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Pathogenesis of Graves' disease focusing on Graves' ophthalmopathy

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Graves' disease (GD) is one of the most common autoimmune diseases in the US, with the prevalence about 1 % (Jacobson and Tomer 2007). Typical clinical features - tachycardia, proptosis, and goiter - were termed in 1840 by Carl-Adolph von Basedow as "Merseburg trias". Pathognomonic histopathophysiological picture of GD consists of typical lymphocytic infiltration of ocular muscles and retrobulbar tissues resulting in the exophthalmus. Epidemiological studies showed a strong influence of multiple genetic/environmental interactions on GD (Tomer and Davies 2003) between susceptibility genes (e.g. CTLA-4, HLA-DR and thyroglobulin-Tg) and environmental triggers (e.g. dietary iodine or infection) which result in the activation of thyroid-autoreactive CD4+ T cells infiltrating the thyroid. The T cells further activate B cells to secrete TSH receptor stimulating antibodies that induce thyrocyte proliferation and secretion of excess thyroid hormones, resulting in the classic hyperthyroid symptoms of GD.

The major genes associated with GD include the major histocompatibility complex (MHC) class II, and more recently, the MHC class I, human leukocyte antigen-C genes (Gough and Simmonds 2007; Simmonds et al. 2007), as well as five, additional, non-MHC genes (Jacobson and Tomer 2007). Grumet et al. (1974) first showed the association between GD and the alleles of MHC class I, with a higher frequency of HLA-B8 in GD patients (47%) as compared to controls (21%). The MHC class II molecules play a critical role in the initiation of adaptive immune responses. Peptide antigens can only be recognized by T cell receptors when they are attached to the binding region of an MHC molecule on the surface of an antigen presenting cell. However, it was not until recently that an arginine residue, at position 74 of the HLA-DRb1 chain (DRb1-Arg74), was found as the critical DR amino acid conferring susceptibility to GD (Ban et al. 2004). Early studies showed association of HLA-DR3 with Graves' disease (GD) (Jacobson et al. 2008). Interestingly, patients with concomitant Graves' opthalmopathy (GO) and/or TSH-binding inhibiting antibodies showed a higher prevalence of HLA-B8 and DR3 compared to a normal control group (Schleusener et al. 1983). Other studies conducted have not replicated this result (Frecker et al. 1988; Kendall-Taylor 1988).

Recently, a significant association of the IL-23R gene variants emerged with GD as well as with GO (Huber et al. 2008). It is possible that these variants may predispose to GO by changing the expression of IL-23R, thereby promoting a proinflammatory signaling cascade. Evidence for genetic/environmental influence in GD patients provides the expression of IFNα and IFNα-inducible genes, particularly MHC class II molecules which may enhance autoantigen presentation of thyroid–stimulating hormone receptor (TSHR) on thyrocytes (Kuang et al. 2010).

The concept that GO might be an autoimmune disease was originally put forward owing to its clinical association with GD, the connective tissue manifestations of which are a consequence of disordered thyroid function reflecting the underlying autoimmune processes. Many patients with GO have antibodies against the TSHR and thyroid peroxidase, and about 50% of the patients have antibodies against the Tg (Bahn and Heufelder 1993). Ophthalmopathy can also occur in some patients with transient thyroiditis, thyroid cancer and Graves' disease many years after treatment of the disease. All these are

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situations where TSHR antibodies are not expected to be present, suggesting that the relationship between TSHR antibodies and the eye disorder has not been established for all cases. Studies show that clinically apparent GO is present in 25% to 50% of patients with GD, and that subclinical evidence of ocular involvement is detectable in most of these patients (Salvi et al. 1990). Conversely, the presence of autoimmune thyroid disease i.e. overt or subclinical Graves' hyperthyroidism or Hashimoto's thyroiditis seems to be necessary, if not sufficient, for the development of GO (Marcocci et al. 1989), including those patients who have been considered to have associated thyroid immunological abnormalities, previously described as euthyroid GD patients. Studies on the chronology of ophthalmopathy in GD disclosed a temporal relationship between the onset of hyperthyroidism and the onset of ophthalmopathy (Bartley et al. 1996). Regardless of which condition occurs first, the other develops within 18 months in 85% of affected patients (Marcocci et al. 1989; Bartley et al. 1996).

Predisposing factors

Autoimmunity affects multiple glands in the endocrine system. Disorders such as type 1 diabetes mellitus, Graves' disease, Hashimoto's thyroiditis, Addison's disease, and many others result from autoimmune mediated tissue inflammation. Each of these disorders can be divided into stages beginning with genetic susceptibility, environmental triggers, active autoimmunity, and finally metabolic derangements with overt symptoms of disease. Animal models and human studies highlight the importance of alleles in HLA-like molecules determining tissue specific targeting that, with the loss of tolerance, leads to organ specific autoimmunity.

Genetic factors. The putative GD susceptibility genes include both immune modifying genes (e.g. HLA, CTLA-4) and thyroid specific genes (e.g. TSHR, Tg). A few studies specifically examined genetic differences between GD patients with GO and GD patients having no apparent eye disease. However, these did not yield any confirmed susceptibility loci for GO because the various polymorphisms offering susceptibility to GD were found in equal proportion in Graves' patients with and without eye disease (Blakemore et al. 1995; Cuddihy and Bahn 1996; Muhlberg et al. 1998; Siegmond et al. 1998). More recent whole genome linkage studies suggested that three interacting loci, found on different chromosomes, induce genetic susceptibility to GD (Tomer et al. 1999). These data did not, however, support a major role for additional familial factors in the development of severe GO in patients with GD (Villanueva et al. 2000). These investigators tested five candidate genes, including HLA, TNFβ, CTLA4, ICAM-1, and TSHR, and found none to be specifically associated with GO (Villanueva et al. 2000; Bednarczuk et al. 2007). Interleukin-1 (IL-1) is known to have an important role in pathogenesis of GO, so far as a positive correlation between polymorphisms in the IL-1α and IL-1RA genes and susceptibility to GO has been demonstrated (Khalilzadeh et al. 2009). On the contrary, the Pro(12)Ala polymorphism of the Peroxisome Proliferator Activated Receptor γ (PPAR γ) gene associated with a modified transcriptional activity, might be affecting less actively GO (Alevizaki et al. 2009). These findings promote further research into genetic correlates of GO. There are many reasons for the lack of reproducibility of association studies in GO, including poor characterization of the studied groups and small sample sizes and different allelotyping methodologies, these studies have yielded contradictory results.Thus, the genetic background of GO remains to be elucidated in future research. It also appears that other factors, rather than major genes, are likely to be the primary predisposing factors to the development of GO. Thus, the possibility that GO may be a genetically heterogeneous disorder cannot be disregarded or that environmental factors, such as smoking, increase the likelihood and severity of GO in advance.

Environmental triggers. Environmental risk factors for AITD (autoimmune thyroid disease- GD or Hashimoto's thyroiditis) may include pollution (including radioactive emissions) and excess iodine intake (through jodbasedow, Wolff-Chaikoff effect, or immunological release of autoantigens) (Martocchia and Falaschi 2007). Smoking has been consistently linked to development or deterioration of GO, in GD patients. Smokers with GD who receive therapeutic dose of radioactive iodine have the highest incidence of unfavorable GO outcome, which is proportional to the number of cigarettes smoked per day. Evidence for a dose-response relationship between smoking and the severity of GO includes the severity of GO being related to the current number of cigarettes smoked per day (Hagg and Asplund 1987; Prummel and Wiersinga 1993; Winsa et al. 1993) and the percentage of heavy smokers being higher in patients with more severe ophthalmopathy (Bartalena et al. 1989). It appears that current, but not lifetime, tobacco consumption constitutes a risk for the incidence of proptosis and diplopia in patients with GO and that

this risk increases in heavy smokers (Pfeilschiftetr and Ziegler 1996).

In addition, other studies have shown that among patients with GO, smokers have more severe eye disease than do nonsmokers (Shine et al. 1990). *In vitro* studies have demonstrated increased glycosaminoglycans (GAGs) production when orbital fibroblasts were cultured under hypoxic conditions (Metcalfe and Weetman 1994), such as in smoking. In addition, tobacco products enhanced IL-1 secretion by these cells, which in turn has been shown to increase GAGs production. Cigarette smoking, which is probably the most important environmental factor associated with GO occurrence and maintenance, might also act, among other mechanisms, by enhancing generation of oxygen reactive species and reducing antioxidant production. Substances such as nicotinamide, allopurinol and pentoxifylline reduce superoxide- or hydrogen peroxide-induced proliferation of fibroblasts, GAG production and HLA-DR expression by GO orbital fibroblasts (Bartalena et al. 2003).

Several studies have reported an association between radioactive iodine treatment for GD and worsening or development of GO independently of smoking (Stan and Bahn 2010). The risk for de novo development of GO was greater in patients treated with radioactive iodine (53 patients) than with medical treatment (23 patients), while worsening of ophthalmopathy was not more common with the administration of radioactive iodine (Metcalfe and Weetman 1994). It is suggested that treatment with radioactive iodine forms a significant risk factor for development of ophthalmopathy in Graves' hyperthyroidism (Stan and Bahn 2010). Despite greater mean TSH levels post radioactive iodine treatment (P = 0.003), there was no increase in the likelihood of developing active ophthalmopathy (OR 0.95; 95% CI 0.56-1.61, P = 0.9) or extra–ocular muscle dysfunction (OR 0.52; 95% CI 0.26-1.06, P = 0.074) in patients with mild or no eye disease at baseline, indeed (Traisk et al. 2009). Interestingly, smokers run the highest risk either for worsening or the development of GO irrespective of the treatment modality (Bartalena et al. 2003).

Among toxins and infections, congenital rubella syndrome is frequently associated with AITD, whereas Epstein-Barr and Yersinia enterocolitica (El-Kaissi et al. 2010), have been investigated as potential triggers of AITD (Martocchia and Falaschi 2007). Infectious processes can potentially initiate autoimmune phenomena if the infecting agent possesses antigens sufficiently similar to host antigens to elicit a cross reactive response. Such molecular mimicry could relate to a B cell (autoantibody) or T cell epitope. Most interest has involved the former. Antigenic cross reactivity between the TSHR and a variety of infectious agents has been reported, including the HIV-1 nef protein (Burch et al. 1991; Tomer and Davies 1993). The most intriguing observations as to the leading infective trigger of GD involve the gram negative bacterium, Yersinia enterocolitica. Antibodies to Yersinia enterocolitica in patients with AITD were first noted in 1974 in Scandinavia (Bech et al. 1974). Because of the high incidence of Yersinia enterocolitica infections in this region, evidence for an association between Yersinia enterocolitica antibodies and thyroid disease was sought, and found, in countries with a low incidence of infections (Shenkman and Bottone 1976; Weiss et al. 1979). Recent studies have found no unique pattern of serological reactivity against Yersinia membrane proteins in patients with autoimmune thyroid disease, raising the possibility that cross reactivity with Yersinia in GD may occur at the T cell epitope level rather than via autoantibodies (Arscott et al. 1992). However, the specific Yersinia enterocolitica proteins and epitopes recognized by antiTSHR autoantibodies (TRAb) have not been fully clarified, resulting in conflicting results from clinical research. Interestingly, the recent identification of the Yersinia enterocolitica protein outer membrane porin F protein (ompF) which was recognized by TRAb and was similar to TSHR certainly offers a great of understanding of the role molecular mimicry plays in the disturbance of immune tolerance and the induction of autoimmunity in both GD and GO (Wang et al. 2010).

Allostatic load in stress response may be a condition favoring the development of AITD. An increasing number of drugs like lithium, amiodarone, interferons (IFNs), and anti-CD52 monoclonal antibody Campath-1H may induce AITD (Weetman and McGregor 1994; Chiovato and Pinchera 1996; Coles et al. 1999; Ruwhof and Drexhage 2001). In particular, preparations of leukocyte derived IFN contaminated with IFN γ were potent inducers of in vivo AITD in humans (Burman et al. 1986).

Immunologic parameters

Diffuse infiltration of lymphocytes, with occasional nests of lymphoid aggregates, is seen within the orbital adipose tissues of patients with GO. A similar, if more sparse, cellular infiltrate is present in the interstitial tissues of the extraocular muscles. The majority of the cells are T lymphocytes, with occasional B lymphocytes seen.

Both helper/inducer ($CD4^+$) and suppressor/cytotoxic ($CD8^+$) T lymphocytes are present, with a slight predominance of the latter (Weetman et al. 1989).

To better understand immune mechanisms operative within the orbit in GO, several groups of investigators have profiled the cytokines secreted by tissue infiltrating T cells. Two groups reported that the majority of retroocular T cell clones produce cytokines involved in cell-mediated T helper cell type 1 (Th1)-type immune processes, namely IL-2, IFN γ, and TNFα, but not IL-4 or IL-5 (Heufelder and Bahn 1993; de Carli et al. 1993). A third group detected mRNA encoding a T helper cell type 2 (Th2)-dominant profile (IL-4, IL-5, and IL-10) characteristic of humoral responses (Hiromatsu et al. 2000), whereas another group of investigators identified clones secreting cytokines characteristic of both subtypes (McLachlan et al. 1994). It appears that Th1 cells may predominate in early disease, whereas Th2 cells may become predominant late in the course of the disease (Aniszewski et al. 2000). Autoimmunity may derive from a decrease of local production of immunosuppressive cytokines like TGFβ or IL-10, a biased production of cytokines by Th1 phenotype cells in respect of Th2 cells or a decreased apoptosis of activated T cells (Martocchi and Falaschi 2007).

$CD4^+$ T cells producing IL-17 (Th17), as distinct from Th1 or Th2 cells, have recently been shown to be associated with autoimmunity, but it is not entirely clear how Th17 cells are generated from naïve T cells. Spanish researchers recently assessed the serum levels and the in vitro synthesis of IL–17 and IL–22 and of different cytokines (IL–6, IL–15, and IL–23) involved in the differentiation of Th17 cells in the peripheral blood and thyroid glands of 26 patients with AITD. Results revealed enhanced levels of T cells synthesizing IL–17 and IL–22 in the peripheral blood from AITD patients (Figueroa-Vega et al. 2010).

Besides participating in T and B cell responses, some Th1 and Th2 cytokines contribute to disease propagation by inducing classical immunomodulatory proteins in orbital and pretibial fibroblasts (Natt and Bahn 1997). Other participating inflammatory mediators and chemokines are elaborated by resident macrophages and fibroblasts. These factors, including IL-1α, IL-6, IL-8, IL-16, TGFβ, RANTES (regulated upon activation normal T-cell expressed, and presumably secreted) which belong to the family of chemotactic cytokines known as chemokines, and prostaglandin E2 (PGE2), trigger T cell migration across activated microvascular endothelium or participate directly in local inflammation (Wang et al. 1986; Smith et al. 1997). The inflammatory cyclooxygenase, COX-2 is usually expressed at extremely low levels under normal basal physiological conditions, but is highly inducible by several factors such as cytokines (Wang et al. 1996; Cao et al. 1998; Han et al. 2002). Cyclooxygenase 2 (COX-2) is expressed at higher levels in the orbital fibroadipose tissues of GO; there is a positive correlation with increasing severity of ophthalmopathy, suggesting a possible relationship with COX-2 expression and orbital inflammation in GO.

A sexual dimorphism has been described in AITD and in other autoimmune diseases, with increased incidence in women (female:male ratio = 4–10:1) (Martocchia and Falaschi 2007). Regarding the mechanisms by which sex steroids affect susceptibility to autoimmunity, it is worth noting that the effects of sex hormones on the immune system may be directly or indirectly exerted through actions of the hypothalamic-pituitary-gonadal or hypothalamic-pituitary-adrenal axis. Sex hormones may directly affect immune mechanisms, including the homing of lymphocytes, the expression of adhesion molecules, the balance between Th1 and Th2 responses, the transcription and translation of cytokine genes, antigen presentation and costimulation (B7.1, B7.2, and CD40), and T cell receptor signalling (Whitacre 2001). A model of interactions between the initiating factors (susceptibility genes, environmental stimuli) and the modulating factors (sex hormones, neuroendocrine influences) has been proposed in the progression of autoimmune diseases. A marked increase in hyperthyroidism was reported during the early years of World War II. More recently, the association between life events and the onset of GD was examined (Mizokami et al. 2004). Circumstantial evidence supports the hypothesis that stress may influence the clinical expression of thyroid autoimmunity in susceptible individuals favouring the development of GD by shifting the Th1/Th2 balance away for Th1 and toward Th2. Conversely, recovery from stress or the immune suppressive effect of pregnancy may induce a Th2 to Th1 "return shift" leading to autoimmune (sporadic) or postpartum thyroiditis, respectively (Tsatsoulis 2006).

Role of 'autoantigens'. The orbital involvement in GD is characterized by lymphocytic infiltration and edema of retrobulbar tissues, resulting in marked swelling of extraocular muscles and orbital fat. Due to the increased volume of orbital contents the retrobulbar pressure rises, interfering with venous drainage (causing lid swelling) and pushing the globe forwards (causing proptosis or exophthalmos) (Krassas and

Heufelder 2001). In severe cases direct pressure on the optic nerve may result in visual loss. The swelling of eye muscles hampers muscle motility, associated with diplopia. The swelling of retrobulbar tissues is largely attributed to excessive secretion of GAGs by orbital fibroblasts (OFs). In vitro studies have shown that OFs are capable of producing GAGs in response to various cytokines. These cytokines are probably released by infiltrating T lymphocytes in the orbit. Accumulating data have led to widely accepted view that the OFs are the primary targets of the autoimmune attack. Regarding the nature of the autoantigen consensus has now been reached that the full length TSHR is expressed at the messenger RNA and protein level in orbital adipose/ connective specimens of GO patients but scarcely in that of controls. The TSHR is functional, as evident from an increase of cAMP in response to TSH. Overall, Krassas and Wiersinga (2005) support that the preponderant concept regarding the nature of the autoantigen is that a subpopulation of OFs may be the target cells in GO, enabling the preadipocytes, when stimulated, to differentiate into nature adipocytes expressing increased levels of TSHR. Recently, several other key antigens have been identified, namely flavoprotein (FP), G2s, and the calcium binding protein calsequesterin (CSQ). It turns out the CSQ is expressed 4.6 times more in extraocular muscle than in other skeletal muscle, which may partly explain the specificity of the skeletal muscle reactions in GD in the orbit (Gopinath et al. 2006). Nevertheless, the presence of alpha-fodrin, an intracellular organ specific cytoskeleton protein, recently associated with Sjogren's syndrome, and probably its overexpression on the surface of lacrimal and orbital target cells may explain the frequent involvement of the lacrimal gland in patients with GO (Kahaly et al. 2005). The fact that 22% only of the subjects with GO were positive for fodrin antibodies does not make alpha fodrin a key autoantigen in this disease. Determination of fodrin antibodies in Graves' patients may be predictive for development of Sjogren's syndrome in the future course of the disease.

In summary, GO is an autoimmune disease in which the nature of all the autoantigens involved in the autoimmune process has not yet been identified. Therefore, there are few effective options for the management of GO, a cell-mediated immune comorbidity of thyroid disease. Somatostatin analogues inhibit lymphocyte proliferation and activation and accumulate in the orbital tissue during the active ophthalmopathy. That is why such therapy is able to inactivate the ophthalmopathy without complications (Coloma-Gonzalez et al. 2007). The results using somatostatin analogues in the treatment of GO seem promising. Krassas (2003) had the opportunity to treat 3 paediatric patients with moderately severe thyroid eye disease. All had increased clinical activity scores (CAS) and were euthyroid on antithyroid drugs at the time of initiation of treatment for GO. They received 20 mg octreotide (Sandostatin LAR) i.m. one injection every 30 days for 4 months. Their ophthalmopathies improved substantially and CAS decreased in all the patients. The potential role of these substances may be due to suppression of proangiogenic molecules, a direct inhibition of proangiogenic signaling at the cell level, an antifibrotic action, thus reducing fibrovascular formations, and at least a partial correction of the systemic GH and IGF-1 dysregulation (Boehm and Lustig 2002).

Despite the importance to GD pathogenesis of antibodies generated against TSHR (Davies 1996), it has been very recently reported that antibodies from these patients can also activate IGF-1 receptor (IGF-1R), an interaction that induces expression of T cell chemoattractants (Pritchard et al. 2002, 2003; Gianoukakis et al. 2006) and hyaluronan (Smith and Hoa 2004). Thus, IGF-1R may represent a second 'autoantigen', in addition to TSHR, relevant to the pathogenesis of GD. The physical association between them, as is implied by our current findings, may play some as yet undetermined role in antibody generation. One possibility concerns potentially, exaggerated exposure of autoreactive T cells and B cells to increased numbers and diversity of antigens, resulting from epitope spread. This process can enhance the amplitude of immune reactivity and promote antigen specific tissue targeting by immunocompetent cells (Kent et al. 2005; Nakayama et al. 2005; Doyle and Mamula 2008). Antigenic presentation and recognition of IGF-1R and TSHR through innate and cognate responses could broaden and intensify those responses. Thus, immunity associated with GD may prove analogous to that occurring in allied diseases such as Type 1 diabetes mellitus and systemic lupus erythematosus, where a stepwise generation of new autoantigens results in multiple disease manifestations (Yu et al. 1996; Bonifacio et al. 1999; Skyler et al. 2005). Since fibroblasts from anatomic regions, not ordinarily manifesting the disease in patients with GD, also overexpress the receptor and engage in signalling that culminates in chemoattractant synthesis, how can we reconcile the localized manifestations found in the orbit? It could be that, while T cell recruitment in this disease might prove global, the very different consequences

of their presence in orbital tissues could result from the differential susceptibilities of orbital fibroblasts to cytokines. Factors such as the differential production of IL-1RA, alluded to previously in connective tissues outside and within the orbit, could account at least in part for the inflammation occurring selectively in that tissue. It is beyond doubt that identifying neo 'autoantigens' relevant to GD and determining whether epitope spreading plays a role in their generation will seek future therapeutic modalities in virtue of counteracting the underlying immune processes.

Animal models

Graves' hyperthyroidism occurs after the loss of tolerance to TSHR and the generation of thyroid stimulatory autoantibodies (TRAb) that mimic the action of TSH (Rapoport and McLachlan 2007). There are no spontaneous animal models of GD. However, in vivo expression of the TSHR cDNA induces TRAb and hyperthyroidism in susceptible mouse strains (Nagayama 2007). The autoimmune regulator (Aire) mediates central tolerance based on negative selection of autoreactive T cells in the thymus (Kappler et al. 1987) for many autoantigens in such a way that autoimmunity occurs spontaneously in Aire deficient humans and mice. Mice genetically engineered to be Aire deficient have decreased self protein levels in stromal medullary thymic epithelial cells (Anderson et al. 2002; Ramsey et al. 2002) and display characteristics of autoimmune polyendocrine syndrome type 1 patients including self reactive T cells and autoantibodies. Importantly, the spectrum of autoimmunity that develops depends on the genetic background of the Aire deficient mice (Jiang et al. 2005). Studies in mice deficient for the Aire and transgenic for the TSHR reveal a role for Aire in tolerance for thyroid autoantigens. On having examined this relationship by applying the TSHR α-subunit expressing adenovirus to Aire deficient mice and wild type mice, hyperthyroidism persisted in a higher proportion of Aire deficient than wild type mice (Misharin et al. 2009).

Alternative immunomodulatory therapeutic agents for GO

Advances in understanding the pathogenesis of autoimmune diseases is leading to development of new targeted therapies, with the resultant improvements in the management of the patients. Autoimmunity to the TSHR is the main cause of GD and components of this disorder are the extrathyroidal complications, including GO. Recently quoted, studies in GO (Pritchard et al. 2003; Tsui et al. 2008), implicate an additional target antigen in the orbital fibroblasts, the IGF-1 receptor which activation by autoantibodies may lead to the cascade of cytokine and chemokines secretion leading to orbital inflammation and swelling. Such immunomodulatory studies are well advanced in terms of clinical phase trials in a number of B cell autoimmune disorders. A variety of new targeted therapies are beginning to come to fruition, such as B cell depleting and modulating monoclonal antibodies, anti cytokine therapies and cellular therapies including regulatory T cells. As we wait for larger scale randomized studies, rituximab (RTX) should be considered experimental and reserved for patients with GO, who are not responding favourably to conventional therapy (Hegedus et al. 2011). This anti CD 20 monoclonal antibody induces transient B cell depletion that may potentially modify the active inflammatory phase of GO (Josseaume and Lorcy 2008). It is the first in what is likely to be a series of new and emerging treatments specifically targeting relevant components of the immune system. On the other hand, although originally implicated in adipocyte differentiation and glucose homeostasis (Santini et al. 2004), PPARγ have recently been shown to be involved in the modulation of inflammatory responses (Marx et al. 2000; Gosset et al. 2001; Schaefer et al. 2005). Recent results indicate that cytokines (IFN γ and TNFα) induced nuclear factor-kappaB activation and chemokine (C-X-C motif) ligand 10 release in GD and GO are modulated by pioglitazone (Antonelli et al. 2011). Further, a pilot study has been conducted on a small series of patients with GO strongly suggesting that an anti TNF factor, etanercept, suppresses the clinical signs in patients with active ophthalmopathy without noticeable short term side effects (Paridaens et al. 2005); yet, a randomized prospective study is needed to clarify if etanercept or other TNF antagonists prove sufficiently effective in reducing the inflammatory symptoms of GO, if they can be administered safely for a prolonged period, and if these side effects compare favourably to those of corticosteroids. To bring this discussion to a close, updated the efficacy of the new anti cytokine and anti lymphocyte treatment of the GO has been revived. We described the first positive results of anti B lymphocyte (anti CD20, RTX) and anti TNFα monoclonal antibodies (i.e. etanercept, infliximab) administration as new therapeutic options in the treatment of patients with active GO.

In summary, it has become apparent that multiple factors contribute to the etiology of GD, including host genetic as well as environmental factors. The same genes that confer susceptibility to GD, both thyroid specific and immunoregulatory, also influence GO although an increasing number of candidate genes with higher impact on orbitopathy are being identified. Smoking, a well known aggravating environmental factor in GO increases both the likelihood and severity of GO developing, in GD patients. The long held hypothesis, of a thyroid cross reactive antigen within the orbital tissues, has recently gained significant support by an animal model of GO and by in vitro and ex vivo studies. On the background of a permissive immunogenetic milieu circulating T cells, in patients with GD, directed against certain antigens on thyroid follicular cells, recognize antigenic epitopes that are shared by tissues contained in the orbital space. Of the cell types residing in these tissues, preadipocytes and fibroblasts, most likely act as target and effector cells of the orbital immune process, respectively. Studies in experimental animals indicate that GD is a slowly progressing disease that involves activation and recruitment of TSHR specific T and B cells and may be due to mimicry of a host antigen by a microorganism, but this remains speculative. This activation eventually results in the production of stimulatory antibodies that can cause hyperthyroidism. Extensive research has provided a clearer understanding of the roles of T and B cells, cytokines and chemokines, as well as of various ocular tissues including ocular muscles and fibroblasts in GO. Results reveal that fibroblasts can act as sentinel cells and initiate lymphocyte recruitment and tissue remodelling.

Conclusion

GD and GO have a multifactorial pathogenesis involving genetic factors and environmental triggers. The TSHR is currently the candidate autoantigen, but there remain uncertainties concerning the source of TSHR in the orbit and its biological activity. Also, the orbital fibroblast remains the most likely target cell and the demonstration of TSHR in the preadipocyte fibroblast population binds together the receptor and this cell type, but the non-preadipocyte fibroblast population could still express important antigens. Nevertheless, it is possible that local factors could somehow amplify an autoimmune response against one ubiquitous protein, such as the differential production of IL-1RA could account at least in part for the inflammation occurring selectively in orbital tissue. Elucidation of the "primary antigen" and how it is recognized by the immune system will be key issues as to the development of new immunomodulatory modalities in the treatment of GO.

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