New understanding of the role of cytokines in the pathogenesis of Graves' ophthalmopathy

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ABSTRACT. Cytokines play a key role in the development of Graves' ophthalmopathy (GO). These molecules are produced in the orbit of GO patients by infiltrating inflammatory cells as well as orbital fibroblasts. Locally produced cytokines stimulate fibroblast proliferation and their production of glycosaminoglycans, which result in accumulation of extracellular matrix and oedema with consequent proptosis. In addition to these direct effects, cytokines can modulate the immune reaction in GO by increasing major histocompatibility complex (MHC) class II, adhesion molecules, CD40, prostaglandin and heat shock protein expression in the orbit, thereby having a role in localising and augmenting the inflammatory response.

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INTRODUCTION

Cytokines play a key role in triggering and coordinating inflammatory and immune responses. They also have a wide array of effects on non-immunological cells, thereby directly affecting organ function.

It is widely accepted now that T cells secreting predominantly interferon-γ (IFNγ) are regarded as Th1 cells, whereas cells producing mainly IL-4 are classified as Th2. Th1 cells promote inflammation, cytotoxicity and delayed type hypersensitivity, while Th2 cells promote B cell differentiation and antibody formation (1). Th3 cells have been also described and produce mainly transforming growth factor-β_1 (TGFβ_1). Th3 cells have an important role in the protection and recovery from autoimmune diseases (2). Separation into distinct subsets can sometimes be difficult and cells producing both Th1 and Th2 cytokines do indeed exist and are termed Th0. Furthermore, established Th2 clones can transiently express IFNγ, which can add some confusion to the definition of Th1 and Th2 cells (1).

Priming towards a Th1 or Th2 response is dependent on a number of factors including the dose and the nature of the autoantigen, the type of antigen-presenting cell, the predominant cytokine at time of antigen presentation and undefined environmental and genetic factors (1). In general, IL-4, produced mainly by lymphocytes, is important in priming towards a Th2 response, whereas IL-12, a product of macrophages and dentritic cells, is the main cytokine responsible for priming towards a Th1 response.

In addition to T cells, many other cells in the immune system produce cytokines, including macrophages, monocytes, B cells and dendritic cells. Finally, cytokines can be produced by non-immune cells, including endothelial cells, keratinocytes and fibroblasts.

CYTOKINES IN ORBITAL TISSUE

The autoimmune process in Graves' ophthalmopathy (GO) involves mainly the extraocular muscles (EOM), although orbital fat and connective tissue are also affected. In the active stage, EOM are infiltrated by inflammatory cells, including macrophages, mast cells, T and B cells with the capabilities to produce cytokines. Cytokine-activated fibroblasts secrete glycosaminoglycans (GAG) into the orbital space, which cause water trapping resulting in oedema and muscle swelling, in turn leading to proptosis (3). Late in the disease, in the inactive stage, the inflammatory infiltrate is minimal and some degree of fibrosis can be detected.

PATHOGENIC MECHANISMS IN GO

Fibroblasts of the EOM appear to be important primary targets of the autoimmune response in GO,

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and therefore this material may yield the greatest insights into the involvement of cytokines in GO pathogenesis. It is less clear what drives the pathogenesis of the small group of patients who have apparently enlarged orbital tissues as a result of fat rather than muscle swelling.

The selective involvement of retrobulbar connective tissue in GO remains an enigma. Earlier studies have shown that IFNγ-induced human leukocyte antigen-DR (HLA-DR) expression is higher in retrobulbar and pretibial fibroblasts compared with abdominal fibroblasts (3), which may partially explain this selective inflammatory response. Furthermore, Graves' disease (GD) IgGs can induce adhesion molecule expression in retrobulbar fibroblasts from GO patients but not controls suggesting a possible mechanism for the orbit-directed inflammatory response in GO (3). Enhanced susceptibility to cytokine-mediated induction of GAG production and prostaglandin (PG) synthesis seems to be a feature of fibroblasts in orbital tissue, which may explain the localisation of the autoimmune process (4).

Humoral immunity

Using immunoblotting, antibodies against eye muscle (55, 64 and 95 kDa antigens) and fibroblast (23 and 66kDa antigens) membranes have been more frequently detected in patients with GO than controls (5). The 64 kDa antigen created major interest as it is shared between eye muscle and thyroid and it was subsequently shown that this protein represents a number of different molecules including D1 protein, calsequestrin and succinate dehydrogenase (6). Other candidate autoantigens in GO include acetylcholinesterase, actin, tubulin, acetyl choline receptor and G2s but none have been confirmed as the key autoantigen (5, 7).

The conventional thyroid antigens TG and TPO do not seem to have a role in GO, although there has been a recent revival in interest regarding TG. In contrast, it is widely believed now that the TSH receptor (TSH-R) represents an autoantigen in GO. Full length TSH-R transcripts have been detected in retrobulbar tissue, including adipose tissue and fibroblasts but not EOM (8, 9). Furthermore, TSH-R expression seems to be upregulated in active GO (9). Others have also detected TSH-R in orbital tissue lesions by immunostaining, confirming that TSH-R mRNA is translated into protein (8). The TSH-R molecule expressed in the orbit is functional, which is supported by increased cyclic adenosine monophosphate (cAMP) levels after recombinant human TSH (rhTSH) stimulation of retrobulbar adipocytes in culture (8). Moreover, immunising mice with TSH-R can induce changes in the orbit similar to those seen in GO (detailed below).

In summary, several lines of evidence strongly suggest that TSH-R is at least one autoantigen involved in GO pathogenesis.

Cellular immunity

Orbital-derived lymphocytes are mainly of the $CD4^+$ phenotype and can proliferate in response to thyroid and EOM extracts and membrane preparations. In addition, GO-derived lymphocytes have been shown to proliferate in response to TSH-R extracellular domain, further suggesting that TSH-R is an autoantigen involved in GO (3).

Analysis of T cell receptor (TCR) gene usage revealed a limited heterogeneity in recent onset GO. Comparison of TCR usage in orbital connective tissue and EOM from the same patient did not show major differences suggesting similar antigenic epitopes in both compartments. Moreover, similarities have been found in the intrathyroidal, pretibial and retrobulbar TCR repertoire usage, albeit in a limited number of patients, indicating recognition of similar antigenic determinants in the different sites of GD complications (3).

CYTOKINE PRODUCTION IN ORBITAL TISSUE AND ORBITAL-DERIVED LYMPHOCYTES

In vivo *cytokine production*

Studies of *in vivo* cytokine expression in GO tissue have been limited due mainly to the difficulties encountered in obtaining tissue samples, in addition to technical problems in analysis related to small sample size. Using immunohistochemistry, IFNγ, IL-1α and tumor necrosis factor (TNF)α have been detected in retroocular connective tissue from patients with GO but not normal controls (10). The more sensitive reverse transcriptase polymerase chain reaction (RT-PCR) has shown both IL-4 and IL-10 expression in 2 of 5 orbital fat specimens and in a single muscle biopsy obtained from GO patients, but failed to detect IFNγ in any of the samples analysed, indicating a Th2-like immune response (11).

Using the same technique, others have investigated a wide range of cytokines in 12 GO-derived EOM biopsies and 5 EOM biopsies taken from patients with no history of thyroid autoimmunity (12). In this study, the majority of GO samples expressed IL-4, IL-8 and TNFα, a minority IL-1α , IL-2 and IL-10, whereas IL-6 was detected in half of the samples. IL-2 expression was found in patients with more severe disease. IL-1β, IL-12, IL-13 and IFNγ were not found in any of the samples analysed. The control group showed expression of IL-6 and IL-8 only. An interesting observation was the detection of multiple cytokine mRNA with failure to detect TCR expression in two samples, indicating that some cytokines in the orbit can be

produced by cells other than lymphocytes *in vivo*. Also, variability was found in cytokine mRNA expression between different EOMs from the same patient. This most probably reflects the patchy inflammatory infiltration associated with GO, as a result of which biopsies might have been taken from an area which was not infiltrated with inflammatory cells. The detection of IL-4 mRNA in multiple samples together with a lack of IFNγ expression again suggested an ongoing humoral immune response in EOM of at least some patients with GO.

In contrast, Hiromatsu et al. (13) showed IFNγ expression in 13 of 14 EOM samples from GO patients, whereas IL-4 and IL-10 mRNA were expressed in one sample only. IL-6 was expressed in the majority and TNFα in 5 samples. However, results were different when retrobulbar adipose tissue was analysed. Eight of 29 samples expressed IFNγ, whereas IL-4 and IL-10 were expressed in 7 and 11 samples respectively. A correlation was detected between TNFα expression and EOM enlargement, whereas IL-6 correlated with retrobulbar fat volume. A negative correlation between fat volume and IL-4/IL-10 expression was also found. A more recent study compared cytokine gene expression in a group of 6 patients with active untreated GO (aGO) undergoing emergency surgery and a group of 11 patients with inactive disease (iGO) (9). IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12 and IL-18 were detected in almost all the samples with aGO, whereas IFNγ was detected in half and TNFα in one third of the samples. In the iGO group, IL-6, IL-8 and IL-18 were expressed in the majority of the samples, IL-10 in two thirds but IL-1β and IFNγ were found only in a minority. IL-4 was not detected in either group whereas IL-13 was detected in half the iGO group and only one aGO sample. This study suggests that patients with active GO have a Th1 cytokine profile in the orbit, whereas Th2 cytokines, including IL-10 and IL-13 but not IL-4, predominate in the inactive stage of the disease.

Taking the above studies together, the following conclusions can be made:

1) Generally, a mixed Th1 and Th2 response is found in retrobulbar tissue from GO patients. The type of immune response may be related to the stage of the disease, but this remains an area to be resolved;
2) A difference in cytokine profile is detected comparing EOM with retrobulbar fat in GO tissue. Furthermore, differences in cytokine profile are found in different EOM samples from the same patient. This can be simply explained by the small sample size harbouring only a limited number of inflammatory cells. This casts some doubt on the accuracy of some of the data, and may explain the contradictory results in different studies;
3) Cytokine mRNA can be detected in GO tissue negative for TCR gene expression indicating that cytokine production in GO *in vivo* is not restricted to lymphocytes;
4) IL-6 seems to have a role in GO as it is expressed in the majority of GO samples analysed and its expression correlates with orbital volume. On the other hand, it can also be detected in some normal EOM, but the above studies used RT-PCR, which is not quantitative and it is possible that IL-6 expression is higher in EOM samples from GO patients compared with controls;
5) TNFα expression is detected in around one half of the GO samples. The expression of this cytokine correlates with retrobulbar volume and seems to play an important role in active GO, probably by augmenting fibroblast proliferation and GAG production;
6) IL-2 mRNA is found in samples with active GO suggesting a central role for this cytokine in the inflammatory process. This is hardly surprising, as this cytokine is produced by activated lymphocytes, which are known to be present in active GO;
7) A negative correlation has been found between orbital content volume and IL-4/IL-10 mRNA expression in GO samples suggesting that these cytokines are associated with remission of the disease. However, animal studies suggest that Th2 cytokines are important for the development of GO as detailed below.

It should be stressed that the above studies used RT-PCR, which investigates mRNA rather than protein expression and it is a semi-quantitative method at best. Therefore, more work is needed to analyse cytokine protein production using quantitative techniques, which would help to shed a brighter light on the role of these molecules in GO.

In vitro *cytokine production*

Initial *in vitro* studies have shown a Th1-like cytokine profile in orbital lymphocytes from GO patients but further work has demonstrated that EOM-derived T cell lines produce both Th1 and Th2 cytokines (14). Pappa et al. (12) have shown IFNγ production in all EOM-derived T cell lines, IL-4 production in 60%, whereas IL-10 was produced by all lines. A mixed Th1/Th2 response was further confirmed in orbital-derived T cells from 6 patients with GO (15). A more recent study on T cells clones from 3 GO patients showed the predominance of a Th1 response, although some clones had a Th0 profile but none had pure Th2 features (16). More recently, Anisewski et al. (17) studied the characteristics of orbital-derived T cell clones from 6 GO patients. A Th1 profile predominated in T cell cultures from patients with recent onset GD (less than 2 yr). In contrast, T cell clones from patients with disease duration of more than 2 yr exhibited a Th2 cytokine profile. The fre-

quency of Th0 cells was similar in both groups. It was concluded that a Th1 response is important in the early stages of the disease and a Th2 response takes over later in the disease process. It is worth noting that a Th1 response was not seen in any patient with inactive disease, but a Th2 response was evident in patients with active GO.

It should be emphasised that culture conditions usually favour the expansion of Th1 cells and may therefore give misleading results by failing to represent the *in vivo* situation. This is shown in the study by Pappa et al. (12), which failed to detect IFNγ expression *in vivo* despite the production of this cytokine by orbital-derived lymphocytes in culture.

Cells other than lymphocytes have the capability of producing cytokines *in vitro*. Retrobulbar fibroblasts can produce IL-1, IL-6, IL-8, IL-16 and regulated on activation, normal T-expressed and secreted (RANTES) and this production can be modulated by cytokines, steroids and Graves' disease IgGs (18, 19). Of particular interest is the production of IL-16 and RANTES by fibroblasts. The former activates $CD4^+$ T cells, as well as monocytes and eosinophils, and can be found in GD thyroid tissue (20). RANTES is known to attract inflammatory cells and it has been implicated in the lymphocytic infiltration found in GD (21). These findings suggest an active role for fibroblasts in perpetuation, if not initiation, of the inflammatory response in the orbit. On the other hand, fibroblasts can produce IL-1RA in culture, which might have a role in dampening the immune response. The production of IL-1RA by fibroblasts can be enhanced by irradiation, offering one mechanism for the beneficial effects of radiotherapy in GO (22).

Taking the *in vitro* studies together, the following conclusions can be drawn:

1) A mixed Th1 and Th2 response is detected in orbital-derived lymphocytes *in vitro*. This may be related to the duration of the disease, with a Th1 response predominating in the early stages and a Th2 response emerging later in the disease process;
2) No clear association has been documented between the type of the immune response in orbital-derived lymphocytes *in vitro* and the clinical severity of GO;
3) Orbital fibroblasts can produce a number of cytokines in culture. This production can be modulated by cytokines, steroids, irradiation and GD IgGs, further emphasising the role of these cells in disease pathogenesis;
4) There are general discrepancies between *in vivo* and *in vitro* data, which is most probably due to culture conditions, where multiple agents are used to stimulate cells, thereby modifying their cytokine pattern. For example, the presence of IL-4 in culture is important for the development of a Th2 response. The majority of culture studies have grown T cells without the addition of IL-4, thereby artificially biasing the cells towards a Th1 response. This potential problem is clearly demonstrated in the study by Aniszewski et al. (17), who have shown a different cytokine pattern in T cells expanded with or without the addition of IL-4, although the difference did not reach statistical significance.

One caveat of the above *in vitro* studies is related to the fact that T cells were grown in culture in the absence of a specific antigen. Therefore, once the orbital autoantigens in GO are firmly established, it would be interesting to study the cytokine profile in orbital-derived T cells grown in the presence of these specific antigens.

IMMUNOLOGICAL AND FUNCTIONAL EFFECTS OF CYTOKINES IN ORBITAL TISSUE

Retrobulbar fibroblast proliferation is stimulated by cytokines, including IL-1α, IL-4 and TGFβ, but not IL-2 or IL-6 (14). The function of these cells is also modulated by cytokines: IFNγ, TNF, TGFβ, IL-1 and leukoregulin all induce GAG production, IFNα and IL-6 have no effect, whereas IL-4 and IL-1RA both inhibit GAG synthesis (14, 22-24). Fibroblast stimulation by these cytokines is further enhanced by hypoxia, which may explain the adverse effects of smoking on thyroid eye disease (14). On the other hand, pentoxifylline inhibits both basal and cytokine-stimulated GAG production, offering a potential new therapeutic approach (25). One mechanism for increased GAG synthesis is the upregulation of hyaluronan synthase by cytokines, and this upregulation can be inhibited by dexamethasone in culture (26). Also, IL-1β has been shown to increase oxygen free radical production in retrobulbar fibroblasts, which correlates with GAG production in these cells. This suggests that oxygen free radicals are directly involved in GAG synthesis (27). The production of hyaluronic acid by fibroblasts involves protein kinase activation, and seems to be calcium-dependent, at least when cells are stimulated by IL-1 (28). Cytokines also stimulate fibroblasts to produce metalloproteinase inhibitors (14) suggesting that excessive accumulation of extracellular matrix in orbital tissue in GO is not only due to increased production but also to impaired degradation.

In addition to these direct effects, cytokines may affect the inflammatory process through augmenting adhesion molecule, CD40, PG synthase, major histocompatibility complex (MHC) class II, heat shock protein (HSP) and TSH-R expression in the retrobulbar tissue. ICAM-1 is overexpressed in disease-affected tissue and its expression in retrobulbar fibroblasts and vascular endothelial cells is stimulated by IL-1α, TNFα and IFNγ *in vitro* (14). Furthermore, these cytokines induce *de novo* expression of endothelial leukocyte adhesion molecule-1 (ELAM-1) and vas-

cular cell adhesion molecule-1 (VCAM-1) in vascular endothelial cells from both GO and normal control patients (14). Increased adhesion molecule expression modulated by cytokines could be a mechanism responsible for orbit-specific lymphocyte recruitment in GO.

CD40 is an important co-stimulatory molecule for both B and T cells. Orbital fibroblasts have been shown to express CD40 and this expression is upregulated by IFNγ treatment. Furthermore, interaction between CD40 on fibroblasts and CD40 ligand on inflammatory cells, results in activation of fibroblasts and cytokine production, thereby augmenting the inflammatory response (29).

PGE_2 is a known modulator of the immune response and can play a role in the development of a Th2 response. PG-endoperoxidase-H-synthase-2 (PGHS-2) is upregulated by leukoregulin and IL-1 in fibroblasts *in vitro* (30). This induction is much higher in orbital fibroblasts compared with skin fibroblasts, which may partly explain the localisation of the disease to the orbit (4).

MHC class II expression in cultured fibroblasts, from both GO and normal control tissue, increases with IFNγ treatment, though this expression is significantly greater in patients with GO. Concomitant treatment with TNFα further enhances class II expression, whereas addition of TNFβ, EGF, IL-6 or pentoxifylline attenuates IFNγ-induced class II expression (3). Interestingly, enhanced class II expression is more prominent in retrobulbar fibroblasts compared with abdominal fibroblasts taken from the same patient. This may explain the selective involvement of the retrobulbar connective tissue in GO (3).

HSPs have immunomodulatory properties and play a role in cell proliferation and protection from stressful stimuli. HSPs are detected, both *in vitro* and *in vivo*, in fibroblasts from GO but not normal orbital tissue, suggesting a role for these molecules in disease pathogenesis (3). Culture studies have shown that IFNγ and TNFα enhance HSP expression in GO-derived fibroblasts but not fibroblasts from normal controls, whereas IL-1, IL-6 and TGFβ increase HSP expression in fibroblasts from both normal and GO tissue (3).

IL-6 increases TSH-R expression in preadipocyte fibroblasts from patients with GO but not controls (31). IL-1 was also studied but failed to show similar effects (8). Studies on IL-4 have been confusing, as one study showed no effect on TSH-R expression (31), whereas others demonstrated increased cAMP production and TSH-R expression in cultured retrobulbar fibroblasts stimulated with IL-4 (8). In contrast, IFNγ, TNFα and TGFβ all inhibit TSH-R expression in cultured orbital-derived preadipocyte fibroblasts (32). The above studies suggest a direct role for IL-6, and possibly IL-4, in the initiation of GO by augmenting the expression of a putative autoantigen in the orbit. The importance of IL-6 in GO pathogenesis is further strengthened by demonstrating high serum levels of this cytokine in patients with active disease (33).

The immunological and functional effects of cytokines in orbital tissue is summarised in Table 1. The potential role of cytokines in GO is shown in Figure 1.

PERIPHERAL CYTOKINE LEVELS IN GO AND THE EFFECTS OF EXOGENOUS CYTOKINES

IL-6 concentration in peripheral blood is significantly increased in patients with GO compared with controls and levels of this cytokine correlate with the clinical activity of the disease (33), although it remains difficult to disentangle a uniquely orbital response in these patients who have autoimmune thyroid disease. Others found that IL-1β, TNFα, sIL-2R, IL-6, sIL-6R and IL-10 levels are elevated in GO patients compared with controls. However, these levels did not correlate with severity, activity, duration of the disease or smoking but were able to predict a beneficial response to therapy (34-36). A positive correlation between IL-1RA levels and a favourable therapeutic outcome has been documented (37). Furthermore, a negative cor-

Table 1 - *Immunological and functional effects of cytokines in the orbit in Graves' ophthalmopathy.*

	IL-1	IL-4	IL-6	IFNγ	TNFα	TGFβ
GAG synthesis	↑	→	→	↑	→	↑
Cell proliferation	↑	↑	→	→	→	↑
Class II expression	↑	NS	↓	↑	↑	NS
Adhesion molecules expression	↑	NS	NS	↑	↑	NS
HSP expression	↑	NS	↑	↑	↑	↑
Metalloproteinase inhibitors production	↑	NS	↑↓	↑	↑	↑
TSH-R expression	→	↑, →	↑	↓	↓	↓
CD40 expression	↑	NS	NS	↑	NS	NS
PGE_2	↑	↑	NS	NS	NS	NS

GAG: glycosaminoglycans; HSP: heat shock protein; PGE_2: prostaglandin E_2; ↑: increase; ↓ decrease; →: no effect; NS: not studied

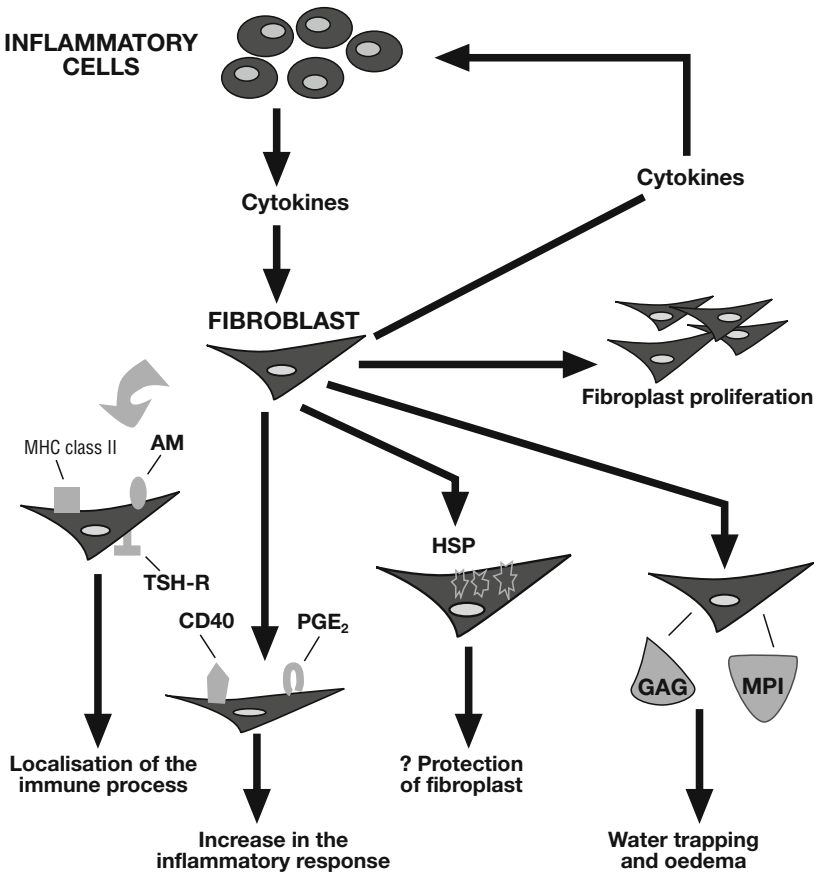

Fig. 1 - *The potential role of cytokines in the pathogenesis of Graves' opthalmopathy. Cytokine, produced by inflammatory cells, stimulate the fibroblasts to proliferate and produce glycosaminoglycans (GAG) and metalloproteinase inhibitors (MPI) resulting in water trapping and oedema. Cytokines stimulate major histocompatibility complex (MHC) class II, adhesion molecules (AM) and TSH receptor (TSH-R) expression on fibroblasts, which helps localisation of the immune process. Cytokines also increase CD40 expression, prostaglandin E_2 (PGE_2) synthesis and cytokine production by fibroblasts, thereby augmenting the inflammatory reaction. Finally, cytokines upregulate heat shock protein (HSP) expression in fibroblasts, which may protect these cells from stressful stimuli.*

relation between IL-1RA and smoking was also found. Therefore, IL-1RA administration, or at least strategies to increase its level, may offer a novel therapeutic approach in the future. An increase in IL-4 and IL-10 levels has been shown after steroid therapy suggesting that these cytokines may have a role in disease remission (34). Also, they may serve as a marker for predicting response to a particular therapy.

Several reports have documented that cytokine administration to humans can affect thyroid function, causing hypo-or hyperthyroidism but GO was not a feature (38). However, severe worsening of GO in a patient receiving IFNα treatment for hepatitis C has been recently reported (39). This suggests that cytokines are directly involved in augmenting the inflammatory reaction and highlights the importance of regular check ups in GO patients receiving cytokine treatment.

CYTOKINE GENE POLYMOPRPHISM

Mapping of susceptibility genes in medical conditions is usually performed using association or linkage analysis. Association studies investigate genes that increase the risk for the development of a particular disease. The frequency of a suspected allele in the disease group is compared with an ethnically similar disease-free group. Although very sensitive, this approach may detect genes that are not directly involved in disease pathogenesis. On the other hand, linkage analysis, albeit less sensitive, detects disease-related genes but it requires large family studies, which are generally difficult to undertake. Genetic variations in genes of the cytokine network, either functional or regulatory, have been investigated as potential risk factors for GO.

An association of GO with a microsatellite dinucleotide repeat polymorphism in the first intron of the IFNγ gene has been documented in Caucasians (40). The frequency of allele 3 of the IFNγ gene in GO was 17.9% compared with 4.3% for patients with GD without GO, whereas the frequency in the control group was 4.2%.

A strong association of GO with a polymorphism of the 5'flanking region of the TNFα gene at positions -1031 and -863 was shown in Japanese patients. Allele frequency of -1031C and -863A was 31.5% and 23.4% respectively in GO patients compared with 13.5% and

11.7% respectively in GD patients with no ophthalmopathy. Furthermore, the strength of -1031C association increased with increasing disease severity (41).
Although a decreased frequency of a polymorphism in the IL-4 promoter region was associated with GD, no association was found with GO (42). Finally, the frequency of a substituition polymorphism in the IL-13 promoter gene at -1055 was significantly decreased in patients with GO (43) compared with GD patients without GO or with normal controls (17.7%, 27.7% and 36.7% respectively).
Cytokine gene polymorphisms represent an interesting group of potential susceptibility markers in GO. The reports described above were based on case control analyses, which can be misleading when disease heterogeneity is present and the number of samples studied is small. The development of automated genotyping, concentration on linkage rather than association studies and more comprehensive association studies should clarify the role of the various cytokine polymorphisms in determining susceptibility to GO.

ANIMAL MODELS OF GD

Several lines of indirect evidence suggest that TSH-R is an autoantigen in GO, as detailed above. Early experiments investigating the role of TSH-R as an autoantigen relied on immunising mice with fibroblasts transfected with full length TSH-R. Some of the animals developed thyroid dysfunction with TSAb or TBII but none had evidence of GO (44).
Some success in modelling GO was achieved by immunising mice with syngeneic TSH-R primed T cells. Two strains of recipients were used: BALB/c and non obese diabetic (NOD). TSH-R antibodies were induced in both strains using this technique and thyroid examination revealed a Th1 profile in NOD and a Th2 profile in BALB/c mice. The orbital tissue of NOD mice exhibited normal histology, whereas 70% of BALB/c orbits showed infiltration with inflammatory cells, accumulation of adipose tissue and destruction of muscle fibres (45). It was concluded that a Th2 response is at least associated with the development of GO. Similar results were obtained by genetic immunisation with TSH-R cDNA in Naval Medical Research Institute (NMRI) outbred mice (46).
These studies clearly highlight the potential role of TSH-R as an orbital autoantigen in GO, and they also emphasise the importance of Th2 response in the development of the disease.

SUMMARY

Cytokines play an important role in GO. T cells are recruited to retrobulbar tissue in GO patients, probably recognising antigens that are shared with thyroid tissue and one of these antigens may well be TSH-R. These antigen-specific T cells, as well as orbital fibroblasts, are activated producing cytokines which result in perpetuation of the inflammatory process through a number of mechanisms. Cytokines result in an increase in HLA class II, HSP, CD40, PG, adhesion molecule and TSH-R expression locally, thereby augmenting the inflammatory response. In addition to these indirect effects, cytokines can directly modulate cellular function in the orbit as they increase fibroblasts proliferation and enhance their production of GAG, which results in accumulation of the extracellular matrix, swelling and oedema with consequent proptosis. Studies indicate that orbital fibroblasts, particularly those present within patients with GO, may be especially sensitive to the effects of cytokines, accounting for the frequent, and relatively selective, involvement of the orbit in GD.
Given the role of cytokines in GO and the fact that treatment of the disease is currently far from ideal, cytokine antagonists may offer a new therapeutic option in the future. Pentoxifylline, which inhibits cytokine action *in vitro*, has been administered to patients with GO with beneficial effects but the lack of a control group made interpretation of the findings difficult (47). An alternative approach by targeting specific cytokines may prove effective. For example, IL-1RA has been shown to inhibit fibroblasts GAG and PG production *in vitro*. Clinical trials in other disorders have shown that systemic administration of IL-1RA does not carry significant toxicity (48), and therefore, future studies are warranted to look at the effect of IL-1RA treatment *in vivo* for patients with active GO.

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