Inducing Graves’ ophthalmopathy

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ABSTRACT. The majority of patients with Graves’ disease (GD) have some degree of ocular involvement and this requires surgical or medical intervention in about 5% of cases. There are autoimmune and inflammatory processes operating in Graves’ ophthalmopathy (GO), which together induce glycosaminoglycan production, edema and adipogenesis resulting in an increase in the volume of the orbital contents. GO is a heterogeneous disorder, i.e.: 1) whilst usually associated with hyperthyroidism it may occur in euthyroid (and even hypothyroid) patients; 2) expansion of orbital tissues may be due to ‘big-fat’ or ‘big muscles’. The heterogeneity is further exemplified by the spectrum of protocols which have succeeded in inducing aspects of the disease both in animal models and in humans including: 1) Production of severe hypothyroidism in guinea pigs by thyroidectomy and administration of pituitary extract (TSH); 2) Induction of T cells autoreactive to the thyrotropin receptor (TSHR) in mice; 3) Depletion of regulatory T cells in humans susceptible to autoimmunity; 4) Modulation of adipose tissue metabolism in mice and men. In addition, identical induction protocols result in different pathological features depending on the environment, e.g. TSHR primed T cells produce thyroiditis and ocular pathology in BALBc mice in Brussels but thyroid stimulating antibodies accompanied by elevated thyroxine in these animals (from the same supplier) in Cardiff. Thus, experiences in the induction of GO have confirmed the polygenic, multifactorial nature of the disorder and highlight the importance of careful disease classification to promote further progress in understanding.

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INTRODUCTION
The majority of patients with Graves’ disease (GD) have some degree of ocular involvement which is usually self-limiting. However 3 to 5% of patients develop Graves’ ophthalmopathy (GO) requiring medical or surgical intervention. Both the thyroid gland in GD and the orbital tissues in GO are infiltrated by lymphocytes, indicating an autoimmune pathogenesis (1).

The main clinical features of GO are proptosis, conjunctival injection, chemosis, ocular muscle restriction and, in extreme cases, corneal ulceration and loss of sight due to compression of the optic nerve. These signs can be explained by the increase in volume of the orbital contents by three mechanisms: 1) edema; 2) production of hydrophilic glycosaminoglycans (GAG) and 3) hypertrophy of the adipose tissue by adipogenesis (2). The first two induce expansion of the extra-ocular muscles (EOM) which are greatly enlarged in GO and have been the focus of investigations to identify the autoantigen in GO and the link with the thyroid gland.

GD is caused by thyroid stimulating antibodies (TSAB) which mimic the action of TSH (3). Since both the growth and function of the thyroid are controlled by TSH (4), TSAB lead to hyperthyroidism and diffuse goitre. The target of the autoimmune response in GD is the thyrotropin receptor (TSHR) (3). There are several lines of evidence suggesting that it may also have a role in GO. Patients with GO tend to have the highest tHs of TSAB (5). TSHR transcripts have been demonstrated, using a variety of techniques, in the orbital adipose compartment (6, 7). Immunocytochemistry, with a spectrum of antibodies, suggests that the transcripts are translated into TSHR protein. Furthermore, depending on the end point measured, the receptors are functional, with high concentrations of TSH required to increase cAMP (8) although in some depots physiological levels were sufficient to stimulate leptin release (9).

The central role of TSAB in GD is exemplified by the transient hyperthyroidism in new borns of mothers with GD, due to their placental transfer (1). However,
very little progress has been made in understanding the mechanisms responsible for breaking immune tolerance and which lead to the production of TSAB. There are no reports of neonates with GO and the proptosis occasionally observed in children harbouring gain of function TSHR mutations resolves once euthyroidism is restored (10). Thus there are no spontaneous models of GO although aspects of the disease can be induced. For a model to mimic GO closely we would expect some or all of the following features: 1) Elevated circulating thyroxine and/or suppressed TSH; 2) Antibodies to the TSHR, at least thyrotropin binding inhibiting immunoglobulins (TBII) and preferably TSAB; 3) Changes in thyroid architecture and size; 4) Lymphocytic non-destructive thyroiditis; 5) Clinical signs of hyperthyroidism such as weight loss; 6) Female animals more susceptible than male and 7) Orbital changes similar to those seen in GO including disordered structure of extra-ocular muscles, edema, infiltration by immune cells and fat accumulation.

MODELS OF GO

From early in the twentieth century attempts were made to develop animal models that recapitulated the signs and symptoms of GO. The first work, in which exophthalmos was convincingly due to an increase in the volume of the orbital contents, rather than to a nervous mechanism, is probably that of Smelser in 1936 (11), who administered pituitary extract to guinea pigs. All animals lost weight, had signs of thyroid hypertrophy and some had slight exophthalmos. When he repeated the experiment, but with the addition of thyroidectomy, the majority developed extreme exophthalmos and a 40% increase in the weight of the orbital contents was observed, when compared with non-injected thyroidectomized controls, predominantly in the orbital fat and lacrimal gland. The orbital tissues were examined histologically and found to be edematous and infiltrated by lymphocytes and eosin stainable mucopolysaccharide. Whilst in Brussels, Sabine Costagliola, Marie-Christine Many and myself achieved some success in modelling GD and GO by transferring TSHR primed T cells to naive syngeneic recipients. We used unfractionated T cells and a CD4+ enriched population with the in vivo TSHR priming step performed using the extra-cellular domain of the receptor produced as a maltose binding protein fusion (ECM-MBP) in bacteria or genetic immunization. In both cases in vivo priming was followed by an in vitro priming period using ECM-MBP. In BALBc and non-obese diabetic (NOD) recipients of syngeneic receptor primed T cells, both strains of mice displayed thyroiditis but with very different histological features (12). In the BALBc mice, B cells and immunoreactivity for interleukin (IL)-4 and IL-10 were found but in the NOD mice there were very few B cells and immunoreactivity for interferon (IFN)-γ, indicating the Th2 and Th1 nature of the induced disease respectively. In later experiments the mouse orbits were also examined (13). All of the NOD recipients of primed and non-primed cells, displayed normal histology with intact well-organised muscle fibre architecture. BALBc orbits of primed (but not non-primed) T cells appeared strikingly different. The muscle fibres were disorganised and separated by periodic acid Schiff positive edema. There was accumulation of adipose tissue and infiltration by immune cells, especially mast cells. These changes were observed in almost 70% of the BALBc recipients of receptor primed cells and did not correlate with thyrotropin binding inhibiting immunoglobulin (TBII) or thyroxine (T₄) levels. However, orbital changes were observed only in mice having the most severe thyroiditis with 25–30% of the gland occupied by interstitium which also correlated with the most skewed Th2 response, B: T cell ratio 1.6–1.9 and IL-4: IFN-γ ratio >2.5. Similar results were obtained by genetic immunization of Naval Medical Research Institute (NMRI) outbred mice (14), 9/30 males displayed signs of hypothyroidism with reduced T4, and 5/29 females developed stable hyperthyroidism with circulating TSAB accompanied by increased thyroxine but undetectable TSH. In addition Th2 thyroiditis and orbital changes, including infiltration by mast cells and macrophages, were induced. Analysis of the major histocompatibility complex (MHC) haplotype of the mice revealed that they were predominantly H2q, irrespective of whether disease had been induced or not.

The BALBc transfer model, with its higher incidence, seemed sufficiently promising to be of use in dissecting the mechanisms operating in GO. However it first required further characterisation including establishing whether or not it follows a Rundle’s curve. Our attempts to establish the model in Cardiff produced very different results. In Brussels, the BALBc mice rarely developed TSAB or hyperthyroidism, irrespective of priming with fusion protein or cDNA. In contrast, in some experimental groups, we induced TSAB and elevated T₄ levels in more than 50% of the mice with both protocols but no thyroiditis. We have repeated the experiments with BALBc mice from the same supplier as well as using water, chow and bedding imported from Brussels. None of these in vivo primed animals or syngeneic recipients of their splenocytes developed thyroiditis or orbital pathology. The experiments in Brussels and Cardiff have been performed in units with standard housing and not specific pathogen free conditions. Therefore we