interfering with TNF-α action have revolutionized the treatment of rheumatoid arthritis and inflammatory bowel disease. Paridaens *et. al.*, in a prospective but uncontrolled study, examined the effects of Etanercept in 10 euthyroid patients with active TAO (75). Following therapy, the mean CAS improved from 4.0 to 1.6 after 12 weeks. The majority of clinical improvement involved the soft tissues but proptosis was unimproved. Overall, 60% of the subjects reported moderate to marked improvement (75). Durrani *et. al.* reported treating a single patient with sight-threatening TAO who may have benefited from therapy with Infliximab (76).

Conclusion

The pathophysiology of Graves' disease remains enigmatic. Absence of a spontaneous or contrived animal model of GD, replete with the inflammatory features characteristic of human disease, has hindered progress in this field. Recent studies have identified multiple genes, molecular pathways and autoantigens which represent potentially exciting avenues of investigation to pursue. They may prove important targets for therapy development.

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