Clin. exp. Immunol. (1990) **79**, 182–188

Thyroid peroxidase and the induction of autoimmune thyroid disease

S. M. McLACHLAN, M. C. ATHERTON*, Y. NAKAJIMA, J. NAPIER*, R. K. JORDAN*, F. CLARK† & B. REES SMITH *Endocrine Immunology Unit, Department of Medicine, University of Wales College of Medicine, Cardiff, Wales; and *Division of Immunology and †Department of Medicine, University of Newcastle upon Tyne, England*

(Accepted for publication 13 September 1989)

SUMMARY

Animal models of autoimmune thyroid disease are associated with thyroglobulin (Tg) as autoantigen whereas in man the autoimmune response to microsomal antigen/thyroid peroxidase (TPO) appears to play a major role in thyroiditis. Consequently, we have compared the ability of TPO and Tg to induce thyroid autoantibodies and thyroid damage in mice known to be susceptible (CBA/J) or resistant (BALB/c) to thyroiditis induced using murine Tg. Groups of three to five mice were immunized twice using Freund's complete adjuvant with 80–100 μg highly purified porcine (p) TPO, pTg, rat (r) Tg, human Tg, bovine serum albumin (BSA) or BSA+0·2 μg pTg (the level of Tg contamination of TPO). Four weeks after immunization with TPO, plasma from CBA/J (but not BALB/c) mice contained IgG class antibodies which bound to TPO-coated tubes in the presence or absence of excess Tg (and could therefore be clearly distinguished from Tg antibodies) but there was no evidence of thyroiditis in either strain of mice. In contrast, in CBA/J mice immunized with rTg and, to a lesser extent in mice that had received pTg, thyroid tissue was infiltrated with lymphoid cells and/or neutrophils and antibodies to pTg (but not pTPO) were present. Our observations demonstrate that induction of TPO antibody alone is insufficient to lead to thyroiditis in CBA/J mice. Further, these studies emphasize the complex interactions between MHC and different thyroid antigens in the processes leading to thyroid destruction.

Keywords thyroid peroxidase thyroid peroxidase antibody thyroglobulin thyroglobulin antibody experimental autoimmune thyroiditis

INTRODUCTION

Animal models of autoimmune thyroiditis are usually characterized by the development of antibodies to thyroglobulin (Tg), either spontaneously as in obese strain (OS) chickens (reviewed by Wick *et al.*, 1985) and BB rats (Like, Appel & Rossini, 1987) or following immunization with thyroid preparations in adjuvant as in mice (reviewed by Kong, 1986). Autoantibodies to cellular antigens such as thyroid microsomal antigen appear to be rare in these animals, although they have been reported in some OS chickens (Khoury *et al.*, 1982) and in Rhesus monkeys immunized with autologous thyroid preparations (Kite, Argue & Rose, 1966). In contrast, thyroid microsomal antibodies occur frequently in human autoimmune thyroid disease and observations *in vivo* and *in vitro* suggest that their presence is closely associated with thyroid destruction and the development of hypothyroidism (Buchanan *et al.*, 1962; Forbes *et al.*, 1962; Khoury *et al.*, 1981; Hirota *et al.*, 1986).

Until recently, relatively little was known about microsomal antigen other than its location on the apical membrane and in the cytoplasm of thyroid epithelial cells (Roitt *et al.*, 1964, Khoury *et al.*, 1981; Fenzi *et al.*, 1982; Khouri, Bottazzo & Roitt, 1984) However, it has now been shown that the microsomal antigen is the enzyme thyroid peroxidase (TPO) (Czarnocka *et al.*, 1985). TPO is a 100-kD glycoprotein (Banga *et al.*, 1985; Czarnocka *et al.*, 1985; Kajita *et al.*, 1985) and the complete gene and amino acid sequences of human and porcine (p) TPO have recently been determined (Libert *et al.*, 1987; Kimura *et al.*, 1987; Magnusson *et al.*, 1987).

We now describe an investigation to determine whether immunization with purified pTPO leads to thyroiditis in mice. Since the ability to induce thyroiditis with Tg is related to the presence of certain MHC antigens, we used CBA/J mice (strain H-2k) and BALB/c mice (strain H-2d) which are 'high' and 'low' responders, respectively, in terms of the pathology of the thyroid lesion produced following immunization with murine Tg (Vladutiu & Rose, 1971; Kong, 1986). Further, we compared the effects induced by immunization with pTPO and Tg from different species (pig, rat and man) on thyroid pathology and the nature of the autoantibodies.

Correspondence: Dr S. M. McLachlan, Endocrine Immunology Unit, Department of Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, Wales.

MATERIALS AND METHODS

Antigens

TPO was prepared from deoxycholate solubilized porcine

thyroid microsomes using affinity chromatography based on monoclonal anti-pTPO antibodies (Ohtaki *et al.*, 1982; Czarnocka *et al.*, 1985). The final product had a specific activity of approximately 300 U/mg of protein when the guiacol method of Hosoya & Morrison (1967) was used to determine enzyme activity and the method of Bradford (1976) to determine protein concentration. The preparations contained trace amounts of Tg (0·2% as determined by radioimmunoassay).

Tg from porcine and human thyroid tissue homogenates was purified by ammonium sulphate precipitation (Derrien, Michel & Roche, 1948; Valenta *et al.*, 1968) followed by gel filtration on sephacryl S-400. Rat and mouse Tg were the gift of Dr N. Parrish, Department of Immunology, Middlesex Hospital Medical School, London. Bovine serum albumin (BSA, Sigma Chemical Co., Poole, UK) was used as a control antigen.

Mice and Immunization protocol

Female CBA/J (H-2k) and BALB/c (H-2d) mice were purchased from the Clinical Research Centre (Harrow, UK) and used at approximately 7 weeks of age. Mice were immunized subcutaneously in the neck region on two occasions, 7 days apart, using pTPO or pTg, rat (r) Tg or human (h) Tg diluted in 150 mM NaCl to give a final concentration of 20, 80 or 100 μg protein in 0·2 ml of a 1:1 emulsion in Freund's complete adjuvant (FCA; Sigma). Control immunizations included BSA at 20 or 100 μg, BSA (100 μg) containing 0·2 μg pTg (to control for the level of Tg contaminating TPO) and saline, all in FCA. Prior to immunization, an aliquot of blood (approximately 1 ml) was collected from the tail vein of each mouse into heparinized tubes to provide pre-immunization plasma samples. Mice were killed 3 weeks after the second immunization; blood was collected from the axillary vein into heparin and both thyroid lobes were removed for histopathology studies.

Analysis of serum antibodies

IgG class antibodies to TPO were measured by a solid-phase radioimmunoassay as follows: duplicate 250-μl aliquots of plasma diluted 1:250 in 20 mM tris-HCl, 150 mM NaCl, 0·5% BSA (pH 7·5) were added to tubes coated with pTPO (10 μg/ml pTPO in 0·1 M $NaHCO_3$, pH 9·2, overnight at 4°C). After incubation for 2 h at 37°C, the tubes were washed twice in serum diluent and 500 μl of affinity purified anti-mouse IgG (Sigma) labelled with ^{125}I (specific activity 10 μCi/μg; 50000 ct/min per tube) were added. After further incubation overnight at room temperature and washing, the tubes were counted for ^{125}I and the results expressed as % binding of total ct/min added. Since even highly purified TPO contained a trace amount of Tg (0·2%), the assay was performed on untreated plasma and plasma pre-incubated overnight at 4°C with pTg at a final concentration of 500 μg/ml Tg. A similar assay using pTg-coated tubes was used to assess the level of pTg autoantibodies.

In addition, IgG class Tg antibodies were measured by ELISA in plasma as previously described for hTg autoantibodies (McLachlan *et al.*, 1982) using ELISA plates coated with pTg, hTg or rTg and goat anti-mouse IgG conjugated to horseradish peroxidase (Sigma). Plasma samples were assayed at serial ten-fold dilutions from 1:100 to 1:100000 and the results reported as the optical density (OD) at 492 nm.

Preliminary studies to determine whether antibodies to pTPO cross-reacted with murine TPO were carried out by indirect immunofluorescence on cryostat sections of murine and porcine thyroid tissue frozen in liquid nitrogen. Tissue sections (6 μm thick, two sections/slide) were incubated for 20 min at room temperature with plasma (diluted 1:10 in phosphate-buffered saline, PBS) from mice immunized with 2 × 100 μg pTPO or pTg. The slides were washed in PBS, exposed to goat anti-mouse IgG conjugated to fluorescein (Becton Dickinson, Oxford, UK) diluted 1:10 in PBS, washed again, mounted using uvinert (Gurr, BDH, Poole, UK) and viewed under u.v. light with a Leitz Ortholux II microscope. Positive controls included murine monoclonal antibodies to pTPO and pTg (assessed against porcine thyroid tissue). Negative controls included plasma from untreated mice and from mice immunised with Tg or BSA.

Histopathology of thyroid glands

When mice were killed, blocks of tissue, including both thyroid glands, were removed and fixed in formol saline and serial sections, prepared from paraffin wax-embedded material, stained with haematoxylin and eosin. In one experiment, cryostat sections were prepared from thyroid lobes frozen in liquid nitrogen.

Statistical analysis

The significance of differences was assessed using unpaired or paired Student's *t*-test as appropriate.

RESULTS

Immunization with a low dose of pTPO

In CBA/J mice immunized twice with a low dose of pTPO (20 μg), antibody binding to pTPO-coated tubes was significantly inhibited by excess pTg ($P < 0·001$, paired *t*-test) as in plasma from mice immunized with 20 μg pTg ($P < 0·001$, Fig. 1a) and similar results were obtained for BALB/c mice (Fig. 1b, $P < 0·03$ for TPO and $P < 0·003$ for Tg). These observations suggested that the antibody binding to TPO-coated tubes was predominantly directed against Tg contaminating the TPO.

The presence of pTg antibodies in CBA/J and BALB/c mice immunized with pTPO was confirmed by radioimmunoassay (using Tg-coated tubes) and by ELISA (Table 1, experiment a). More Tg antibody was induced in mice immunized with 2 × 20 μg pTg than with 2 × 20 μg pTPO as shown by significantly higher levels of binding to pTg-coated tubes and ODs in ELISA (Table 1, unpaired *t*-test, $P < 0·05$–$0·001$).

Antibody responses in mice immunized with high and low doses of TPO

On the basis of the above experiment, a pilot study was carried out in which groups of three to five animals were immunized twice with high (80 μg) or low (20 μg) doses of pTPO or pTg. CBA/J mice which received 2 × 80 μg pTPO developed antibodies which bound to TPO-coated tubes in the presence and absence of excess Tg (Fig. 2a, paired *t*-test, not significant). A lower level of binding was observed in CBA/J mice immunized with 2 × 20 μg TPO and, as in the previous study, this binding was inhibited by Tg ($P < 0·03$). The levels of binding by plasma from CBA/J mice immunized with either 2 × 20 or 80 μg pTg were high, comparable with those in 80-μg-TPO-treated animals, but the binding was inhibited by excess Tg ($P < 0·001$, Fig. 2a). Immunization of BALB/c mice with high or low doses of

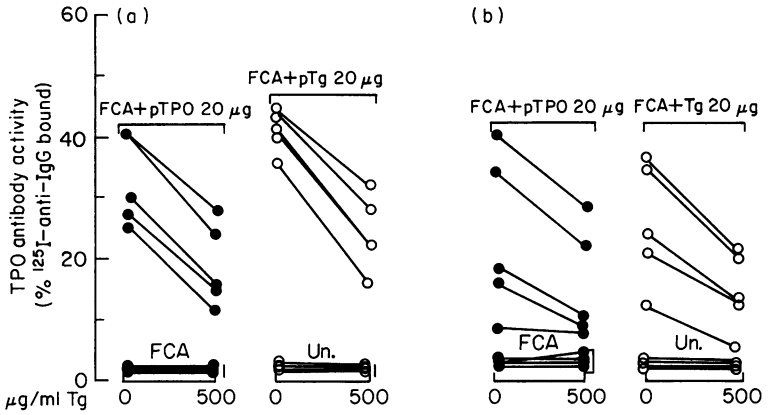

Fig. 1. Inhibition of IgG class antibody binding to thyroid peroxidase (TPO) coated tubes by excess thyroglobulin (Tg) in plasma from (a) CBA/J and (b) BALB/c mice, immunized twice with 20 μg porcine TPO or Tg in Freund's complete adjuvant (FCA). Control mice were untreated (Un.) or immunized with saline in FCA.

Table 1. IgG class antibodies to porcine thyroglobulin (Tg) measured by radioimmunoassay (RIA) and ELISA in CBA/J and BALB/c mice immunized twice with low or high doses of porcine thyroid peroxidase (TPO) or porcine TG in Freund's complete adjuvant (FCA)

	Tg antibody		
	RIA	ELISA	
	% ^{125}I-anti IgG bound	OD 492 nm	*n*
(a) Immunization with low antigen doses			
CBA/J			
TPO (20 μg)	35·5±3·5	0·33±0·09	5
Tg (20 μg)	51·6±0·3†	0·96±0·07‡	5
Saline	3·5±0·8	0·00±0·00	5
BALB/c			
TPO (20 μg)	25·6±4·5	0·12±0·03	5
Tg 20 (μg)	44·5±4·3*	0·54±0·09†	5
Saline	5·4±2·2	0·00±0·00	5
(b) Immunization with low and high antigen doses			
CBA/J			
TPO (20 μg)	39·1±2·8	0·32±0·04	3
(80 μg)	46·2±0·8	0·53±0·06	3
Tg (20 μg)	53·5±0·7*	0·83±0·05‡	5
(80 μg)	51·0±1·0†	0·80±0·03*	5
Saline	2·7±0·4	0·00±0·00	3
BALB/c			
TPO (20 μg)	39·5±2·9	0·23±0·01	3
(80 μg)	38·4±1·7	0·16±0·04	3
Tg 20 μg	55·9±0·8*	0·64±0·03†	4
80 μg	56·0±0·5†	0·75±0·02‡	5
Saline	4·6±1·6	0·00±0·03	3

Low antigen doses, 20 μg; high antigen doses, 80 μg.

Control mice received saline + FCA. Results are mean ± s.e.m. for groups of three to five mice (*n*) in plasma diluted 1:250 for RIA and 1:1000 for ELISA.

Values significantly higher than those obtained for mice immunized with 20 or 80 μg TPO: * $P<0·05$; † $P<0·001$; ‡ $P<0·001$ (unpaired Student's *t*-test).

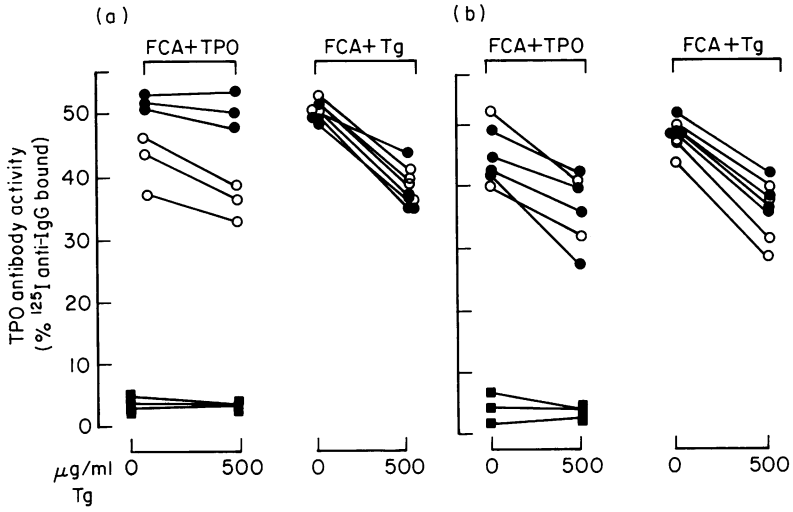

Fig. 2. Comparison of IgG class antibody binding to thyroid peroxidase (TPO) coated tubes, with or without excess thyroglobulin (Tg), in plasma from (a) CBA/J and (b) BALB/c mice, immunized twice with high doses (80 μg, closed circles) or low doses (20 μg, open circles) of porcine TPO or Tg. Full squares, mice immunized with saline + Freund's complete adjuvant (FCA).

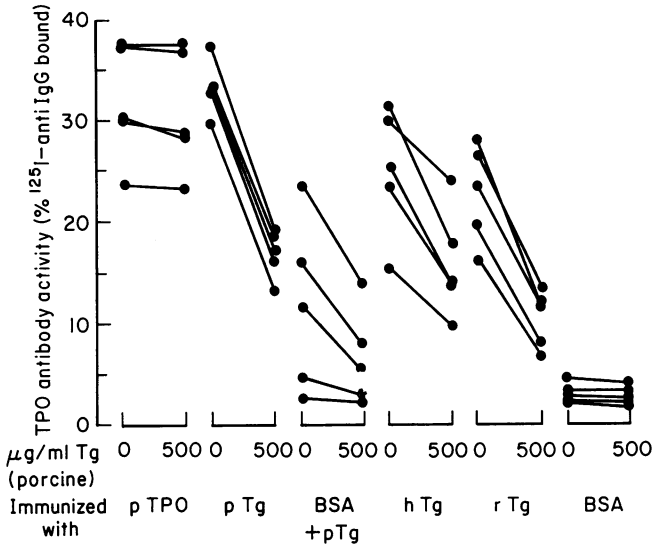

Fig. 3. Induction of thyroid peroxidase (TPO) antibody activity which was not inhibited by excess thyroglobulin (Tg) in plasma from CBA/J mice immunized twice with 100 μg porcine (p) TPO but not in mice immunized with porcine, human (h) or rat (r) Tg, or BSA containing 0·2 μg pTg. Control mice were immunized with BSA (2 doses of 100 μg) only.

TPO or Tg resulted in antibody binding which was inhibited by 500 μg/ml Tg ($P < 0{\cdot}02$–$0{\cdot}01$, Fig. 2b).

The inhibition by Tg of plasma binding to TPO-coated tubes was incomplete and the lack of inhibition in the case of plasma from CBA/J mice immunized with 80 μg Tg (Fig. 2a) could conceivably have been due to the presence of very high levels of Tg antibody. Therefore Tg antibody levels were investigated in more detail by ELISA. Both CBA/J and BALB/C mice immunized with Tg (2 × 20 or 80 μg) had significantly higher levels of Tg antibodies than did mice immunized with 2 × 80 μg pTPO (unpaired *t*-test, $P < 0{\cdot}05$–$0{\cdot}001$) as shown in Table 1 for plasma diluted 1:1000 (experiment b). Further, Tg antibody activity in ELISA was reduced to 15–20% by absorption with 100 μg/ml Tg in sera diluted 1:1000 but only 45–70% in sera diluted 1:100 (data not shown).

Overall, therefore, the observations in Fig. 2 and Table 1 (experiment b) suggest that in CBA/J (but not BALB/c mice) immunization with 2 × 80 μg (but not 20 μg) pTPO induces the development of antibodies to pTPO which can be clearly distinguished from those to pTg. Therefore further experiments were restricted to CBA/J mice using a high dose of pTPO.

Immunization of CBA/J mice with high doses of pTPO or pTg, hTg or rTg

CBA/J mice immunized twice with 100 μg pTPO developed TPO antibodies as shown by plasma binding to TPO-coated tubes in the presence or absence of Tg (Fig. 3, paired *t*-test, not significant). In contrast, mice which received 2 × 100 μg pTg developed antibody which bound to a comparable extent to TPO-coated tubes in the absence of pTg but to a significantly lower extent ($P < 0{\cdot}001$) in its presence. Immunization with two doses of 100 μg hTg or rTg generally induced lower levels of antibody binding to pTPO-coated tubes and this binding could also be inhibited by Tg ($P < 0{\cdot}001$ for both antigens). A similar pattern of binding, inhibitable by Tg ($P < 0{\cdot}05$) developed in mice immunized with 0·2 μg pTg + 100 μg BSA (to control for Tg contamination of TPO), but the levels were much lower and more variable than in plasma from mice immunized with 100 μg of pTg, hTg or rTg (Fig. 3).

Although CBA/J mice appeared to develop TPO antibodies following immunization with pTPO, thyroid glands from those animals showed only low levels of lymphoid infiltration, comparable with those in mice receiving BSA. Lymphoid infiltrates were also minimal in BALB/c mice immunized with 2 × 80 μg TPO. In contrast, extensive lymphoid and/or neutrophil infiltration and thyroid destruction was evident in CBA/J mice which were immunized twice with 2 × 100 μg rTg and to a lesser extent in mice which received 2 × 100 μg pTg (data not shown).

In studies using indirect immunofluorescence on sections of murine and porcine thyroid tissue, it was not possible to determine conclusively whether antibodies to pTPO cross-

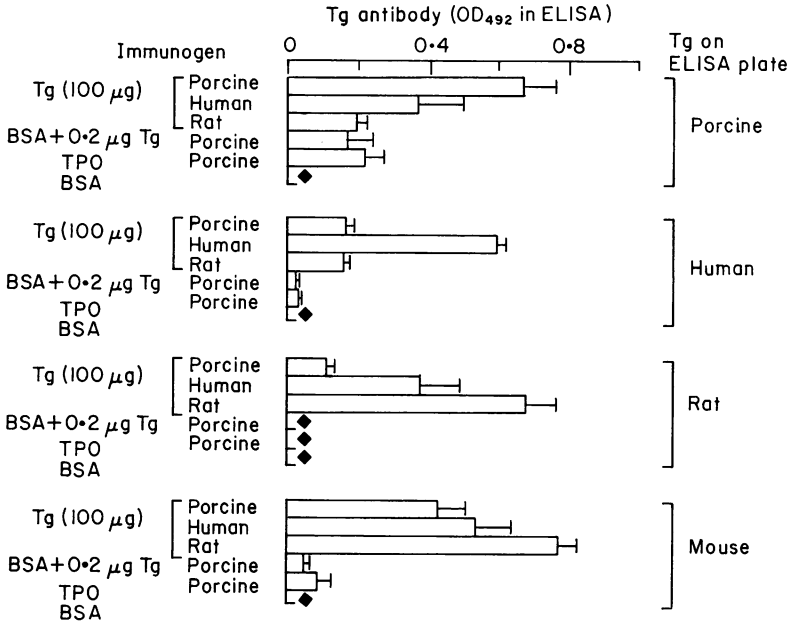

Fig. 4. IgG class thyroglobulin (Tg) antibody activity to Tg from different species in plasma (diluted 1:1000) from CBA/J mice immunized twice with 100 μg porcine, rat or human Tg; with 100 μg porcine thyroid peroxidase (TPO), 0·2 μg Tg + 100 μg bovine serum albumin (BSA); or 100 μg BSA alone. Antibody activity was measured by ELISA using plates coated with porcine, human, rat or murine Tg and the results are expresed as the mean OD (492 nm) + s.e.m. for groups of five mice. ◆, undetectable.

reacted with murine TPO. Background levels of fluorescence were relatively high, reducing the contrast between positive and negative results. However, fluorescent labelling of the apical borders of murine thyroid follicles was observed using plasma from one CBA/J mouse immunized with 2 × 100 μg pTPO and similar labelling was seen in porcine thyroid tissue with plasma from a second CBA/J mouse. These patterns, although of lower intensity, resembled those obtained using a murine monoclonal antibody raised against pTPO. In contrast, plasma from mice immunized with Tg interacted with material in the thyroid follicle and plasma from control mice (untreated or immunized with BSA) stained the basement membrane.

Comparison of Tg antibody reactivity to pTg, hTg or rTg

Tg antibody reactivity to Tg from different species was investigated in sera diluted 1:100, 1:1000 and 1:10000 using ELISA plates coated with pTg, hTg, rTg or mouse Tg. In mice immunized with Tg from these three species, striking differences were observed and the results for plasma diluted 1:1000 are shown in Fig. 4. The highest levels of activity against pTg were observed in mice immunized with 100 μg pTg and much lower levels were present in mice immunized with 100 μg hTg or rTg. Similarly, immunization with 100 μg hTg induced greater amounts of antibody to hTg than did immunization with 100 μg pTg or rTg and comparable observations were made for mice immunised with rTg, namely high levels of reactivity to rTg in rTg-treated animals and lower levels in mice which received 100 μg pTg or hTg. Mice immunized with pTPO had only small amounts of activity against pTg (and virtually none against rTg or hTg) and these levels were almost identical to those observed in mice treated with 0·2 μg Tg + 100 μg BSA—the level of Tg contaminating the TPO preparation. The pattern observed for antibody activity against mouse Tg was similar to that obtained for rTg, with the highest levels being observed in mice immunized with rTg (Fig. 4).

DISCUSSION

Immunization with two doses of 80–100 μg (but not 20 μg) highly purified TPO in FCA induced IgG class antibody to pTPO in CBA/J mice but not in BALB/c mice. In some instances, immunization with pTPO led to the production of antibodies cross-reactive with lactoperoxidase and horseradish peroxidase as judged by solid-phase assays and Western blotting (Nakajima, Petersen & Rees Smith, 1988) and similar cross-reactivity has been observed between human autoantibodies to TPO and lactoperoxidase, myeloperoxidase and horseradish peroxidase (Banga *et al.*, 1989).

Although autoantibodies to TPO were readily detectable in CBA/J mice, lymphoid infiltration of the thyroid and epithelial cell damage was minimal. In contrast, in CBA/J mice immunized with rTg, as well as in some animals which received pTg, lymphoid and/or neutrophil infiltration and thyroid destruction was evident as observed by others (reviewed by Kong, 1986). Our inability to induce thyroiditis (as opposed to TPO autoantibodies only) in CBA/J mice by immunization with pTPO could have been due to one of the following four factors:

(i) Inappropriate immunization schedule. Several approaches have been used to induce thyroiditis with Tg including different routes of antigen administration and adjuvants other than FCA (Kong *et al.*, 1985). The approach we followed, namely subcutaneous immunization with TPO in FCA on day 0 and day 7, has been used in a number of studies for Tg, and thyroiditis can be demonstrated 28 days after the initial injection (Romball & Weigle, 1987).

(ii) A lack of cross-reactivity between the relevant epitopes on porcine and murine antigens. Homologous TPO may be more effective in inducing thyroiditis than heterologous TPO in mice, as suggested by the minimal thyroid damage induced by human microsomal antigen compared with autologous thyroid fractions in Rhesus monkeys (Andrada, Rose & Kite, 1968).

In a comparison of Tg antibody cross-reactivity in mice immunized with pTg, rTg or hTg we observed high levels of antibody to the immunizing Tg and lower levels of antibodies reactive with Tg from the other species, although antibody reactivity against rTg and mouse Tg were comparable. Similar observations were made by Tomazic & Rose (1976) using mouse, rabbit and human Tg in high (C57Br/cd) and low (C57/BL/10) responder mice. Further, in a panel of 10 murine monoclonal antibodies produced by immunization of CBA/Ca (H-2k) mice with mouse Tg, all antibodies cross-reacted with rTg but only two recognized Tg from other species including humans (Champion *et al.*, 1987). Despite the lower levels of antibody to mouse/rat Tg in animals immunized with porcine rather than rat Tg, we observed mild thyroiditis in mice immunized with pTg and these observations are in agreement with other studies using rabbit or hTg (Tomazic & Rose, 1976), pTg (Salamero *et al.*, 1987) and bovine Tg (Romball & Weigle, 1984). Therefore, it seems unlikely that the absence of any thyroid damage could be attributed solely to the use of pTPO. Further, our preliminary studies using immunofluorescence suggest that antibody activity to murine TPO was induced.

(iii) Failure to induce cytotoxic T cells and/or delayed-type hypersensitivity (DTH) response. It is conceivable that the immunization schedule we followed and the use of pTPO may not have induced either cytotoxic T cells, which appear to play a role in experimental murine thyroiditis (Creemers, Rose & Kong, 1983) or DTH responses, which may contribute to thyroid destruction in OS chickens (Wick *et al.*, 1985).

(iv) Inappropriate mouse strain. Recently Kotani et al. (1988) reported that pTPO induced thyroiditis in C57/B16 (H-2b) but not in mice of strains H-2k or H-2d (including CBA/J and BALB/c). Overall, therefore, it seems likely that a major factor in our inability to induce thyroiditis using pTPO was the selection of an inappropriate mouse strain.

In their studies, Kotani *et al.* (1988) observed that the development of thyroiditis in C57/B16 mice did not appear to be clearly related to TPO autoantibody levels. Further, although the MHC was important in determining thyroiditis, other genetic factors (not necessarily associated with immune responses) were involved. These observations, together with the results of our study, emphasize the complex nature of the interactions between MHC and different thyroid antigens in the processes leading to thyroid destruction. In addition, they are consistent with the role of at least three genetic loci in the development of spontaneous thyroiditis in OS chickens (Wick *et al.*, 1985).

The nature of the thyroid lesion induced experimentally with Tg is variable, even within the susceptible H-2k strain. A granulomatous thyroiditis, resembling de Quervain's thyroiditis develops in CBA/J mice whereas lymphocytic thyroiditis, resembling Hashimoto's thyroiditis, occurs in C3H mice (Imahori & Vladutiu, 1983). Detailed analysis of Tg-specific cell clones derived from a single mouse strain (CBA/CAJ) demonstrated that some clones induce thyroid infiltrates characterized by lymphoid cells, whereas others induce polymorph infiltrates (Romball & Weigle, 1987). The preliminary report by Kotani *et al.* (1988) suggests that thyroiditis induced by TPO in C57/B16 mice closely resembles Hashimoto's thyroiditis. Consequently, detailed studies of T cell responses to TPO, including proliferation and cytoxicity against thyroid-specific antigens on thyroid cells could be carried out at different time intervals following TPO immunization. Observations of these T cell responses, and their relation to the presence of TPO autoantibodies, are likely to contribute substantially to our understanding of the mechanisms involved in autoimmune thyroid destruction in humans.

ACKNOWLEDGMENTS

We would like to thank Mrs Gwyn Pyle, Mr E. Waggott and Mr B. MacFarlane for technical assistance and Dr N. Parrish, Department of Immunology, Middlesex Hospital Medical School, London, for her generous gift of rat and mouse thyroglobulin. The work was supported financially by the Wellcome Trust, RSR Ltd., Cardiff and the Research Committee of the Newcastle upon Tyne Health Authority.

REFERENCES

ANDRADA, J.A., ROSE, N.R. & KITE, J.H. (1968) Experimental thyroiditis in the rhesus monkey. The role of thyroglobulin and cellular antigens. *Clin. exp. Immunol.* **3,** 133.

BANGA, J.P., PRYCE, G., HAMMOND, L. & ROITT, I.M. (1985) Structural features of the autoantigens involved in thyroid autoimmune disease: the thyroid microsomal/microvillar antigen. *Mol. Immunol.* **22,** 629.

BANGA, J.P., TOMLINSON, R.W.S., DOBLE, N., ODELL, E. & MCGREGOR, A.M. (1989) Thyroid microsomal/thyroid peroxidase autoantibodies show discrete patterns of cross-reactivity to myeloperoxidase, lactoperoxidase and horseradish peroxidase. *Immunology*, **67,** 197.

BRADFORD, M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal. Biochem.* **72,** 248.

BUCHANAN, W., KOUTRAS, D.A., CROOKS, J., ALEXANDER, W.E., BRASS, W., ANDERSON, J.R., GOUDIE, R.B. & GRAY, K.G. (1962) The clinical significance of the complement fixation test in thyrotoxicosis. *J. Endocrinol.* **24,** 115.

CHAMPION, B.R., PAGE, K., RAYNER, D.C., QUARTEY-PAPAFIO, R., BYFIELD, P.G. & HENDERSON, G. (1987) Recognition of thyroglobulin autoantigenic epitopes by murine T and B cells. *Immunology*, **62,** 255.

CREEMERS, P., ROSE, N.R. & KONG, Y.M. (1983) Experimental autoimmune thyroiditis: *in vitro* cytotoxic effects of T lymphocytes on thyroid monolayers. *J. exp. Med.* **157,** 559.

CZARNOCKA, B., RUF, J., FERRAND, M., CARAYON, P. & LISSITZKY, S. (1985) Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. *FEBS Lett.* **190,** 147.

DERRIEN, Y., MICHEL, R. & ROCHE, J. (1948) Recherches sur la preparation et les proprietes de la thyroglobuline pure. *Biochim. biophys. Acta*, **2,** 454.

FENZI, G.F., BARTALENA, L., CHIOVATO, L., MARCOCCI, C., ROTELLA, C.M., ZONEFRATI, R., TOCCAFONDI, R. & PINCCHERA, A. (1982) Studies on thyroid cell surface antigens using cultured human thyroid cells. *Clin. exp. Immunol.* **47,** 336.

FORBES, I.J., ROITT, I.H., DONIACH, D. & SOLOMON, I.L. (1962) The Thyroid cytotoxic autoantibody. *J. clin. Invest.* **41,** 996.

HIROTA, Y., TAMAI, H., HAYASHI, Y., MATSUBAYASHI, S., MATSUZUKA, F., KUMA, K., KUMAGAI, L.F. & NAGATAKI, S. (1986) Thyroid function and histology in 45 patients with hyperthyroid Graves' disease in clinical remission more than 10 years after thionamide drug treatment. *J. clin. Endocrinol. Metab.* **62,** 1065.

HOSOYA, T. & MORRISON, M. (1967) The isolation and purification of thyroid peroxidase. *J. biol. Chem.* **242,** 2828.

IMAHORI, S.C. & VLADUTIU, A.O. (1983) Autoimmune granulomatous

thyroiditis in inbred mice: resemblance to subacute (de Quervain's) thyroiditis in man. *Proc. Soc. exp. Biol. Med.* **173,** 408.

KAJITA, Y., MORGAN, D., PARKES, A.B. & REES SMITH, B. (1985) Labelling and immunoprecipitation of thyroid microsomal antigen. *FEBS Lett.* **187,** 334.

KHOURY, E.L., BOTTAZZO, G.F. & ROITT, I.M. (1984) The thyroid 'microsomal' antigen revisited. Its paradoxical binding *in vivo* to the apical surface of the follicular epithelium. *J. exp. Med.* **159,** 577.

KHOURY, E.L., BOTTAZZO, G.F., PONTES DE CARVALHO, L.C., WICK, G. & ROITT, I.M. (1982) Predisposition to organ-specific autoimmunity in Obese strain (OS) chickens: reactivity to thyroid, gastric, adrenal and pancreatic cytoplasmic antigens. *Clin. exp. Immunol.* **49,** 273.

KHOURY, E.L., HAMMOND, L., BOTTAZZO, G.F. & DONIACH, D. (1981) Presence of the organ-specific 'microsomal' autoantigen on the surface of human thyroid cells in culture: its involvement with the complement mediated cytotoxicity. *Clin. exp. Immunol.* **45,** 316.

KIMURA, S., KOTANI, T., MCBRIDE, O.M., UNICKI, K., HIRAI, K., NAKEYAMA, T. & OHTAKI, S. (1987) Human thyroid peroxidase: complete cDNA and protein sequence, chromosome mapping and identification of two alternately spliced mRNA's. *Proc. natl Acad. Sci. USA*, **84,** 5555.

KITE, J.H., ARGUE, H. & ROSE, N.R. (1966) Experimental thyroiditis in the rhesus monkey. I. Cytotoxic, mixed-agglutinating and complement-fixing antibodies. *Clin. exp. Immunol.* **1,** 139.

KONG, A.M. (1986) The mouse model of autoimmune thyroid disease. In *Immunology of Endocrine Diseases* (ed. by A. M. McGregor) p. 1. MTP Press, Lanchester.

KONG, Y.C., AUDIBERT, F., GIRALDO, A.A., ROSE, N.R. & CHEDID, L. (1985) Effects of natural or synthetic microbial adjuvants on induction of autoimmune thyroiditis. *Infect. Immun.* **49,** 40.

KOTANI, T., UMEKI, K., MORITA, M. & OHTAKI, S. (1988) Experimental allergic thyroiditis induced by porcine thyroid peroxidase in mice. In *8th International Congress of Endocrinology, Kyoto, Japan.* (Abstract). p. 270. Japan Convention Services.

LIBERT, F., RUF, J., LUDGATE, M., SWILLENS, S., ALEXANDER, N., VASSART, G. & DINSART, C. (1987) Complete nucleotide sequence of the human thyroid peroxidase-microsomal antigen cDNA. *Nucl. Acids Res.* **15,** 6735.

LIKE, A.A., APPEL, M.C. & ROSSINI, A.A. (1982) Autoantibodies in the BB/W rat. *Diabetes*, **31,** 816.

MAGNUSSON, R.P., GESTAUTAS, J., TAUROG, A. & RAPOPORT, B. (1987) Molecular cloning of the structural gene for porcine thyroid peroxidase. *J. biol. Chem.* **262,** 13885.

MCLACHLAN, S.M., CLARK, S., STIMSON, W.H., CLARK, F. & REES SMITH, B. (1982) Studies of thyroglobulin autoantibody synthesis using a micro-ELISA assay. *Immunol. Lett.* **4,** 27.

NAKAJIMA, Y., PETERSEN, V.B. & REES SMITH, B.R. (1988) Monoclonal antibodies to human thyroid microsomal antigen and porcine thyroid peroxidase which cross react with lactoperoxidase and horseradish peroxidase. In *8th International Congress of Endocrinology, Kyoto, Japan.* p. 273. Japan Convention Services. (Abstract).

OHTAKI, S., MAKAGAWA, H., NAKAMURA, M. & YAMASAKI, I. (1982) Reactions of purified hog thyroid peroxidase with H_2O_2, tyrosine and methylmercaptoimidazole (goitrogen) in comparison with bovine lactoperoxidase. *J. biol. Chem.* **257,** 761.

ROITT, I., LING, N.R., DONIACH, D. & COUCHMAN, K.G. (1964) The cytoplasmic autoantigen of the human thyroid. I. Immunological and biochemical characteristics. *Immunology*, **7,** 375.

ROMBALL, C.G. & WEIGLE, W.O. (1984) T cell competence to heterologous and homologous thyroglobulins during the induction of experimental thyroiditis. *Eur. J. Immunol.* **14,** 887.

ROMBALL, C.G. & WEIGLE, W.O. (1987) Transfer of experimental autoimmune thyroiditis with T cell clones. *J. Immunol.* **138,** 1092.

SALAMERO, J., REMY, J.J., MICHEL-BECHET, M. & CHARREIRE, J. (1987) Experimental autoimmune thyroiditis induced by a 5–10 kDa tryptic fragment from procine thyroglobulin. *Eur. J. Immunol.* **17,** 843.

TOMAZIC, V. & ROSE, N.R. (1976) Autoimmune murine thyroiditis. VIII. Role of different thyroid antigens in the induction of experimental autoimmune thyroiditis. *Immunology*, **30,** 63.

VALENTA, L., ROQUES, M., TORRESANI, J., ROLLAND, M. & LISSITSKY, S. (1968) Human thyroglobulin. Physico-chemical properties in relation to iodine content. *Biochim. biophys. Acta*, **168,** 507.

VLADUTIU, A.O. & ROSE, N.R. (1971) Autoimmune murine thyroiditis. Relation to histocompatibility (H-2) type. *Science*, **174,** 1137.

WICK, G., MOST, J., SCHAUENSTEIN, K., KROMER, G., DIETRICH, H., ZIEMIECKI A., FASSLER, R., SCHWARZ, S., NEU, N. & HALA, K. (1985) Spontaneous autoimmune thyroiditis: a bird's eye view. *Immunol. Today*, **6,** 359.