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IgG subclass distribution of thyroglobulin antibodies in patients with thyroid disease

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SUMMARY

The IgG subclass distribution of thyroglobulin antibodies (TgAb) has been studied in Hashimoto and Graves' patients by several investigators with conflicting results, in part explainable by methodological problems. We have recently developed a quantitative ELISA to measure in absolute terms the serum concentration of TgAb subclasses. The aim of the present study was to apply this method in a large series of patients with autoimmune as well as, for the first time, non-autoimmune thyroid diseases. We examined 28 patients with Hashimoto's thyroiditis, 30 with Graves' disease, 21 with thyroid carcinoma and 18 with non-toxic goitre, all selected for the presence of TgAbs. The results indicated that TgAbs in thyroid diseases were not restricted to any particular isotype, but comprised all four IgG subclasses. IgG1 was represented similarly in the four groups. The same was true for IgG3, even though its contribution to the total antibody content was very small. IgG4 was the dominant subclass in patients with Graves' disease, thyroid carcinoma and non-toxic goitre, probably reflecting a prolonged antigenic challenge. In Hashimoto's thyroiditis IgG2 was dominant, possibly because T helper lymphocytes infiltrating the thyroid are typically Th1 type.

Keywords: thyroglobulin antibodies IgG subclasses

INTRODUCTION

Circulating antibodies directed against thyroglobulin (TgAb) are principally of the IgG isotype [1]. The major Fc-mediated functions of IgG include the activation of complement via the classical pathway, and the targeting of bound antigen for elimination through phagocytosis and antibody-dependent cell-mediated cytotoxicity (ADCC) [2]. Among the four IgG subclasses, IgG1 and IgG3 have the highest affinity for C1q [3] and the strongest ADCC [4]. Protein antigens preferentially induce IgG1 and IgG3 or IgG1 and IgG4, whereas the response to polysaccharides is restricted in most cases to IgG2 [5].

The IgG subclass distribution of TgAbs has been studied, with conflicting results. The early reports of Hay & Torrigiani [6] and Spiegelberg [7], using a co-precipitation radioimmunoassay with radiolabelled thyroglobulin and polyclonal rabbit anti-IgG subclass sera, showed that TgAbs in Hashimoto patients were not restricted to a particular subclass, but closely paralleled the relative amount of each subclass in normal serum. On the other hand, a second group of investigators [8–12] suggested a restriction to some IgG subclasses in response to thyroglobulin. Using an ELISA with a panel of mouse MoAbs directed against the four IgG subclasses, they reported that TgAbs in Hashimoto and also Graves' patients are restricted to IgG1 and IgG4. A third group of investigators did not find an absolute restriction of the TgAbs to any particular subclass, with IgG1, IgG2 and IgG4 in roughly equivalent amount as whole sera [13]. This finding was confirmed using individual subclasses purified on affinity columns [14].

None of the studies reported above expressed the concentrations of the four IgG subclasses in absolute terms, due to a lack of a standard containing defined amounts of IgG1, IgG2, IgG3 and IgG4 for TgAbs. We have recently reported the development of a quantitative ELISA to measure in absolute terms the concentrations of TgAb IgG subclasses in human sera [15]. With this assay, we showed that TgAbs in Hashimoto patients were not restricted to IgG1/IgG4, being distributed among the IgG1, IgG2 and IgG4 isotypes.

The aims of this study were to expand our previous work on subclass distribution of TgAbs in Hashimoto patients, to

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analyse a number of well characterized patients with Graves' disease (GD), and to study for the first time patients with non-autoimmune thyroid diseases: non-toxic multinodular goitre and differentiated thyroid carcinoma.

PATIENTS AND METHODS

Study population

The study population included 97 patients all selected on the basis of positive TgAbs. The non-toxic multinodular goitre group included 18 patients (one male, 17 females, mean age 45·8 years) submitted to partial thyroidectomy for the presence of cervical obstructive signs and symptoms. Diagnosis was based on the presence of an enlarged thyroid, not associated with hyper- or hypothyroidism, containing two or more echographically defined nodules, benign on fine needle aspiration (FNA) biopsy. All sera were obtained after thyroidectomy, during L-thyroxine therapy, and in all cases TgAbs were present before, and persisted after, thyroidectomy. Histological examination showed multinodular macrofollicular goitre and no evidence of coexistent Hashimoto's thyroiditis (HT).

The differentiated thyroid carcinoma group included 21 patients (18 papillary and three follicular; three males and 18 females, mean age 36·7 years). Sera were obtained in euthyroidism ($n = 12$) during L-thyroxine therapy or in hypothyroidism ($n = 9$) after withdrawal of L-thyroxine for ^{131}I total body scan, 1–13 years after total thyroidectomy. None of these patients had a history of HT before the diagnosis of cancer, and in all cases TgAbs were present before (and persisted after) thyroidectomy.

The GD group included 30 patients (four males, 26 females; mean age 37·9 years). Eight patients were untreated with active thyrotoxicosis, 18 were euthyroid on methimazole treatment, and four hypothyroid after ^{131}I administration. Twelve patients had no eye involvement, while 18 had evidence of severe Graves' ophthalmopathy, assessed according to Mourits *et al.* [16].

The HT group included 28 patients. Twenty-two patients had the classic (or goitrous) variant of thyroiditis (all females; mean age 46·3 years). Diagnosis was based on the presence of a firm, symmetrically enlarged, thyroid, associated with positive TgAbs and/or thyroid peroxidase antibodies. Ultrasound scan of the thyroid revealed in all cases a hypoechogenic pattern. Primary hypothyroidism was present in the 17 out of 22 patients; the remaining five were euthyroid. FNA biopsy showing lymphocytes was available in six hypothyroid and in all euthyroid patients. Six patients had the atrophic variant (idiopathic myxedema) of thyroiditis (one male, five females; mean age 49·5 years). Diagnosis was based on the presence of long-standing primary hypothyroidism associated with positive TgAbs and/or thyroid peroxidase antibodies. Thyroid echography showed in all cases the atrophy of the gland.

Serological and clinical examinations

Serum samples were kept frozen at −20°C until use, and repeated thawing and freezing was avoided. TgAbs were determined utilizing in all cases two different methods previously described: chromic chloride haemagglutination [17] and ELISA [18]. Free thyroxine, free triiodothyronine, thyroid-stimulating hormone (TSH) and thyroid peroxidase antibodies were assayed by commercial kits (free thyroxine and free triiodothyronine by Lysophase, Technogenetics S.p.A., Milan, Italy; TSH by Incstar Corporation, Stillwater, MN; thyroid peroxidase antibodies by Sorin biomedica S.p.A., Saluggia, Italy).

Thyroid ultrasound examinations were performed by one of us (P.C.) with a real time instrument (Aloka SSD 121, Aloka Co. Ltd., Tokyo, Japan).

Thyroglobulin preparation

A thyroid gland was obtained at autopsy from a man with no history of thyroid diseases and stored at −70°C until use. The thyroid was sliced in borate buffer and centrifuged at 45 000 ***g*** for 90 min. The insoluble material was discarded and the thyroglobulin was purified on a Sephacryl S-400 column (Pharmacia Fine Chemicals, Uppsala, Sweden). After filter sterilization, aliquots were frozen at −70°C until use.

Subclass-specific monoclonal reagents and human thyroglobulin affinity standard

The specificity of the mouse monoclonal anti-human subclass-specific reagents utilized has been extensively documented in an IUIS/WHO international collaborative study [19]. These monoclonals (HP 6001 and HP 6069 for IgG1; HP 6002 and HP 6014 for IgG2; HP 6047 and HP 6050 for IgG3; HP 6020 and HP 6025 for IgG4) were used in pairs for each IgG subclass, at 1:1000 dilution, in order to increase the sensitivity of the assay. Furthermore, for IgG2 and IgG4 the monoclonals were directed against spatially distinct epitopes, allowing a cooperative reactivity. At least one monoclonal for each subclass was in the list recommend by IUIS/WHO.

The human thyroglobulin affinity standard was previously generated [15] combining high titered plasma samples from three Hashimoto patients, chosen based on preliminary work that indicated that antibodies from all four subclasses were represented. The concentration of each IgG subclass for this standard diluted 1:300 was: 187 mg/*l* for IgG1; 629 mg/*l* for IgG2; 34 mg/*l* for IgG3, and 94 mg/*l* for IgG4. The standard was run at eight doubling dilutions in each plate.

ELISA specific for IgG subclasses of TgAbs

This assay was performed as previously described [15]. Briefly, 96-well plates (Corning, Corning, NY) were coated with 10 μg human thyroglobulin and blocked with PBS–1% bovine serum albumin (BSA). Serum samples, tested in duplicate at four doubling dilutions (starting at 1:100), were incubated, and after extensive washing, subclass-specific monoclonals were added. After incubation with goat anti-mouse IgG alkaline phosphatase conjugate, the *p*-nitrophenyl phosphate was added at 1 mg/ml in diethanolamine buffer at pH 9·8. Colour development at 405 nm (single wavelength endpoint) was measured with a V-Max ELISA reader (Molecular Devices, Menlo Park, CA). A standard curve was generated using the eight doubling dilutions of the affinity-purified standard [15]. To eliminate interplate variability, the standard was included in each plate together with a normal control serum to monitor non-specific binding. Subclass concentration was interpolated from the standard curve using a spline fit program (SoftMax, by Molecular Devices) which statistically analyses the variation of individual data points and the data in relation to the standard. The program computes the concentration of antibody based on the affinity-purified standard control values as well as assessing parallelism.

Statistical analysis

To assess the differences among the four patient groups (goitre, cancer, GD and HT) regarding the mean concentration of IgG specific for thyroglobulin, we have initially used the analysis of variance (ANOVA). We have then performed the *post hoc* comparisons between pairs of groups using the Scheffe's test. The level of significance chosen to reject the null hypothesis was 0·05. Pearson product–moment correlation was used to determine the association between the titre of TgAbs and the total concentration of thyroglobulin-specific IgG. Statistics was carried out using StatView 4.0 for Macintosh (Abacus Concepts Inc., Berkeley, CA).

Table 1a. IgG subclass concentrations of thyroglobulin antibodies in serum of patients with non-toxic multinodular goitre

Patient no.	ELISA titre	HA titre	IgG1 (mg/*l*)	IgG2 (mg/*l*)	IgG3 (mg/*l*)	IgG4 (mg/*l*)	Total IgG (mg/*l*)
1	3	0	0·4	0·5	0·0	0·2	1·2
2	5	0	0·2	0·6	0·0	0·1	0·9
3	6	1	0·5	0·8	0·0	0·1	1·4
4	6	5	4·8	1·5	0·6	3·5	10·4
5	7	10	0·8	2·1	0·0	14·7	17·6
6	8	0	0·7	1·0	0·3	1·0	3·0
7	8	6	1·1	0·5	0·1	0·4	2·0
8	9	0	4·1	0·1	0·0	1·0	5·3
9	10	6	0·9	0·8	0·6	2·9	5·2
10	10	13	5·2	0·3	0·6	2·6	8·6
11	11	0	0·5	1·1	0·1	2·1	3·8
12	11	9	2·5	1·0	0·2	24·5	28·2
13	11	9	16·5	7·1	0·6	3·5	27·7
14	11	11	1·3	1·3	0·3	26·1	29·0
15	11	11	5·5	0·8	4·5	5·7	16·5
16	12	9	0·5	5·1	0·2	53·9	59·6
17	13	10	10·8	6·6	0·1	51·1	68·5
18	13	13	9·5	7·5	0·8	79·3	97·1
Mean			3·7	2·2	0·5	15·1	21·4
s.d.			4·5	2·5	1·0	23·3	27·3

Table 1b. IgG subclass concentrations of thyroglobulin antibodies in serum of patients with differentiated thyroid carcinoma

Patient no.	ELISA titre	HA titre	IgG1 (mg/*l*)	IgG2 (mg/*l*)	IgG3 (mg/*l*)	IgG4 (mg/*l*)	Total IgG (mg/*l*)
19	4	0	0·3	0·1	0·1	0·1	0·6
20	6	1	0·4	0·4	0·1	0·1	1·0
21	7	6	0·3	1·6	0·0	3·1	5·1
22	7	6	0·4	0·3	0·0	0·1	0·8
23	7	8	0·5	0·9	0·1	0·4	1·8
24	7	10	3·5	2·0	0·0	2·3	7·9
25	8	10	1·7	0·2	0·0	3·2	5·1
26	10	5	0·3	0·9	0·1	4·3	5·5
27	10	8	1·2	2·0	0·1	20·0	23·3
28	10	9	1·9	1·0	0·0	0·8	3·8
29	10	10	1·1	1·5	0·1	13·2	15·8
30	10	11	3·7	1·7	0·0	0·4	5·8
31	10	12	9·0	7·3	0·1	3·3	19·6
32	11	7	2·8	2·4	0·1	44·5	49·7
33	11	9	2·1	2·4	0·0	20·7	25·2
34	11	11	3·2	0·6	9·0	2·1	14·8
35	11	11	11·0	0·8	0·5	0·4	12·7
36	11	12	13·1	3·4	0·1	3·3	19·8
37	12	8	0·2	1·1	0·0	3·3	4·5
38	18	14	78·1	20·4	1·0	78·2	177·7
39	19	17	1·8	17·3	8·2	94·7	122·0
Mean			12·4	6·2	1·8	27·1	47·5
s.d.			16·8	5·4	2·6	26·4	44·0

RESULTS

Anti-thyroglobulin subclass concentration

Tables 1 and 2 report the TgAbs, measured by ELISA and haemagglutination, and the subclass distribution of IgG specific for thyroglobulin in the individual patients, subdivided according to the clinical diagnosis.

We have initially analysed the contribution of each IgG subclass to the total concentration of thyroglobulin-specific

Table 2a. IgG subclass concentrations of thyroglobulin antibodies in serum of Graves' patients, with or without ophthalmopathy

Patient no.	Status	ELISA titre	HA titre	IgG1 (mg/l)	IgG2 (mg/l)	IgG3 (mg/l)	IgG4 (mg/l)	Total IgG (mg/l)
40	↑	7	4	0·2	0·2	0·0	0·8	1·2
41	↑	7	5	0·2	0·2	0·0	1·0	1·3
42	↑	7	13	17·4	4·5	0·2	18·5	40·7
43	↑e	8	0	2·6	0·1	0·0	1·2	4·0
44	↑	8	0	1·4	2·5	0·0	0·5	4·5
45	↑	10	5	2·0	0·1	0·0	0·1	2·2
46	↑	10	8	0·2	0·3	0·0	0·2	0·7
47	↑e	12	12	4·7	4·1	0·1	0·6	9·5
48	–e	1	0	0·1	0·1	0·0	0·0	0·2
49	–e	7	12	16·1	4·3	0·3	48·6	69·3
50	–	9	7	2·2	0·7	0·0	2·1	5·0
51	–	18	19	77·4	15·1	0·5	303·5	396·5
52	–e	4	0	1·7	0·1	0·0	1·1	2·9
53	–e	5	0	0·7	0·8	0·1	0·1	1·7
54	–	10	8	21·2	1·4	0·0	28·3	50·9
55	–e	10	0	0·4	0·0	0·0	0·1	0·5
56	–e	10	3	1·5	0·1	0·9	0·1	2·5
57	–e	10	5	0·5	0·7	0·1	0·3	1·6
58	–e	10	9	3·9	0·3	0·0	3·1	7·3
59	–e	10	9	1·3	1·0	0·1	15·2	17·4
60	–	10	10	2·3	1·0	1·0	0·4	4·7
61	–e	11	12	2·7	5·0	0·9	13·9	22·4
62	–	12	5	2·3	4·9	0·2	52·7	60·1
63	–e	12	10	1·6	1·7	0·0	25·9	29·3
64	–	14	13	23·9	7·6	0·3	18·7	50·5
65	–e	19	17	111·3	69·5	7·4	223·1	411·3
66	↓e	2	0	0·7	0·3	0·8	0·5	2·3
67	↓e	10	6	1·7	6·3	0·0	30·3	38·3
68	↓e	17	18	24·5	123·5	0·4	121·3	269·7
69	↓e	19	19	29·8	48·8	0·3	89·1	168·0
Mean				11·9	10·2	0·5	33·4	55·9
s.d.				24·4	26·1	1·4	69·4	110·2

↑, Hyperthyroidism; –, euthyroidism; ↓, hypothyroidism; e, ophthalmopathy.

Table 2b. IgG subclass concentrations of thyroglobulin antibodies in serum of patients with goitrous Hashimoto's thyroiditis (HT) and idiopathic myxedema (IM)

Patient no.	Status	ELISA titre	HA titre	IgG1 (mg/l)	IgG2 (mg/l)	IgG3 (mg/l)	IgG4 (mg/l)	Total IgG (mg/l)
70	↓	7	11	2·4	1·7	1·0	0·3	5·3
71	↓	10	15	0·8	0·7	0·0	0·1	1·6
72	↓	11	11	53·1	10·6	2·7	2·0	68·5
73	↓	12	12	1·1	1·9	0·5	2·6	6·1
74	↓	14	14	28·0	39·3	2·8	21·7	91·7
75	↓	14	13	1·5	5·0	0·1	2·9	9·5
76	↓	15	16	1·3	13·4	0·1	1·5	16·3
77	↓	16	17	54·2	124·5	1.1	10·6	190·4
78	↓	16	15	70·2	1001·0	8·5	353·0	1433·1
79	↓	16	14	11·8	7·9	0·1	0·5	20·2
80	↓	16	20	2·2	117·2	0·7	2·7	122·8
81	↓	17	20	16·6	110·2	0·2	12·4	139·4
82	↓	18	18	139·1	19·1	0·1	0·2	158·5
83	↓	18	15	26·5	54·2	0·1	14·3	95·2
84	↓	19	17	24·7	4·2	0·2	5·7	34·7
85	↓	20	17	140·3	124·5	4·2	11·3	280·3
86	↓	20	20	523·5	14·9	4·4	545·0	1087·7
87	–	8	1	0·8	0·8	0·0	0·1	1·7
88	–	10	8	1·8	0·9	1·1	4·1	7·9
89	–	10	8	0·9	0·3	0·0	0·1	1·3
90	–	13	16	11·1	14·3	0·4	7·2	32·9
91	–	14	14	7·2	2·5	0·1	0·4	10·2
92	im	2	4	0·2	0·4	0·0	0·1	0·8
93	im	10	3	1·2	0·5	0·0	0·1	1·8
94	im	15	15	9·0	9·3	0·1	0·6	18·9
95	im	17	14	1·1	11·8	0·1	44·7	57·7
96	im	17	15	1·9	9·1	0·2	0·7	11·9
97	im	18	19	18·8	61·9	1·1	4·0	85·8
Mean				41·1	62·9	1·1	37·5	142·6
s.d.				101·8	188·4	1·9	119·5	326·5

↓, Hypothyroidism; –, euthyroidism.

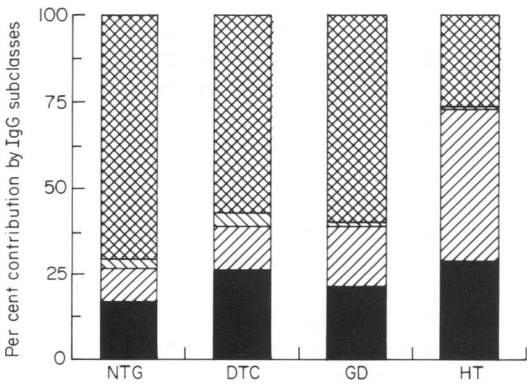

Fig. 1. Percentage of contribution by IgG subclasses of thyroglobulin antibodies in patients with non-toxic goitre (NTG), differentiated thyroid carcinoma (DTC), Graves' disease (GD) and Hashimoto's thyroiditis (HT). ■, IgG1; ▨, IgG2; □, IgG3; ▩, IgG4.

IgG in the different diagnostic categories. Figure 1 illustrates that IgG1 was represented almost identically in the four groups; the same was true for IgG3, even though the contribution of this subclass to the total was very low. The contributions of IgG2 and IgG4 were similar in patients with non-toxic goitre, thyroid carcinoma and GD. In these diseases IgG4 was the prevalent isotype. In contrast, in patients with HT IgG2 was the subclass preferentially used by TgAbs.

Figure 2 summarizes the distribution of the absolute concentrations of TgAb IgG subclass in the different diagnostic categories. The four groups of patients had similar levels of IgG1, IgG3 and IgG4. IgG2 was the only subclass that differed among the patients; it was significantly greater in HT than in all the other groups: $P = 0{\cdot}0005$ *versus* GD, $P = 0{\cdot}007$ *versus* thyroid carcinoma, and $P < 0{\cdot}005$ *versus* non-toxic goitre.

Correlation between IgG subclass concentrations and TgAb ELISA titre

Figure 3 illustrates the correlation between the titre of TgAbs assessed by ELISA and the total concentration of thyroglobulin-specific IgG. When the data were logarithmically transformed, to normalize the skewness to the right of the subclass distribution and the presence of outlying values, there was a significant positive correlation between these two variables: $r = 0{\cdot}79$, $P < 0{\cdot}0001$.

DISCUSSION

This study confirms in a large series of patients with thyroid diseases the observation that TgAbs are not restricted to any particular isotype, but comprise all four IgG subclasses [6, 13, 14]. This finding is in sharp contrast with the TSH-receptor antibodies which are mainly of the IgG1 subclass [20]. In addition, TgAbs do not fix complement. In our study, IgG3, the most potent isotype in complement fixation, is the least represented (only 1–4% of the total). Even though IgG1 constitutes a discrete proportion (17–29%), TgAbs are still unable to bind C1q [13]. This probably reflects the fact the epitopes on the thyroglobulin molecule are widely spaced, not allowing the binding of two or more of the C1q globular heads. Thus, polyclonality and inability to activate the complement cascade strengthen the notion that TgAbs have little pathogenic significance, representing only a marker of thyroid disease.

In patients with GD the most represented IgG subclass of TgAbs is G4, in agreement with all the previous studies that have analysed GD patients [8, 9, 13]. We have also shown for the first time that the same pattern is present in patients with non-toxic goitre and thyroid carcinoma. G4 in man is the least abundant IgG subclass, representing approximately 2% of the total IgG in the serum of adults [21]. G4 is poor at complement fixation, failing to bind C1q [4], and it tends to be functionally monovalent, being not able to effectively cross-link antigen molecules [22]. It has low affinity for Fcγ receptors, making it very weak in mediating ADCC [23]. The reason why IgG4 is over-represented in patients with GD, thyroid carcinoma and non-toxic goitre is not clear, but may reflect the nature of the particular epitopes of the thyroglobulin antigen. It has been shown that IgG4 predominates with prolonged antigenic challenges, such as in hyposensitization therapy for allergy and in chronic parasitic infections [24, 25]. G4 over-representa-

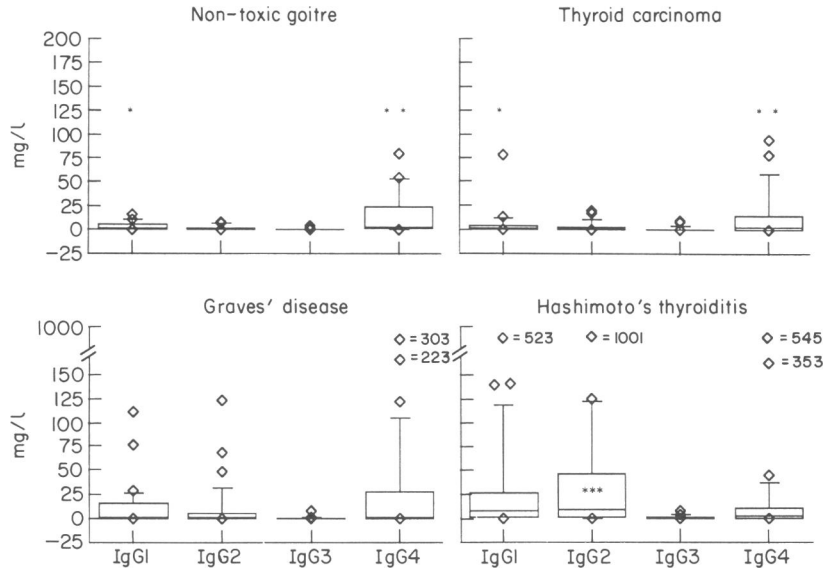

Fig. 2. Concentration and distribution of IgG subclass of thyroglobulin antibodies in sera from patients with various thyroid diseases. Each box represents the central 50% of the sample distribution, the top corresponding to the 75% percentile and the bottom to the 25% percentile. The horizontal line inside the box indicates the median. The ends of the upper and lower straight lines represent the largest and the smallest observation, respectively. ***$P = 0{\cdot}0005$ *versus* Graves', $P = 0{\cdot}007$ *versus* thyroid carcinoma, $P < 0{\cdot}005$ *versus* non-toxic goitre.

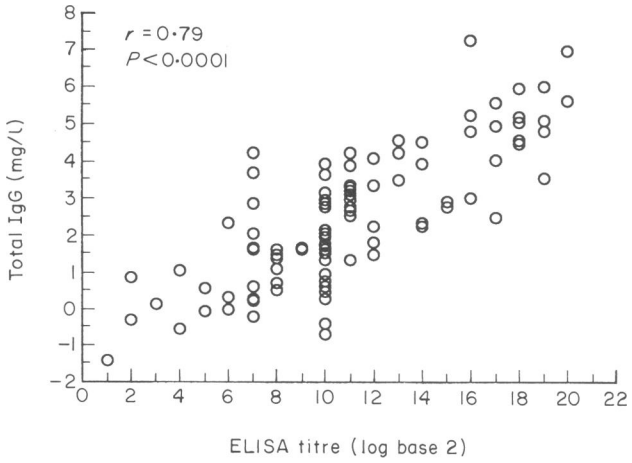

Fig. 3. Correlation between the concentration of total thyroglobulin-specific IgG subclass and the whole thyroglobulin antibody titre, assessed by ELISA.

tion in these forms of thyroid diseases may reflect the chronic nature of these disorders.

This study confirms previous observations [6, 13–15] that TgAbs in patients with HT are not restricted to IgG1 and IgG4 as suggested by other investigators [8–12]. We clearly demonstrate a preferential usage of IgG2 in Hashimoto's patients. Several explanations can be offered for this discrepancy. First, as initially pointed out by Weetman & Cohen [13], the IgG2 reagent (HP 6009) used by certain investigators [8–12] was in fact the least reactive of the monoclonals tested against IgG2, and was not recommended by WHO/IUIS study group as a reagent for IgG2 [19]. Second, it is possible that the different genetic backgrounds of the patients influences a different subclass usage. The patients in this study were Southern European, whereas the patients studied by the second group of investigators were mainly Northern European. The dominance of G2 in HT, as opposed to the dominance of G4 in the other thyroid diseases, could be explained by the peculiar infiltrate of the thyroid gland. Previous studies have shown that the T helper lymphocytes infiltrating the thyroid gland are mainly of the Th1 type [26], a subset characterized by the production of IL-2 and interferon-gamma (IFN-γ) [27]. The factors that control the switching to IgG isotypes have been partially identified in mice, but are largely unknown in humans. In mice, the switching to the G2b isotype (analogous to the human G2) is strongly suppressed *in vivo* by IL-4 [28] and enhanced by IFN-γ [29]. Even if attempts to parallel the regulation and function of analogous isotypes in mice and humans may be misleading, we can speculate that if the T helper lymphocytes infiltrating the thyroid gland produce mainly IFN-γ, the end results are a reduced production of G4 and a proportional over-expression of G2. This may be particularly important, as it is known that the thyroid is probably the primary source of thyroid autoantibody production [30].

In conclusion, this study has shown in a large series of patients that: (i) TgAbs in thyroid diseases are polyclonal, that is, not restricted to any particular IgG subclass; (ii) IgG4 is the most represented subclass in patients with GD, thyroid carcinoma and non-toxic goitre; (iii) IgG2 is the most represented subclass in HT, possibly reflecting the fact that T helper lymphocytes infiltrating the thyroid are Th1 type.

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