The EMBO Journal Vol.2 No.10 pp.1821 – 1826, 1983

Specificity of monoclonal antibodies against human thyroglobulin; comparison with autoimmune antibodies

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Communicated by S.Lissitzky
Received on 11 July 1983

Ten monoclonal antibodies (mAb) directed against human thyroglobulin (hTgb) were produced, purified and characterized. The mAb avidity for hTgb ranged from 10^{-10} to 10^{-6} M. The species specificity of the mAb was as follows: eight mAb reacted with monkey Tgb, three with dog Tgb and one with pig Tgb; none with bovine and ovine Tgb. The binding of mAb to hTgb was not significantly inhibited in the presence of Tgb carbohydrate moieties, tyrosine, iodotyrosines and iodothyronines. The topology of the antigenic determinants recognized by the 10 mAb on hTgb was explored by inhibition of Tgb binding of radiolabeled mAb by the other antibodies. Six distinct clusters of reactivity were described. Localization of the antigenic determinants recognized by mAb on hTgb was attempted using tryptic fragments of hTgb to inhibit the binding of mAb to hTgb. The inhibitory effect of hydrolysis products was different for each mAb but exhibited partial analogies between mAb of the same cluster of reactivity. Anti-hTgb autoimmune antibodies (aAb) purified from sera of Graves patients cross-reacted essentially with mAb of one out of the six clusters. These results demonstrate that the large number of antigenic determinants presented by the hTgb are not disseminated on the molecule but are clustered in antigenic regions. Furthermore, from the six antigenic regions evidenced in this paper, only one is involved in autoimmune antibody production in Grave's disease.

Key words: thyroglobulin/monoclonal antibody/autoimmune antibodies/antigenic determinants

Introduction

Current concepts of autoimmunity emphasize the role of antibodies in the development and maintenance of most autoimmune disorders (Allison, 1977). In the thyroid diseases, autoantibodies are directed against various components of the gland, such as thyroglobulin (Tgb), the microsomal antigen, the second colloid antigen, the cell-surface antigen(s), the antigen related to the thyrotropin receptor and the thyroid hormones, thyroxine and 3,5,3'-triiodothyronine (Pinchera *et al.*, 1980). Since the majority of autoantibodies reactive with self-components are polyclonal, a detailed study of their structure, specifity and pathogenic role using conventional hetero-antisera has been found to be very difficult. The hybridoma technology offers a unique opportunity to study the actual products of autoimmune diseases in molecular detail. Hybridomas resulting from the fusion of mouse myeloma cells with spleen cells from mice immunized with plasma membranes from human thyroid produced numerous monoclonal antibodies (mAb) reacting with human thyroid antigens. Several mAb were found to be directed against human Tgb (hTgb) which is a large glycoprotein specific to the thyroid (Lissitzky, 1981). The present report defines the characteristics of these mAb and their comparison with autoimmune antibodies (aAb).

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Results

Interaction of mAb with hTgb and related molecules

Culture supernatants of the 10 selected clones contained mAb able to bind the hTgb molecule as shown in Figure 1. Radioimmunoassay of the hTgb revealed that the mAb avidity for this molecule varied widely (Figure 1 lower part). Two mAb, J7C9.3 and J7C76.20, which were slightly displaced by hTgb, were also tested against hTgb with different iodine content to check if the antigenic determinants of the molecule recognized by these mAb were dependent on their iodination level. J8A53.9 mAb was taken as control. No difference was observed between the various Tgb which reacted similarly with each of the three mAb (data not shown). The Tgb molecule (660 000 daltons) is formed of two identical peptide chains of ~300 000 daltons. It also comprises 8 – 10% carbohydrates according to species, and iodotyrosines and idothyronines (Lissitzky, 1981), which represent potential antigenic determinants. No significant inhibition was detected with various concentrations of tyrosine, iodotyrosines and iodothyronines in the range 3 – 3000 μg/ml. Likewise, Tgb oligosaccharide units purified after pronase digestion of hTgb, which releases all the N-oligosaccharidic units (Cheftel *et al.*, 1964), were devoid of any significant effect on the binding of the mAb on hTgb at concentrations up to 30 μg/ml (data not shown). These results clearly indicated that all 10 mAb were directed against the peptide moiety of the hTgb molecule.

Species specificities of the mAb

As determined by Scatchard plot analysis, the equilibrium dissociation constants of mAb interaction with hTgb ranged from 8.2×10^{-11} M to 9.4×10^{-7} M (Table I).

The reactivity of the mAb was further studied with Tgb from simian, canine, porcine, bovine and ovine origin. As shown in Table I, eight mAb reacted with simian Tgb, three with canine and only one with porcine Tgb. Bovine and ovine Tgb were constantly ineffective at concentrations up to 10^{-5} M. With respect to hTgb affinity, mAb could be put into two groups: the first including J8B89.5, J7C44.6, J8A32.13, J7C73.7, J7B49.15 and J8A53.9 mAb which displayed a high K_D (8.2×10^{-11} M – 5.5×10^{-9} M) and the other being composed of J7C9.3, J7C6.20, J8B45.5 and J8B6.12 mAb which showed a weak avidity (2.3×10^{-8} M – 9.4×10^{-7} M). In the first group, two mAb exhibited a restricted specificity for hTgb; the other four mAb cross-reacted only with simian Tgb with an identical or a decreased affinity (as compared with hTgb). In the second group, all mAb cross-reacted with simian Tgb while J7C76.20, J8B45.5, J8B6.12 mAb cross-reacted with canine Tgb and only J8B45.5 with porcine Tgb; J7C9.3, J8B45.5

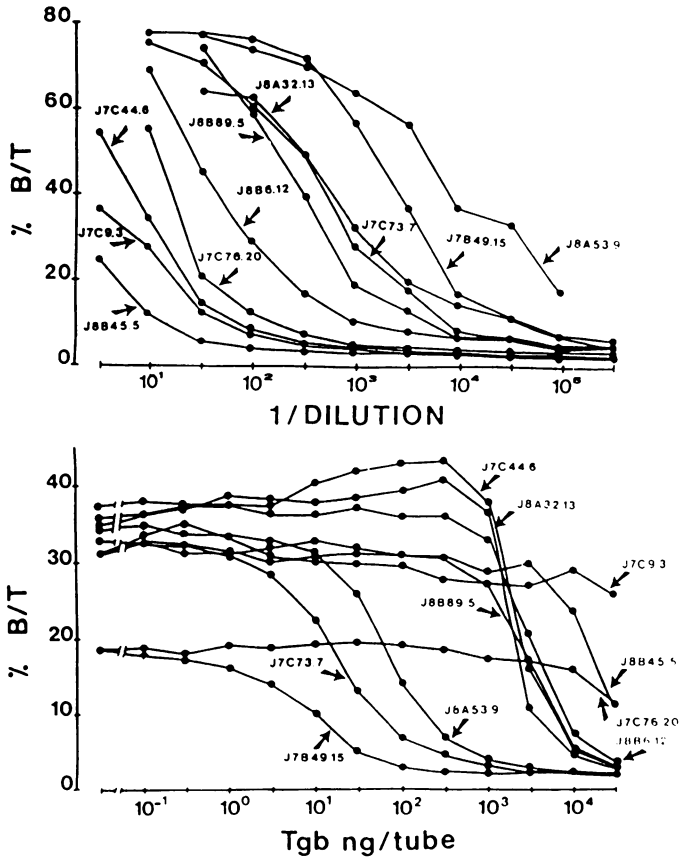

Fig. 1. Titration curves of the culture supernatants of the 10 clones producing anti-hTgb mAb **(upper part)**, and inhibition curves of radioiodinated hTgb binding to mAb by unlabeled hTgb **(lower part)**. Radioimmunoassay was carried out in a tube with the use of precipitating antiserum to separate the bound and the free [^{125}I]hTgb. Figures represent the arithmetic mean of duplicate assays.

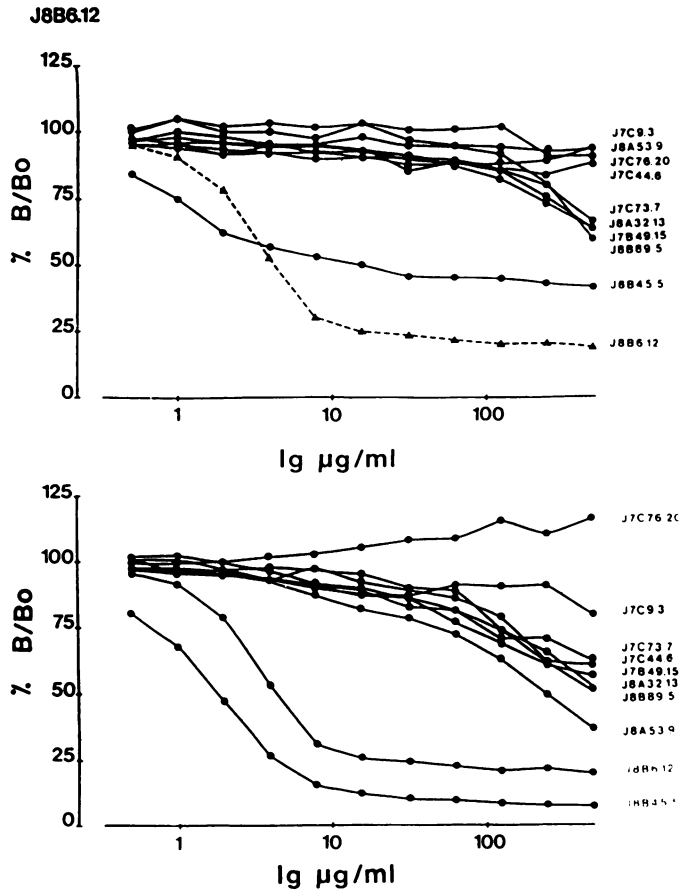

Fig. 2. Inhibition curves of radioiodinated J8B6.12 mAb binding to hTgb by the 10 unlabeled purified mAb **(upper part)**, and inhibition curves of the 10 radioiodinated mAb binding to hTgb by unlabeled purified J8B6.12 mAb **(lower part)**. Solid phase radioimmunoassay was carried out in purified hTgb-coated wells of microtiter plates. Results are expressed according to the maximal probe binding obtained with buffer instead of mAb inhibitor and are the arithmetic mean of duplicate assays.

Table I. Immunoglobulin class and species specificity of mAb

mAb	Ig class	Avidity for Tgb of various species (K_DM)			
		Human	Simian	Canine	Porcine
J7C9.3	γ1	7.5×10^{-7}	7.7×10^{-7}	(−)	(−)
J7C76.20	γ2b	9.4×10^{-7}	1.5×10^{-7}	8.5×10^{-7}	(−)
J8B45.5	γ1	3.4×10^{-7}	3.8×10^{-7}	4.4×10^{-6}	1.1×10^{-6}
J8B6.12	γ1	2.3×10^{-8}	3.0×10^{-8}	2.1×10^{-7}	(−)
J8B89.5	γ1	3.2×10^{-9}	3.0×10^{-9}	(−)	(−)
J7C44.6	γ1	5.5×10^{-9}	2.2×10^{-6}	(−)	(−)
J8A32.13	γ1	1.2×10^{-9}	(−)	(−)	(−)
J7C73.7	γ2a	4.0×10^{-10}	1.2×10^{-7}	(−)	(−)
J7B49.15	γ1	8.2×10^{-11}	(−)	(−)	(−)
J8A53.9	γ1	1.2×10^{-10}	3.1×10^{-9}	(−)	(−)

(−): $>1 \times 10^{-5}$ M.

and J8B6.12 mAb, showed the same avidity for human and simian Tgb and J7C76.20 mAb for human and canine Tgb.

Identification of different antigenic determinants on the hTgb molecule

In order to define the topology of the antigenic determinants or epitopes recognized by the different anti-hTgb mAb, cross-inhibition experiments were carried out as indicated in Materials and methods. Typical results are illustrated in Figure 2: the upper part represents the displacement curves of Tgb-bound radioiodinated mAb from clone J8B6.12 by the 10 mAb. Reciprocally the lower part represents the displacement curves of all Tgb-bound radioiodinated mAb by the unlabelled J8B6.12. This kind of experiment was carried out for the 10 mAb and the results are summarized in Figure 3. Displacement curves were classified into three groups: ineffective inhibition of binding at concentrations up to 500 µg/ml, partial inhibition which occurs at high mAb concentrations (>100 µg/ml), and complete inhibition occurring at low mAb concentrations (<100 µg/ml). On the figure chart each column represents an experiment as depicted in Figure 2 (upper part) and each line an experiment as depicted in Figure 2 (lower part). Those mAb capable of completely cross-inhibiting each other while their binding to Tgb was either partially or not inhibited by the other mAb were grouped in a cluster. Accordingly, six clusters can be distinguished. Cluster I comprises four mAb (J8A32.13, J7C73.7, J7B49.15, J8A53.9), cluster II two (J8B45.5, J8B6.12) and cluster III two (J8B89.5 and J7C44.6). The remaining mAb J7C9.3 and J7C76.20 were not completely displaced by any of the other mAb and consequently formed two individual clusters (V and VI). Cluster IV appears clearly by the juxtaposition of clusters I and III and includes four mAb (J7C44.6, J8A32.13, J7C73.7, J7B49.15). This indicates the inter-overlapping of various antigenic regions defined by the clusters of reactivity.

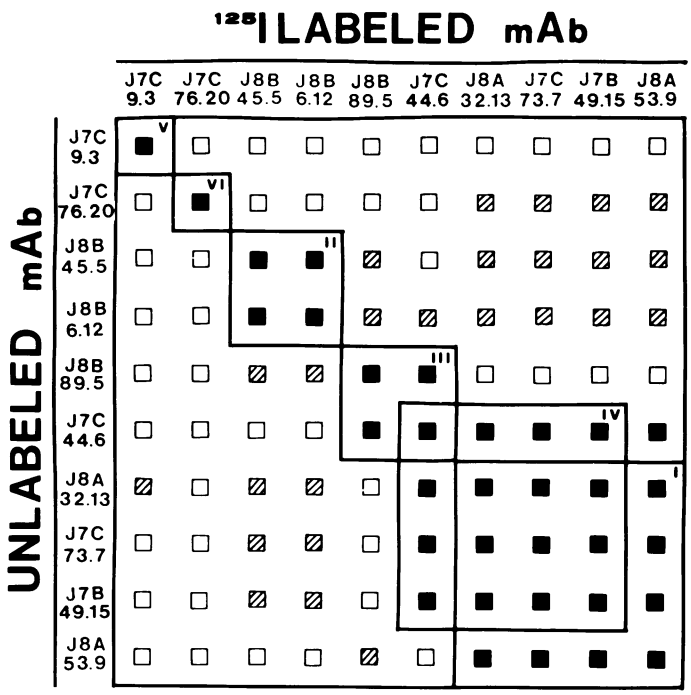

Fig. 3. Schematic representation of the inhibition curves of the 10 radioiodinated mAb by the 10 unlabeled mAb. Data obtained in Figure 2 are summarized by the fourth column and the fourth row. ■ refers to a complete inhibition; ▨ refers to a partial inhibition; □ refers to an absence of inhibition. Reactivity with identical antigenic regions are suggested by mAb which reciprocally achieved complete inhibition and accordingly are grouped in cluster (numbered solid line); interpenetration of antigenic regions is suggested by mAb which are located in several clusters (intersection of solid lines).

mAb of cluster I and IV failed to be grouped in the same cluster because J8A53.9 mAb displayed an asymmetric cross-inhibition pattern with mAb J7C44.6. This observation suggests that an asymmetric conformational change of the hTgb molecule or asymmetric steric hindrance between two mAb may occur after the binding of one of them. Likewise, J8B89.5 and J7C44.6 mAb which were grouped in the same cluster exhibited different patterns of cross-reactivity with mAb J8A32.13, J7C73.7, J7B49.15, J8A53.9; thus it was concluded that the antigenic determinants recognized by the two mAb of cluster III were different. Finally, symmetrical patterns of partial cross-inhibition were observed between mAb of cluster II and J8B89.5, J8A32.13, J7C73.7, J7B49.15. This strongly suggests the proximity of the various antigenic domains defined by cluster II on the one hand and clusters I, III, IV on the other, and is probably a consequence of the spatial localization of the distinct sites recognized by the mAb. These findings suggest that mAb are directed against various antigenic determinants on the hTgb molecule which appear rather distant (no cross-reactivity) or, in contrast, very close together (cluster).

Attempts to identify the antigenic determinants recognized by the mAb in the hydrolysis products of hTgb

The localization of the antigenic determinants detected by the various mAb was further studied by inhibition experiments using hydrolysis products of hTgb (see Materials and methods). The fractions obtained by gel filtration were tested for their ability to inhibit the binding of mAb. The activity profiles shown by the mAb differ slightly for the mAb of cluster III and widely for the other clusters IV, V and VI. The profiles obtained with the four mAb of cluster I were at least partially different (Figure 4). J8A32.13 mAb

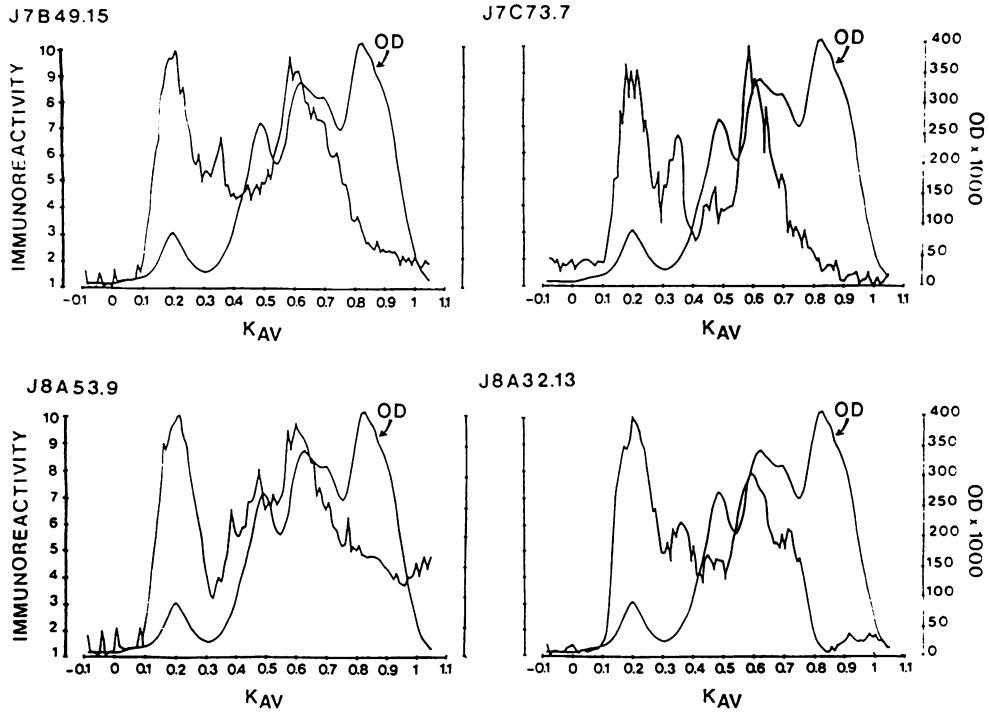

Fig. 4. Immunoreactivity profiles of the fractions obtained by gel filtration of hTgb tryptic digest with the mAb of cluster I. The tryptic hydrolysate was layered on a 1.6 x 82 cm ACA 34 column. Fraction volume was 1.2 ml. An aliquot of each fraction was tested for its inhibiting activity in solid phase radioimmunoassay using radiolabeled mAb. Determination of the optical density at 280 mm of each fraction was reported. Mol. wt. of each O.D. peak determined by calibration procedure was 549 500, 190 500, 114 800 and 57 500, respectively. K_{AV} of intact hTgb is 0.

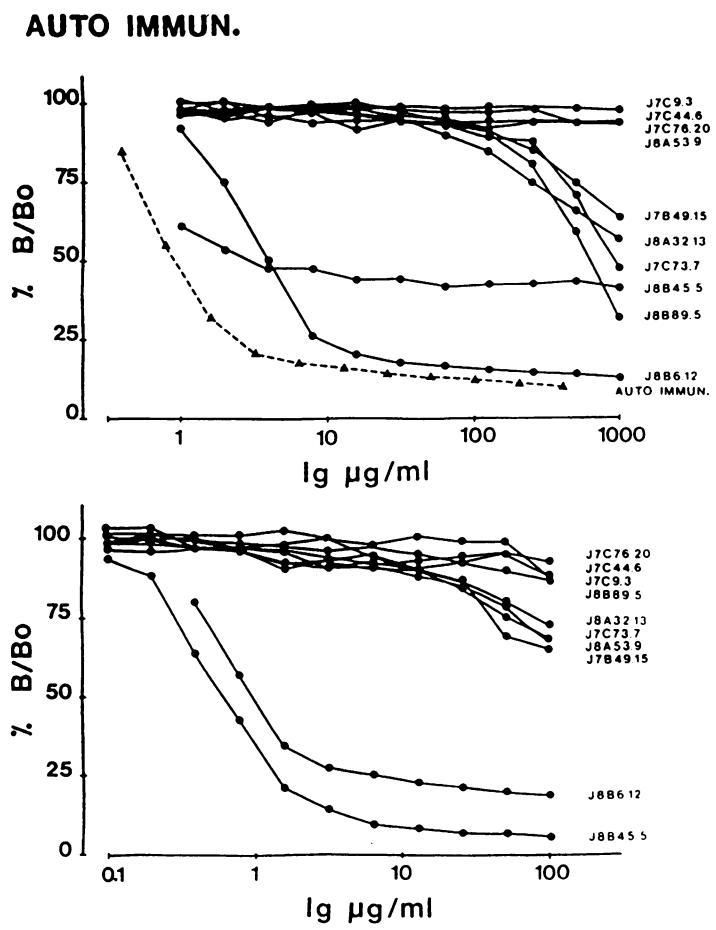

Fig. 5. Inhibition curves of radioiodinated aAb binding to hTgb by the 10 unlabeled purified mAb **(upper part)**, and inhibition curves of the 10 radioiodinated mAb binding to hTgb by unlabeled purified aAb **(lower part)**. See legend of Figure 2 for details.

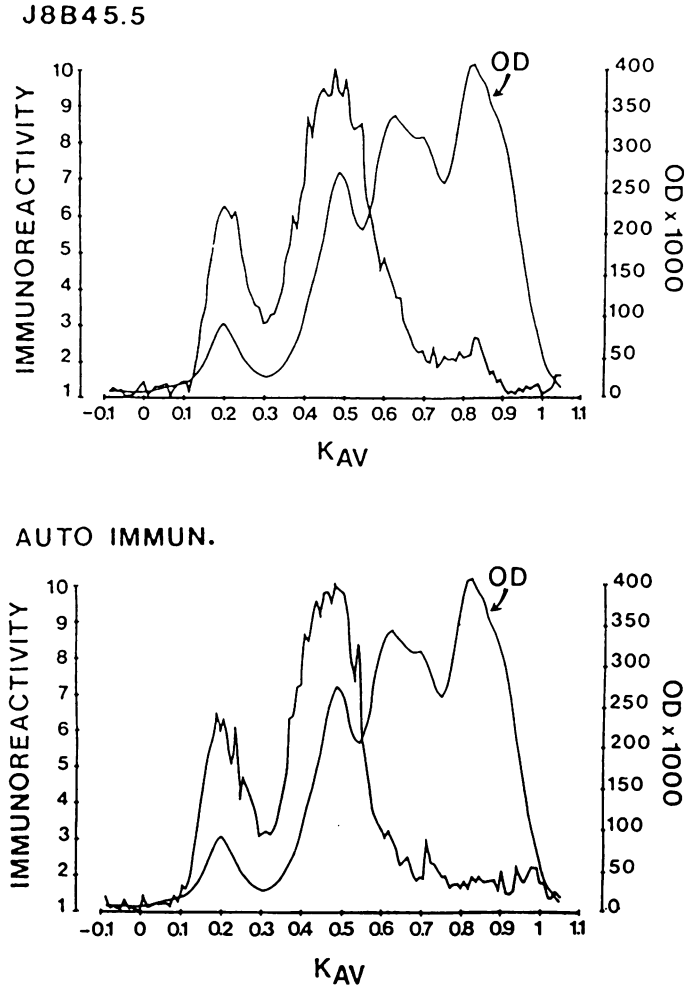

Fig. 6. Immunoreactivity profiles of the fractions obtained by gel filtration of hTgb tryptic digest with J8B45.5 mAb **(upper part)** and aAb **(lower part)**. See legend of Figure 4 for details.

discriminated five peaks of K_{AV} 0.20, 0.35, 0.48, 0.60 and 0.72, respectively. The other mAb were only able to detect four peaks in the case of J7C73.7 mAb and three in the case of J7B49.15 and J8A53.9 mAb.

Inhibition experiments performed with hTgb fragments obtained after 60 min incubation with trypsin showed increased differences among mAb (data not shown). The first O.D. peak disappeared and the corresponding fractions were not able to react as strongly as previously. These data confirmed that mAb from the same cluster (reacting with the same antigenic region) may be directed against different antigenic determinants. As shown in Figure 4, J7B49.15, J7C73.7, J8A53.9 and J8A32.13 mAb that exhibited bidirectional cross-inhibition of binding, thereby defining a cluster of determinants (called cluster I), seem to be directed against adjacent but separate antigenic sites.

Identification of the autoimmune antigenic determinants on the hTgb molecule

aAb directed against Tgb were purified from a pool of sera from patients with Graves' disease. To find which mAb interact with the antigenic regions recognized by the aAb, cross-inhibition experiments were undertaken. As shown in Figure 5 (upper part), the binding of radioiodinated aAb to hTgb was inhibited at low Ig concentrations (1 – 10 µg/ml) only by mAb of cluster II. Inhibition by mAb J8B89.5, J8B45.5, J7C73.7, J8A32.13 and J8B49.15 from other clusters was obtained at higher Ig concentrations (100 – 1000 µg/ml). The remaining mAb J7C79.3, J7C744.6, J7C76.20 and J8A53.9 were completely ineffective in displacing labeled aAb. Reciprocally, the hTgb binding of the 10 radioiodinated mAb by aAb (Figure 5, lower part) was observed at low Ig concentrations (<10 µg/ml) in the case of mAb from cluster I, whereas only partial inhibitions occurred at concentrations up to 10 µg/ml for J8A32.13, J7C73.7, J8A53.9 and J7B49.15 mAb. No inhibition of the other mAb was observed at aAb concentrations up to 100 µg/ml. The identification of the binding site of aAb was further studied by inhibition experiments of aAb binding to hTgb by hydrolysis products of hTgb (see Materials and methods). The hTgb fragments obtained by tryptic digestion for 30 min displayed an immunoreactivity profile with aAb which closely paralleled that obtained with J8B45.5 mAb (Figure 6) and differed to various extents from those observed with the other mAb (compare Figures 4 and 6).

Discussion

A precise characterization of the antigens observed in autoimmune thyroid diseases is possible and may be very important for the understanding of their pathogenesis. Regarding

Tgb, a macromolecule which, after heterologous immunization in rabbit, yields antibodies directed against up to 40 determinants (Roitt *et al.*, 1958), the situation is very complex and it is obvious that mAb may be usefully employed to characterize the heterogeneity of antigenic determinants. The interaction of the 10 mAb with hTgb and related molecules indicated that the antigenic determinants recognized by the mAb were part of the peptide moiety of the molecule excluding the N-oligosaccharide units, which are known to be poorly antigenic, as well as thyroid hormones. The fact that these latter did not interfere in the hTgb binding of the mAb was possibly due to the immunogen chosen for immunization. It is possible that the human thyroid membrane preparations injected into mice contained poorly iodinated Tgb, as is the case with Tgb encapsulated in exocytotic vesicles. This is consistent with data suggesting that exocytotic vesicles contribute to the membrane material of the apical cell surface (Ekholm *et al.*, 1975). Scatchard plot analysis showed that some mAb displayed the same avidity for two different Tgb species and consequently could be directed against phylogenetically conserved determinants. Conversely, differences between K_D values give evidence for disparities in the protein structure between species. However, it is noteworthy that none of our 10 mAb showed the same species cross-reactivity pattern in terms of mAb avidity. Mapping of the determinants recognized by mAb was carried out by cross-inhibition analysis. Similar experiments were previously reported in studies on the mouse Ia antigens (Pierres *et al.*, 1981). In these experiments, if the binding of one mAb is inhibited by the prior binding of another mAb, it can be proposed from the steric hindrance observed that the antigenic determinants reacting with those mAb are close to each other. Conversely, the total absence of steric hindrance in the binding of two mAb on the same molecule proves that the antigenic determinants are different and distant from one another. This analysis allowed us to describe six clusters of antigenic reactivity on the hTgb molecule using our 10 mAb. Inhibition experiments with various hydrolysis products of hTgb have confirmed the heterogeneity of the fine antigenic specificity of the mAb. Taking into account the whole of the data on each mAb described in this report, it is apparent that all mAb interact with different antigenic determinants on hTgb and that several polymorphic areas topologically distant from each other probably exist on the Tgb gene product. This study shows a much less limited repertoire of antigenic determinants than was previously suspected by heterologous antisera; it can be postulated that additional mAb should reveal other antigenic determinants. Of the large number of potential antigenic determinants on Tgb, aAb to Tgb are directed towards a restricted number of antigenic determinants (Roitt *et al.*, 1958; Shulman and Witebsky, 1960; Nye *et al.*, 1980). The positive identification of well defined antigenic determinants capable of binding to autoantibodies with reasonable affinities strongly suggests, but does not prove, the role of these determinants in triggering autoantibody formation.

Anti-Tgb aAb of patients with Graves' disease interacted essentially with mAb of the cluster II which represents one of the six antigenic regions evidenced by the mAb. Only such a characterization of the fine antigenic specificity of antibodies can determine the extent of diversity of these autoantibodies in thyroid disorders. Preliminary experiments suggested that the cross-reactivity pattern of the 10 mAb with unique aAb were changed according to the patients and to their disease. However, mAb of cluster II always cross-reacted strongly with aAb (data not shown). Thus, in all cases, aAb did not interfere with the binding of several mAb. This offers a unique possibility for the assay of hTgb in sera containing anti-Tgb aAb which are known to interfere in all the Tgb radioimmunoassays already described (Pacini *et al.*, 1980; Schneider and Pervos, 1978). Establishment of stable hybridomas secreting mAb to Tgb is the first step in the production of various anti-thyroid mAb and such material will provide invaluable probes for the study of the native structure of this protein and for investigation of the nature of Tgb in various thyroid disorders.

Materials and methods

Production of the anti-Tgb mAb

The mAb used in this study were derived from two distinct fusions, J7 and J8. For fusion J7, BALB/c mice were hyperimmunized i.p. using three successive injections of 300 μg of purified human thyroid plasma membrane in 5% Maalox (aluminium and magnesium hydroxide, Rorer, Paris, France) and 25% heat-killed *Bordetella pertussis* vaccine (Michigan Department of Public Health, Lansing, MI) at 15 day intervals. For fusion J8, we employed complete and then incomplete Freund adjuvant (v/v) in i.d. injections for the first three injections of membranes. Three days after a last i.v. boost of 50 μg of immunogen in saline, immune spleen cells were hybridized with the non-secreting mouse myeloma x63-Ag8-653 (Kearney *et al.*, 1979) according to a previously described technique (Galfré *et al.*, 1977) with minor modifications (Pierres *et al.*, 1979; Ruf *et al.*, 1983) in a myeloma/spleen cell ratio of 1:10. After fusion, the cells were seeded in 384 wells of 96 well microtiter plates (Greiner, Bischwiller, France) with BALB/c mouse peritoneal macrophages as feeders. The selected medium was removed 5 days after fusion and the supernatants from growing hybrid cultures were screened at days 17 to 30 using first a membrane binding radioimmunoassay (see below). Selected hybridomas were cloned by limiting dilution. Cloned hybridomas were grown as ascites in pristane primed BALB/c mice. mAb were purified from defibrinated ascitic fluid precipitated by ammonium sulfate on a protein-A Sepharose column (Pharmacia, Uppsala, Sweden), for IgG_{2a} or IgG_{2b} mAb, or DEAE Trisacryl column (IBF, Villeneuve la Garenne, France) for IgG_1 mAb. After elution, mAb were dialyzed against phosphate bufferd saline (PBS) pH 7.3. The Ig class of the mAb was determined by the Ouchterlony immunodiffusion technique using a set of class-specific rabbit anti-mouse IgG sera.

Screening procedures

Indirect membrane binding radioimmunoassay was performed in a 96 V-form wells plate (Greiner, Bischwiller, France). 50 μl of culture supernatant were mixed with 26.9 μg of ammonium sulfate-treated human thyroid membranes (Pekonen and Weintraub, 1980) suspended in 50 μl of PBS-0.2% bovine serum albumin (BSA). After 2 h at room temperature, the wells were filled with 100 μl of PBS buffer containing 10% polyethylene glycol. The plate was centrifuged for 30 min at 1500 *g* and the supernatant discarded. 50 μl of ^{125}I-labeled immunopurified $F(ab')_2$ goat anti-mouse Ig antibody was added to each pellet (~150 000 c.p.m./50 μl) and incubated for 1 h at room temperature. The unbound radioactivity was then removed by centrifugation and the pellets were washed four times with PBS-0.2% BSA. Each pellet was transferred in microtubes and the bound radioactivity counted.

Thyroglobulin solid phase radioimmunoassay was carried out in 96 wells of flexible polyvinyl chloride microtiter plates (Linbro, S-MRC 96, Flow Lab., Hamden, CO). The wells were coated overnight at 4°C with 50 μl of a solution of 50 μg/ml of purified human Tgb in PBS. The protein solution was then removed and kept at −20°C for further use. The wells were filled for 1 h at room temperature with 150 μl of PBS-1% BSA and plates were washed with PBS-0.2% BSA, dried and immediately used for the test. Each Tgb-coated well was incubated with 50 μl of hybridoma culture supernatant for 90 min at room temperature. The wells were then washed and incubated for 90 min at room temperture with 50 μl of ^{125}I-labeled immunopurified $F(ab')_2$ goat anti-mouse Ig antibody (~150 000 c.p.m./50 μl), washed again, heat dried, cut into individual tubes and counted. Irrelevant mAb and PBS-0.2% BSA served as controls.

^{125}I-labeled human Tgb immunoassays

Direct binding of radiolabeled hTgb to anti-Tgb mAb was carried out in 5 ml plastic tubes filled with 100 μl of PBS-0.2% BSA containing various dilutions of lyophilized culture supernatant, brought to 70 mg/ml and 100 μl of the hTgb (~15 000 c.p.m.) labeled by the lactoperoxidase method. Incubation was performed overnight at 4°C. Normal mouse serum and goat anti-

mouse immunoglobulin serum were then added at appropriate dilutions in a final volume of 400 μl to form immunoprecipitating complexes. After a second overnight incubation at 4°C, 2 ml of PBS-0.2% BSA containing 1.5 g/l of corn starch was added to each tube before centrifugation at 1000 *g* for 20 min. The supernatant was then carefully pipetted off and radioactivity was counted.

Inhibition of binding of radiolabeled hTgb to mAb was assessed in the same way but with preliminary incubation of the mAb overnight at 4°C at given concentrations (20–40% B/T) with various concentrations of inhibitor prior to addition of the probe.

Enzymatic digestion and separation of hydrolysis products

Pronase treatment was performed on the hTgb molecule (250 mg) in 40 mM ammonium bicarbonate pH 8.00 at a final enzyme to substrate ratio of 1:10 (w/w) for 3 days at 37°C. After freeze-drying, hydrolysis products were separated on a Sephadex G-25 column and the first peak was shown (orcinol test) to contain the carbohydrate components of Tgb. Purification of this material was achieved by chromatography on a G-100 column to remove pronase. 20 mg of oligosaccharide units were purified (i.e. 8% of the starting material).

Digestion by trypsin was performed on 10 mg hTgb in 50 mM Tris pH 8.00 at 37°C for 30 min at an enzyme to substrate ratio of 1:100 (w/w). The reaction was stopped by addition of 0.5 M final phenylmethylsulfonyl fluoride. Hydrolysis products were separated on an Ultrogel ACA 34 column equilibrated in Tris buffer. The column was calibrated with Tgb (660 000 daltons), ferritin (440 000), catalase (23 200) and aldolase (15 800). The elution fractions were expressed in K_{AV} calculated as follows: $x - v_o / v_t - v_o$ (with v_o, void volume; v_t, total volume of the gel and x, the fraction number).

^{125}I-labeled mAb immunoassays

To investigate whether the determinants on the hTgb molecule recognized by the mAb were different, the binding of each radiolabeled mAb on hTgb-coated plates was measured after previous incubation (90 min at room temperature) with various concentrations of the same or other unlabeled purified mAb. This study was completed by inhibition experiments of the radiolabeled mAb on hTgb-coated plates with hydrolysis products of hTgb tryptic digestion. The solid phase radioimmunoassay used in these experiments was described above (see screening procedures).

Materials

Human thyroid plasma membranes (d = 1.16 g/ml) were prepared from surgically removed Graves' goiters by discontinuous sucrose gradient centrifugation (Carayon *et al.*, 1979). Tgb from man and other species were prepared as described (Marriq *et al.*, 1977) and were the generous gift of C.Marriq. Autoimmune antibodies against hTgb were prepared from a pool of plasma from 48 patients with Graves' disease, precipitated with ammonium sulfate and purified by affinity chromatography of hTgb coupled to CNBr-activated Sepharose 4B. Elution buffer was 0.1 M glycine-HCl, 0.5 M NaCl pH 2.00.

Acknowledgements

The authors wish to thank Mireille Ferrand for expert technical assistance and Nicole Hastert for excellent secretarial help.

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