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Antithyroglobulin monoclonal and autoantibodies cross-react with an orbital connective tissue membrane antigen: a possible mechanism for the association of ophthalmopathy with autoimmune thyroid disorders

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SUMMARY

The possibility that Graves' ophthalmopathy and autoimmune thyroid disorders may be associated because of autoimmune reactions against antigens shared between human orbital and thyroid tissues was investigated using anti-thyroglobulin (Tg) monoclonal and autoantibodies. Eleven of 16 mouse monoclonal antibodies (MCAB) tested reacted, in an enzyme-linked immunosorbent assay (ELISA), with an antigen in human orbital connective tissue membranes (OCTmem), but not with the OCT soluble fraction, or with membrane or soluble fractions of human eye muscle, lacrimal gland or skin connective tissue. The anti-OCTmem activity was absorbed by OCTmem and Tg, but not by liver membranes or bovine serum albumin (BSA). In preliminary studies four out of 113 human MCAB against thyroid or orbital tissue antigens showed reactivity restricted to Tg and OCTmem. Sera from approximately 50% of patients with autoimmune thyroid disorders, with or without ophthalmopathy, also reacted with OCTmem. The autoantibody activity correlated closely with serum titres of antithyroglobulin but not with the presence, duration, or severity of the eye disease. The OCTmem reactivity was absorbed by Tg, thyroid membranes, and OCTmem but not liver membranes, membranes prepared from other orbital tissues, or BSA. The OCTmem-Tg shared antigen site appeared not to be native thyroglobulin since, (i) MCAB and serum autoantibodies did not react with the cytosol fraction of OCT, and (ii) because the membrane antigen was not solubilizable. Because not all patients with ophthalmopathy have detectable anti-Tg antibodies and, conversely, because not all patients with detectable anti-Tg antibodies develop opthalmopathy it is unlikely that autoimmunity against a OCTmem-Tg shared antigen is the primary mechanism of Graves' ophthalmopathy, although this possibility has not been excluded. On the other hand the reaction of anti-Tg autoantibodies with OCT membranes may be a model for other autoimmune reactions against other thyroid-orbital tissue-shared antigens. While the pathogenesis of Graves' ophthalmopathy is likely to be multifactorial, humoral and cellular reactions against primary orbital antigens, thyroid-orbitol tissue shared antigens, or both, are likely to play important roles.

Keywords monoclonal antibodies thyroglobulin antithyroglobulin Graves' ophthalmopathy Graves' disease

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INTRODUCTION

While it is generally accepted that Graves' ophthalmopathy (GO) is immunologically mediated (Doniach & Florin-Christen, 1975; Volpé. 1977; Wall *et al.*, 1981; Gorman 1983; Wall, 1984) the mechanism for its close association with Graves' hyperthyroidism and, to a lesser degree, Hashimoto's thyroiditis, is unclear. Possible mechanisms for the association of ophthalmopathy with autoimmune thyroid disorders include, (i) an effect of hyperthyroidism or hypothyroidism on the immune system of predisposed patients precipitating a second autoimmune disorder, for which there is considerable evidence (Balazcs *et al.*, 1980; Easton & Wall, 1981; Wall, Twohig & Chartier, 1981). (ii) a putative metabolic effect of thyroid hormones or TSH on orbital tissue cells such as endothelial cells, adipocytes, fibroblasts or extraocular muscle cells (Sisson, Kothary & Kirchick, 1973), (iii) an enhanced association of organ-specific diseases in patients with a common genetic background or (iv) autoimmunity against thyroid-orbital tissue shared antigens (Goerman, 1983).

Although the recent findings of anti-eye muscle antibodies in the serum of patients with Graves' ophthalmopathy (Kodama *et al.*, 1982; Atkinson *et al.*, 1984; Kendall-Taylor, Atkinson & Holocombe, 1984) suggests that the eye disease is a separate autoimmune disorder associated with specific antigens it is possible that the antibody activity represents primary thyroid reactive antibodies cross-reacting with shared antigens in orbital tissue. The possibility of cross-reactive autoantibodies being one mechanism for the association of organ-specific autoimmune disorders was raised as a result of recent findings from several laboratories (Haspel *et al.*, 1983a,b; Satoh *et al.*, 1983) that monoclonal antibodies (MCAB) against purified human proteins often cross-reacted with other human-derived proteins and polypeptides. Certainly, the high prevalence of ophthalmopathy in patients with past or present Graves' hyperthyroidism would be well explained by a common factor such as an orbital tissue-thyroid shared antigen(s). We have investigated this possibility using, as probes, mouse and, more recently, human MCAB produced against human thyroglobulin (Tg), thyroid membrane antigens, and orbital tissue antigens, many of which were subsequently shown to cross-react with shared antigens in orbital, or thyroid tissues, respectively. Of particular interest was the finding of an antigenic site shared between human thyroglobulin and human orbital connective tissue membrane against which both anti-Tg MCAB and autoantibodies from patients with thyroid disease reacted.

MATERIALS AND METHODS

Clinical Subjects. Sera from, (i) 23 patients with active Graves' ophthalmopathy (classes 3–6 American Thyroid Association classification (Werner, 1969; Werner, 1977), one male and 22 females aged 20–73 years (mean age 43 years) 20 of whom had associated hyperthyroidism, presently (four cases) or in the past (16 cases), three of whom had associated Hashimoto's thyroiditis (diagnosed from the characteristic goitre, significant titres ($\geqslant 1/250$) of thyroid antibodies and aspiration needle biopsy evidence of lymphocytic infiltration and Hürthle cells, (ii) eight patients with Graves' hyperthyroidism without eye disease, one male and seven females aged 21–60 years (mean age 35 years), and (iii) seven patients with Hashimoto's thyroiditis without eye disease, one male and six females aged 22–67 years (mean age 49 years), were studied for antibodies against Tg and orbital connective tissue membrane antigens. Sera from 10 normal subjects, four males and six females aged 21–55 years (mean age 35 years), were studied as controls.

Antigen Preparation. Normal human orbital tissue (eye muscle, orbital connective tissue (OCT) and lacrimal gland), skin and liver were obtained at autopsy less than 4 h after death. Normal thyroid tissue was obtained at operation from patients undergoing surgery for removal of single (non-malignant) nodules. Tissues were rinsed, cleansed of connective tissue, finely minced using scissors and homogenized in a mechanical blender. For the preparation of soluble and membrane fractions homogenates were first centrifuged at 400 ***g*** for 15 min to separate whole cells, debris and nuclei, and the supernatant centrifuged at 100,000 ***g*** for 30 min. The final supernatant ('cytosol') was retained and the pelled ('membane') washed twice, in phosphate buffered saline. Cytosol and membrane fractions of human thyroid, liver and orbital tissues were prepared in the same way,

aliquoted, and stored at −70°C. In the case of OCT membranes, several different preparations were tested for reactivity with anti-Tg MCAB. Thyroglobulin was prepared from the soluble (cytosol) fraction of normal human thyroid tissue following the method of Van Herle *et al.* (1973) in which soluble proteins are precipitated by ammonium sulphate and the washed pelled chromatographed on Sephadex G-100. The first peak, containing thyroglobulin, is collected, concentrated on Sephadex G-75, and rechromatographed on Sepharose CNBr. The second peak (Tg) is collected and stored at −70°C.

Monoclonal Antibody Production. Mouse monoclonal antibodies against thyroglobulin, human thyroid membrane antigens, and human orbital tissue antigens were produced as described previously (Kodama *et al.*, 1982). Briefly, mice were immunized weekly for up to 12 weeks with Tg, thyroid membranes, or cytosol and membrane fractions of OCT and eye muscle, being given a final booster 3 days before fusion which was carried out when serum antibody levels had reached peak levels. Spleen cells (10^8), were fused with SP-20 mouse myeloma cells (10^7) in 30% polyethylene glycol (PEG) according to the classical method of Köhler & Milstein (1975). All mouse hybridomas were repeatedly subcloned, by limiting dilution, at from 3 to 10 cells/well to establish true monoclonality, and maintained in culture for 6 months prior to use to establish stability of cell growth morphology and antibody production. Seven MCAB-Tg (A1, A3, A7, A18, A25, A53 and A71), whose characteristics were described in detail previously (Kodama *et al.*, 1982), were used for most of the present studies. In addition, we have tested a further nine mouse MCAB-Tg provided by one of us (JR) whose nature, binding affinities, and immunological characteristics have been detailed in a recent publication (Ruf *et al.*, 1983). All 16 monoclonal antibodies gave single precipitation lines when incubated with human thyroglobulin in Ouchterlony immunodiffusion tests. All reacted strongly in an ELISA, by immunofluorescence, and in radioimmunoassays, with all forms of human Tg tested.

Human MCAB were produced by fusion of peripheral blood lymphocytes (PBL) from patients with ophthalmopathy, with or without associated autoimmune thyroid disorders, with a human myeloma cells line (GM 4572B NIG MS, Camden, New Jersey, USA), using a similar procedure the only modification being that, in order to non-specifically enhance antibody-producing B cells, the PBL were infected with Epstein Barr virus (EBV) prior to fusion. One EBV infected cell line expanded in culture for 12 weeks has been subcloned, twice, by limiting dilution (10 cells/well) and 30 subclones (G1–30) maintained in Costar plates, and then small flasks, for up to 10 weeks. Supernatants from more than 30 other EBV-transformed cell lines were also tested, from 3 to 4 weeks after infection, for antibodies against thyroid and orbital tissue antigens. These cell lines have not yet been cloned. Recently hybridization have been carried out between 25 of these EBV-transformed cells lines and the human myeloma cells at a ratio of one lymphocytes to one GM 4572B cell. Clones from the first seven of these have been tested for antibody production and are presently being expanded prior to cloning by limiting dilution, and further characterization. EVB-infected cell lines and mouse and human clones were screened for specific antibody production using an ELISA (described below). All thyroid or orbital antibody-producing cell lines and hybridomas were further characterized, and studied for orbital tissue-thyroid cross-reactivity.

Enzyme-linked Immunosorbent Assays. Immunoreactivity of mouse and human MCAB, human EBV-infected cell lines, and serum autoantibodies (aAB) with Tg, thyroid membranes, and orbital antigens was determined using an ELISA. Standard tests were used. Briefly, antigen was absorbed, at 4°C overnight, to the surface of microtitre plates previously treated, in the case of membranes, with glutaraldehyde (0·1%). After washing, uncoupled antigen reactive sites were blocked by incubation with 3% bovine serum albumin (BSA). Antigen was then incubated with MCAB or serum for 2 h at 37C. After washing second antibody (anti-mouse or anti-human IgG-conjugated to alkaline phosphatase, Boehringer Manheim Biochemicals, No. 60527, West Germany) was added and the plates incubated for a further 2 h at room temperature. Finally, substrate (dinitrophenol) was added and the reaction arrested after 30 min, for soluble antigens, and after 20 min, for membrane antigens. Antigen concentration was, generally, 50 μg/ml. In some experiments dose response studies were performed with antigen concentrations ranging from 0·1 to 100 μg/ml.

MCAB and serum dilution was 1/100 which was found, in preliminary experiments, to be optimal for reaction with Tg and orbital connective tissue membrane antigens. Tests, which were set

up in triplicate were read at 420 nm in a micro ELISA reader and results expressed as optical density (o.d.). A positive tests was determined as an o.d. of more than twice background (tests with complete medium for MCAB and PBS for sera). This was 0·30 for MCAB and 0·25 for serum antibodies.

Other Methods. Protein concentrations were determined by the Biorad method using human gamma globulin as a standard. Serum T4 and T3 were measured using standard radioimmunoassays. Tg and microsomal antibodies were also measured using standard commercial haemagglutination test kits (thymune 'M', thymune 'T', Burroughs Wellcome Ltd, Beckenham, UK).

Statistical Analysis. Serum antibody levels measured in patients and normal subjects were compared for statistical significance using Student's *t*-tests. Correlation between anti-Tg and anti-OCT membrane antibody levels was determined, as a correlation coefficient (r), significance being expressed as $P < 0{\cdot}05$.

RESULTS

Sixty-six mouse MCAB, 44 against human thyroid antigens and 22 against human orbital tissue antigens, and 113 human MCAB, have been tested for thyroid and orbital tissue reactivity. Thirty-three (50%) of the mouse MCAB and 16 (14%) of the human MCAB reacted with one or more antigen sites shared between thyroid gland and orbital (eye muscle, connective tissue, lacrimal gland) tissue. Seventeen mouse MCAB against Tg were extensively studied and form the basis for this report. These MCAB were tested, in an ELISA, for reactivity against cytosol and membrane fractions of various human tissues, including OCT, lacrimal gland, eye-muscle, thyroid and liver. Surprisingly, a large proportion of these antibodies cross-reacted with an antigen in OCT membranes (OCTmem). For reasons that are unclear reaction of anti-Tg MCAB with OCTmem prepared from differential normal subjects was variable. For example, while 10 of the MCAB reacted with OCTmem (1), only three reacted with OCTmem (2) (Fig. 1). Using MCAB A3 and J8A3213 as probes, tests were positive with four of 14 preparations tested, while borderline reactions (o.d. 0·20–0·30) were demonstrated in a further five cases (Table 1). Overall, all but one MCAB reacted with at least one of the 14 preparations of OCTmem tested. The OCTmem antigen reactive with anti-Tg MCAB was different from that reactive with MCAB no. 50–24, a MCAB against a ubiquitous membrane antigen which reacted strongly with all preparations of OCTmem tested including OCTmem (1) and OCTmem (2) (Fig. 1). None of the 'A' series MCAB reacted with

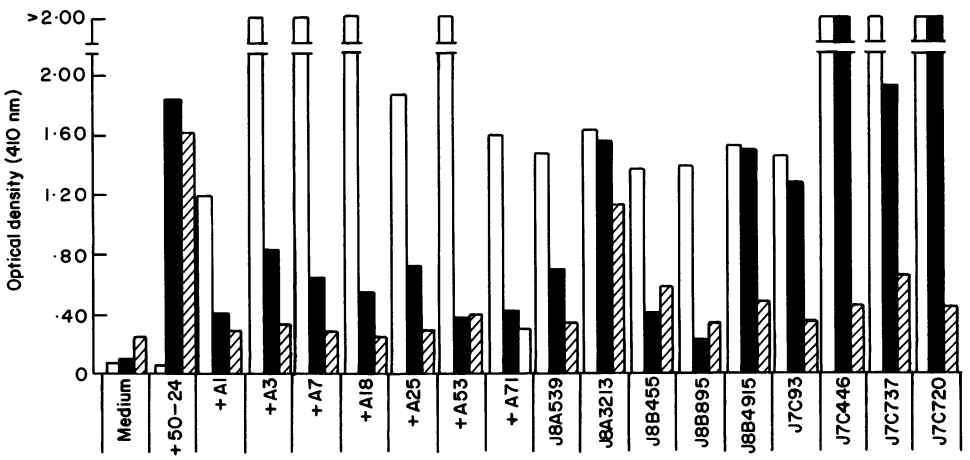

Fig. 1. Reactivity of mouse monoclonal antibodies against human thyroglobulin with Tg (□), human OCTmem (1) (■) and OCTmem (2) (■) assessed using an ELISA. Results expressed as o.d. at 410 nm. no. 50–24 = MCAB against a ubiquitious membrane antigen. no. A1–A71 = MCAB against Tg. J. Series = MCABs against Tg produced in Marseille by one of us (JR). Medium was tested as negative control. A positive test was taken as an o.d. >0·30.

Table 1. Reactivities of mouse antithyroglobulin monoclonal antibodies with human orbital connective tissue membrane and soluble fractions, before and after absorption with orbital connective tissue membranes and thyroglobulin.

	Monoclonal antibody													
	A3											J8A3213		
	Orbital connective tissue mem preparation													
Treatment	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
OCTmem	0·95*	0·21	0·03	0·10	0·26	0·16	0·16	0·15	0··25	0·24	0·40	0·38	0·21	0·66
Inhibition by OCTmem	7·6%†	63·1%	nd	nd	nd	nd	nd	nd	nd	nd	42·9%	30·0%	81·03%	47·9%
Inhibition by Tg	4·8%	10·5%	nd	nd	nd	nd	nd	nd	nd	nd	11·4%	18·8%	18·8%	4·5%

* Results expressed as o.d. in an ELISA (positive = > 0·30).

† Percentage to OCTmems [OCT (1), (2), (3) etc.) expressed as [o.d. with Inhibitor]-[Background]/[o.d. without Inhibitor]-[background] × 100 after absorption with the same preparation of OCT membranes and thyroglobulin. A positive absorption test was taken as percentage binding of < 50%.

nd = Not done.

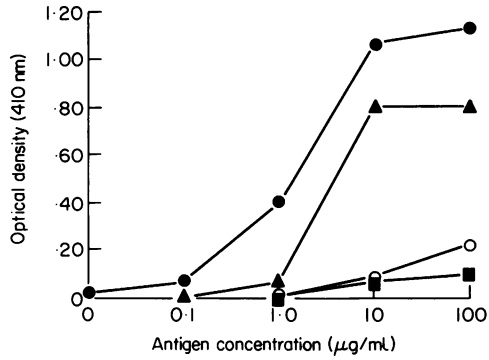

Fig. 2. Reactivity assessed in an ELISA, of MCAB Tg (A3) with increasing concentrations of Tg (●), OCTmem (1) (▲), liver membrane (○), and OCTmem (2) (■). Results expressed as optical density at 410 nm.

membrane or cytosol fractions prepared from eye muscle, lacrimal gland, or thyroid membranes (not shown). Reactivity of MCAB (A3) with OCTmem (1) was dose related (Fig. 2) and the activity was completely absorbed by incubation, at 37C overnight, with OCTmem (1) and Tg but not OCTmem (2) (Fig. 3). Although none of these MCAB-Tg reacted with connective membranes prepared from bowel or skin, all (except A3) reacted strongly with all preparations of liver membranes tested (data not shown). Of the 9 'J' series monoclonal antibodies against Tg four cross-reacted with OCT membranes only, four reacted with membrane preparations from all tissues testing including eye muscle, lacrimal, liver and OCT while only one (J8B455) showed reactivity restricted to thyroglobulin. The Tg-OCT shared reactive site was not native Tg since, (i) activity was not found in the cytosol fraction, and (ii) it was not solubilizable. There were no significant statistical correlations between the anti-Tg and anti-OCTmem activities (expressed as o.d.) for the 16 MCAB when either anti-OCTmem (1) ($r = 0{\cdot}37$, $P = \text{NS}$), or anti-OCTmem (12) ($r = 0{\cdot}107$, $P = \text{NS}$), were used as source of the OCTmem antigen. While mouse MCAB against other thyroid

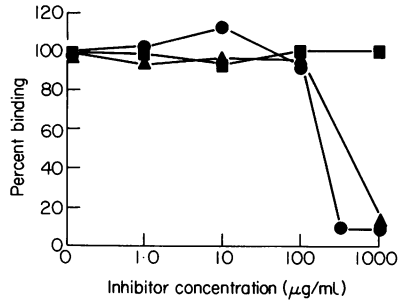

Fig. 3. Reactivity, assessed in an ELISA of MCAB Tg (A3) with OCTmen after absorption, at 37°C overnight, with OCTmen (1) (▲), OCTmem (2) (■), and Tg (●). Results expressed as percentage binding, i.e. [(o.d. with inhibitor) − (background)/(o.d. without inhibitor) − (background)] × 100, at increasing inhibitor concentrations.

or orbital tissue antigens often showed cross reactivity (as described above) this was usually against an antigen found in all tissues tested and reaction against antigens shared between thyroglobulin and OCTmem was not demonstrated.

EBV-infected human cell lines and human hybridomes produced from PBL from patients with Graves' ophthalmopathy have also been tested for cross-reactivity between thyroid and orbital tissues. Of 30 EBV-infected cell lines cloned by limiting dilution from a single cell line 20 reacted against an antigen shared between thyroglobulin, OCTmem, lacrimal gland membrane, eye muscle membrane and liver membrane. In no case was reaction restricted to OCTmem and thyroglobulin. While uncloned EBV-cell lines showed widespread reactivity between thyroid and orbital tissue monoclonality of these lines has not been established. Twenty-five fusions of EBV-infected cell lines with human myeloma cells have been performed. Seven of these have been tested for cross-reactivity. Of the 113 clones secreting antibody reactive with thyroid or orbital antigens 16 (14%) showed reactivity against one or more shared antigens, in four cases between thyroglobulin and OCTmem, in 10 cases between thyroglobulin and one or more other orbital tissue preparations and in two cases between thyroid membrane and OCTmem antigens. Monoclonality of these hybridomas has not yet been established.

Sera from patients with Graves' ophthalmopathy and autoimmune thyroid disorders were next tested for reactivity with OCT membranes. Tests were positive (o.d. > 0·25) in 18 of 23 patients with GO, in six of eight patients with Graves' hyperthyroidism and no associated eye disease, in six of eight patients with Hashimoto's thyroiditis and no eye disease, and in two of eight normal subjects (Fig. 4). Mean ±s.e. levels of anti OCTmem (1) antibody in patients with ophthalmopathy ($0{\cdot}2 \pm 0{\cdot}04$, $n=23$) and Graves' hyperthyroidism without eye disease ($0{\cdot}40 \pm 0{\cdot}75$, $n=8$) were not significantly different (t-tests, $P=$NS), while mean s.e. levels in patients with Hashimoto's thyroiditis without eye disease ($0{\cdot}54 \pm 0{\cdot}08$, $n=8$) was significantly greater than that in both of these groups of patients ($P > 0{\cdot}05$ and $P > 0{\cdot}05$, respectively). There was no close correlation between serum levels of antibody against the OCT membrane antigen with the presence, duration, or severity of the eye disease. There was, however, a close positive correlation between levels of anti-Tg antibody and anti-OCTmem (1) antibody ($r=0{\cdot}97$, $P > 0{\cdot}001$) (Fig. 5). It seemed likely, therefore, that the serum autoantibodies reactive with the OCT membrane antigen were anti-Tg. This was confirmed by absorption experiments, activity against OCTmem being completely absorbed by preincubation with Tg, thyroid membrane and OCTmem (1), but not skin membrane, eye muscle membrane, lacrimal membrane or OCTmem (2), (Fig. 6).

Finally, OCT membranes and cytosol prepared from OCT obtained from a patient with Graves' ophthalmopathy were tested for reactivity with anti-Tg MCAB and serum autoantibodies. In an attempt to quantitate the amount of OCTmem-Tg shared antigen in pathological tissue control tests were set up with OCT membranes and cytosol prepared from the same weight of orbital connective tissue from a normal subject. Although tests were positive, with OCT membranes (but

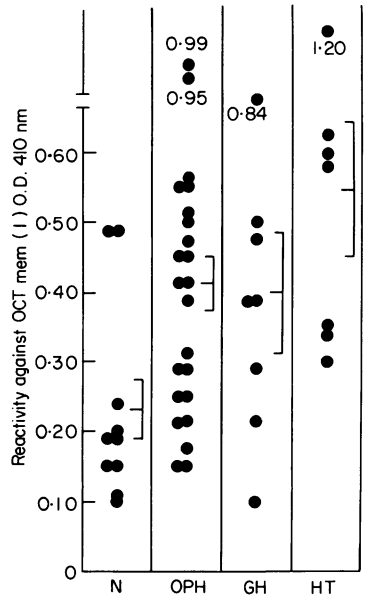

Fig. 4. Levels of serum antibody against OCTmem (1) in patients with Graves' ophthalmopathy (OPH), Graves' hyperthyroidism without eye disease (GH), Hashimoto's thyroiditis without ophthalmopathy (HT) and normal subjects (N), assessed in an ELISA. Horizontal bars (±s.e.) represent mean values. Results expressed as optical density at 410 nm. A positive test was taken as an o.d. >0·25.

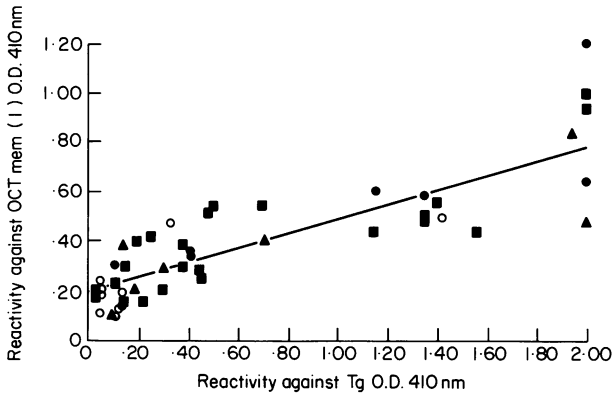

Fig. 5. Correlation between antibody activities against OCTmem (1) and Tg, of sera from patients with Graves' ophthalmopathy (■) Graves' hyperthyroidism without eye disease (▲), Hashimoto's thyroiditis without eye disease (●), and normal subjects (○). Correlation coefficient $r=0{\cdot}85$; gradient $Y=0{\cdot}29x+0{\cdot}20$; $P<0{\cdot}001$.

not cytosol), indicating the presence of the OCTmem-Tg shared antigen, for both MCAB (A3) and sera from two patients with Graves' ophthalmopathy, reactivities were similar for both test and control tissues (data not shown).

DISCUSSION

To summarize the main results, we have shown the existence of an antigen-reactive site in human OCT membrane which is shared with human thyroglobulin, against which anti-Tg monoclonal antibodies and anti-Tg autoantibodies from patients with autoimmune thyroid disorders, with or

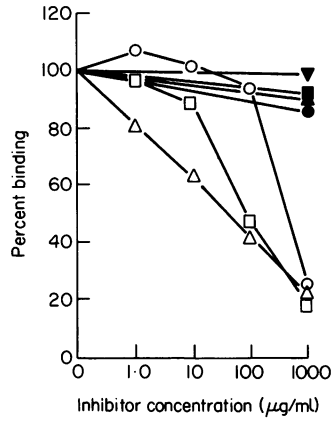

Fig. 6. Reactivity, assessed in an ELISA, of serum from a patient with GO, and detectable antibody against OCTmem (1), with OCTmem (1) after absorption, at 37°C for 1 h with OCTmem (1) (□), Tg (△), THYmem (○), OCTmem (2) (●), LACmem (▲), Eye muscle (EM)mem (■) and skin connective tissue (CT mem) (▼). Results expressed as percentage binding, i.e. [(o.d. with inhibitor) − (background)/(o.d. without inhibitor) − (background)] × 100 at increasing inhibitor concentrations.

without Graves' ophthalmopathy, react. The OCT membrane-Tg shared antigen was not native Tg deposited in the tissue (which was prepared from normal subjects) since the antigen activity was not found in the soluble (cytosol) fraction and was not solubilizable by protein solubilizing agents. On the other hand, the reactive site is clearly an important part of the Tg antigenic site since all but six of the 16 MACB tested reacted with at least one preparation of OCT membranes and both the serum (auto) and monoclonal antibody activities were completely absorbed by native Tg. Thus it seems likely that the OCT membrane reactive site is a small part of Tg and integral with its membrane structure. Since most MCAB and many sera also reacted with liver membranes, the shared protein must also be present in this organ. The finding that not all preparations of OCT membranes contained the antigen reactivity could be explained on the basis that, by chance, positive OCT preparations were derived from subjects with Graves' ophthalmopathy or predisposed to the disease or that the OCT-Tg shared antigen was variably unmasked as a result of enzymatic activity post mortem or in preparation of the membranes. The latter appears the more likely since there is little evidence that abnormal antigen is the basis for the autoimmune reaction (Volpé 1977; Leclerc *et al.*, 1984). Moreover, our failure to demonstrate close correlations between ELISA activities of MCAB-Tg against Tg and OCT membranes suggests that the availability, in positive preparations of OCT, of the Tg component reactive with MCAB, was different for each MCAB. Indeed in the one patient with GO tested in this study there was no increased content of the OCT-Tg shared antigen.

Although this is the first report of cross-reactivity against antigens shared between two target organs in autoimmune disease there is considerable evidence for existence of antigens shared between self antigens and foreign (usually infectious) antigens. In particular, the finding of antigens shared between thyroid membranes and *Yersinia enterocolitica* (Beck, Nerup & Larsen 1977a, Beck *et al.*, 1977b) is most interesting. While this is not directly relevant to the question of multiple organ specific autoimmunity it does suggest a mechanism for the development of thyroid disease in predisposed subjects. There is, of course, no evidence for infection being directly associated with the development of ophthalmopathy.

Graves' ophthalmopathy comprises two major components, a diffuse periorbital inflammation and an extraordinary eye muscle inflammation. The former is associated with proptosis and, occasionally, optic nerve compression, while the latter with abnormal eye movement and double vision. In the early phase of eye muscle disease the muscles are greatly enlarged and contribute to the increase in retro-orbital content and raised intraocular pressure (Kroll & Kuwabara, 1966; Riley, 1972). The mechanism for tissue damage in Graves' ophthalmopathy is not clear but probably involves both humoral and cellular mechanism (Volpé, 1977; Wall *et al.*, 1981; Wall, 1984a,b).

Although anti-Tg does not not usually fix complement its reaction with antigen in the loose connective and associated cellular influx would likely be sufficient to cause increase in orbital contents, proptosis and optic nerve pressure. Because not all patients with thyroid disease and detectable anti-Tg antibodies develop ophthalmopathy a direct role appears unlikely although, if one could detect anti-Tg autoantibodies against individual epitopes expressed in OCTmem, such an association may be shown.

The eye muscle damage which is likely to be the primary abnormality in Graves' ophthalmopathy (Wall *et al.*, 1981; Wall, 1984a), is probably mediated by K cells and cell surface-directed antibody, as is the case for thyroid cell-cytotoxicity (Bogner, Schleusener & Wall, 1984). Kendall-Taylor *et al.* (1984) have shown that antibodies against an eye membrane (microsomal) antigen can be detected in 60% of patients with eye disease, but not in patients with Hashimoto's thyroiditis or Graves' hyperthyroidism without eye disease, although we have not been able to confirm this (Sikorska, Miller & Wall, 1984). Using mouse and human MCAB against thyroid membrane antigens we are currently testing the possibility that thyroid membrane-directed autoantibodies react against a shared antigen on the surface of eye muscle cells. An interesting possibility is that such antibodies may be cytotoxic to both cell targets. Regardless of the nature of the orbital tissue reaction(s) it is likely that Graves' ophthalmopathy is multifactorial. In particular, the tendency to develop ophthalmopathy may be HLA associated, patients who develop orbital connective tissue or eye muscle inflammation having a genetically determined (HLA associated) predisposition to an effect of thyroid antibodies on specific thyroid-orbital tissue shared epitope(s). Indeed there is evidence that patients with ophthalmopathy have a significantly greater prevalence of DrW3 than patients with hyperthyroidism and no eye disease (Farid, Stone & Johnson, 1980; deLange *et al.*, 1984).

To conclude, even if autoreactivity against the OCTme-Tg shared antigen is shown not to be directly relevant to the development of Graves' ophthalmopathy this reaction is clearly of great importance as a model for thyroid-orbital tissue reactivity.

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