Kappa/Lambda Immunoglobulin Distribution in Graves' Thyroid-stimulating Antibodies

Simultaneous Analysis of Cλ Gene Polymorphisms

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Abstract

From patients with untreated Graves' disease 11 sera showing high cAMP release in the FRTL-5 cell assay were studied for relative proportions of kappa or lambda Ig molecules showing cAMP releasing activity. Immunoabsorption of γ-globulins was performed using monoclonal murine anti-kappa or anti-lambda antibodies linked to cyanogen bromide-activated sepharose. Specific kappa- or lambda-adsorbed fractions were also eluted from immunoabsorbents using chaotrophic thiocyanate buffers and equilibrated with pH 7.4 low salt buffer by dialysis. Immunoabsorption and elution experiments showed that five Graves' sera contained predominant cAMP-releasing activity within lambda Ig fractions, whereas two Graves' sera showed predominant cAMP-releasing activity in kappa Ig fractions. Four sera showed cAMP release approximately equally divided between kappa and lambda Ig both after immunoabsorption and specific anti-kappa or anti-lambda eluates were studied.

Cλ genotypes were examined by Southern blotting and restriction fragment length polymorphism analysis of Eco RI-digested genomic DNA from 158 patients with Graves' disease in parallel with 112 normal controls and 29 patients with autoimmune hypothyroidism. Notable shifts in proportions of 8/8 and 18/18 genotypes were present when Graves' patients were compared with normal controls. Allelic frequencies and ratios of genotype 8 to 18 were significantly different ($P < 0.05$) when Graves' patients were compared either to normal controls or to patients with autoimmune hypothyroidism.

Introduction

A selected number of human antibodies appear to exhibit various degrees of natural clonal restriction. Among the first to be recognized were the IgM cold agglutinins which have long been defined as predominantly IgM kappa antibodies (1–3). Many naturally occurring antibodies to human factor VIII have been restricted largely to the relatively rare IgG-4 subclass (4, 5), and recently human monoclonal IgM rheumatoid factors of kappa type appear to share a number of interesting structural and idiotypic similarities (6–10). In 1986, Knight and co-workers (11) reported that human sera derived from patients with high levels of long-acting thyroid stimulator (LATS)[1] activity showed an almost complete lambda Ig molecule restriction. 10 out of 11 such sera appeared to contain LATS or LATS-protector (LATS-P) antibody of primarily lambda class as established by selective immunoabsorption. These authors suggested that this was evidence in favor of the "forbidden clone" hypothesis. Earlier, a report by Zakarija (12) had also provided evidence for what appeared to be relative oligoclonal restriction in autoantibodies occurring in the serum of Graves' disease patients. The present study was therefore directed at an examination of thyroid-stimulating antibodies (TsAb) as measured in a sensitive in vitro bioassay for their possible kappa or lambda restriction. This was initiated because it seemed important to investigate further the striking results obtained with the in vitro LATS bioassay. Moreover, a number of observations regarding anti-thyrotropin-stimulating hormone (TSH) receptor antibodies support the concept that such γ-globulins might include a spectrum of different but related specificities (13–16). In addition, during the present study, genomic DNA from a large sample of patients with Graves' disease was examined directly using restriction fragment length polymorphism (RFLP) analyses in an attempt to uncover possible linkage disequilibrium in germ-line immunoglobulin lambda genes among such patients which might somehow be related to the restriction of TsAb activity to IgG of lambda type.

Methods

Genomic DNA was prepared from peripheral blood samples collected from 119 Graves' disease patients attending thyroid clinics at four London Hospitals: Royal Free, Guy's, St. Thomas', and University College. In addition, a group of 39 patients manifestating severe Graves' ophthalmopathy, followed in the Thyroid Clinic of Moorfields Eye Hospital, were also studied. A second control group of 29 patients with primary autoimmune hypothyroidism followed at Royal Postgraduate Medical School, Hammersmith Hospital, was diagnosed as primary hypothyroidism requiring replacement therapy with thyroxine and showed presence of elevated thyroglobulin or microsomal antibodies. None had ever shown a thyrotoxic phase to their illness.

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1. *Abbreviations used in this paper:* LATS, long-acting thyroid stimulator; RFLP, restriction fragment length polymorphism; TsAb, thyroid-stimulating antibody(ies).

DNA was extracted as previously described (17) from 10-ml samples of blood collected in EDTA tubes. Briefly, cells were lysed in lysis buffer (10 mM Tris-HCl pH 7.5, 5 mM $MgCl_2$, 1% (vol/vol) Triton X-100, 0.32 M sucrose) at 4°C followed by centrifugation at 1,000 *g* for 10 min. The pelleted nuclei were resuspended in 5 ml of resuspension buffer (0.075 M NaCl, 0.029 M EDTA, pH 8.0) followed by immediate addition of 0.5 ml 5% (wt/vol) SDS containing 2 mg/ml of proteinase K. The mixture was incubated for 2–4 h at 37°C and then extracted twice with redistilled phenol saturated with 20 mM Tris pH 8.0 and twice with chloroform-isoamylalcohol (24:1). The DNA was precipitated from the aqueous phase by addition of NaOAc to 300 mM and of 2 vol of ethanol, and was redissolved at 1 mg/ml in 10 mM Tris-HCl pH 7.5, 1 mM EDTA buffer (TE).

Isolation of lymphocytes from Graves' disease thyroid glands. In three instances DNA from peripheral blood mononuclear leukocytes (PBMC) was compared with that simultaneously collected from lymphocytes infiltrating fresh thyroid glands removed at therapeutic subtotal thyroidectomy. In these cases, the fresh thyroid tissue collected at the operating theatre in ice-cold sterile saline was immediately minced in a sterile petri dish on ice in the presence of 20 ml of RPMI plus 10% fetal calf serum (FCS). The minced tissues were strained through gauze and a wire mesh tea strainer into a sterile beaker, and the 40 ml of strained material was spun at 1,400 rpm and 4°C for 10 min. Cell pellets were then resuspended in 10 ml of fresh RPMI containing 10% FCS and cells separated on Ficoll gradients by centrifugation at 1,400 rpm for 30 min. Cells from such Ficoll gradients were 95–98% small lymphocytes as estimated by Geimsa stain, as well as by staining with monoclonal anti-pan B (CD19) and anti-PAN T (CD3) murine monoclonal antibodies after cytospin preparation. DNA was prepared from thyroid-derived lymphocytes as described above for PBMC DNA extraction.

RFLP studies. 10-μg DNA samples from individual patients were digested with Eco RI (Boehringer Mannheim Biochemicals, Indianapolis, IN), electrophoresed on 0.6% agarose gels, transferred to Gene Screen Plus filters (Du Pont Co., Wilmington, DE) and hybridized with a Cλ probe (provided by Dr. Stanley Korsmeyer, Howard Hughes Institute, Washington University Medical Center, St. Louis, MO) which was ^{32}P-labeled by nick-translation (18) to 10^8 cpm/μg. Hybridization and washing were performed according to the manufacturer's instructions, the final wash being in 0.2× SSC (30 mM NaCl, 3 mM trisodium citrate) at 65°C. Blots were autoradiographed at −70°C using Kodak XAR-5 film and Du Pont Cronex intensifying screens. The allelic frequencies of the polymorphic Eco RI fragments detected by this Cλ probe have previously been described (19, 20). These were compared in the 158 Graves patients and the 112 normal healthy controls derived from the same population group as well as in the 29 patients with primary autoimmune hypothyroidism.

Studies of TsAb and absorption using solid-phase immunoabsorbents. A group of 11 sera was studied from patients with active untreated Graves' disease consistently showing marked cAMP release in the FRTL-5 cell in vitro bioassay. This assay had been sensitized by the use of low salt medium as described previously by Rapoport et al. (21, 22) and Kasagi and coworkers (23). γ-Globulins precipitated from serum using 15% polyethylene glycol 4000 were used in the FRTL-5 cell bioassay using the protocol previously described (24, 25). Positive controls were provided by standard dilutions of TSH (Armour Laboratories, Kankakee, IL) and also by including an IgG preparation from an aliquot taken from a single large serum collection from a patient with Graves' disease which had previously been shown to have potent TsAb activity in this bioassay. Positive cAMP release was judged to be at least 150% of values obtained using γ-globulins from a normal serum donor pool.

Immunoabsorption procedures. Samples from Graves' sera showing high levels of TsAb by the FRTL-5 cell assay were subjected to immunodepletion of kappa or lambda Ig molecules using solid-phase immunoabsorbents in which various mouse monoclonal antibodies had been linked to cyanogen bromide (CNBr) activated sepharose. Murine monoclonals 312H (anti-lambda) and 72/2C10 or QE11 (anti-kappas) prepared in the Department of Immunology, University of Birmingham were first precipitated with 40% ammonium sulfate, dialyzed free of ammonium sulfate and then dialyzed against 0.2 M $NaHCO_3$/0.5 M NaCl for 24 h at 4°C before coupling to activated CNBR-sepharose. γ-Globulin preparations were prepared from test serums with 15% polyethelene glycol 4000 and put on immunoabsorbent columns for 1–2 h, followed by washing with 0.1 M pH 7.4 phosphate-buffered saline (PBS). The absorbed γ-globulins were then eluted with 3 M sodium thiocyanate and eluates concentrated and dialyzed to equilibrate them with PBS, pH 7.4. Absorbed and eluted γ-globulin preparations were adjusted to the same protein concentration before assay in the FRTL-5 cell system. Samples were assayed before and after immunoabsorption. Completeness of immunodepletion after absorption procedures was monitored by ELISA determination using a standard curve established with known amounts of kappa and lambda Ig. Control immunoabsorption procedures included passage of the same Graves' sera over Sepharose linked to a monoclonal mouse IgG without defined antibody activity. After washing such control columns with PBS, eluates were prepared in the same fashion as with the specific anti-kappa or anti-lambda immunoabsorbents. These controls were directed at estimating the relative amounts of nonspecific adsorption involved in the immunodepletion procedures.

Results

Absorption studies. Graves' disease patients' sera that were strongly positive in the cAMP-release FRTL-5 cell bioassay were absorbed for kappa or lambda molecules using the monoclonal murine anti-kappa or anti-lambda antibodies linked to sepharose. Results of such immunoabsorption procedures showed remarkable light chain class (lambda or kappa Ig restriction) of immunoglobulins producing cAMP release in some Graves' sera. 5 of 11 Graves' sera showed cAMP-releasing activity predominantly associated with Ig lambda molecules. Examples of representative relative kappa or lambda immunodepletion in parallel with FRTL-5 cell line assay results are shown for Graves' serum A in Table I and Fig. 1. Kappa and lambda ELISA quantitations showed virtually complete elimination (99%) of respective kappa or lambda molecules immunodepleted with < 1% residual contamination of depleted class molecules. The cAMP response of 800% above normal in the control-absorbed Graves' patient sample was markedly diminished following lambda immunodepletion, whereas absorption with anti-kappa immunoabsorbents actually resulted in an increased potency to 1,200% above normal. Since the kappa-absorbed sample was relatively enriched for lambda, this increased potency would be expected. Eluates from the control immunoabsorbent columns which contained undetectable IgG, did not stimulate significantly above the control. However, eluates from the anti-lambda immunoabsorbent induced > 1,200% above normal cAMP release. In parallel, the anti-kappa immunoabsorbent eluate showed only slight activity. This same profile of results was reproduced in entirely different experiments with a fresh serum sample from this patient using complete repetition of the entire immunodepletion and cAMP assay procedure. Two other Graves' disease sera (F and G in Table II) with high cAMP-releasing activity also showed a completely consistent overwhelming lambda predominance in cAMP-releasing activity confirmed in the same pattern as that shown for Graves' serum A in Fig. 1. Moreover, the same lambda predominance pattern of cAMP release was completely reproducible when the entire process—immunoabsorption, elution, and cAMP-releasing assays—was repeated in different experiments.

Table I. Quantitative ELISA Assays of Kappa and Lambda γ-Globulins from Graves' Patients A, B, C, and D before and after Immunodepletion Using Monoclonal Anti-Lambda or Anti-Kappa Linked to Sepharose

Graves' patient studied	γ-globulins	OD at 280 nm	Kappa	Lambda
			μg/ml	
A	Absorbed with anti-kappa	0.78	.01	840
	Absorbed with anti-lambda	0.78	1123	0.05
	Control absorption	0.78	459	165
	Eluate from anti-kappa	0.31	325	0.3
	Eluate from anti-lambda	0.31	7.5	250
	Eluate from control column	0.18	8.7	4.5
B	Absorbed with anti-kappa	0.742	16	400
	Absorbed with anti-lambda	0.742	125	7
	Control absorption	0.742	245	350
	Eluate from anti-kappa	0.30	220	1.3
	Eluate from anti-lambda	0.30	1.2	62.0
	Eluate from control column	0.10	0.6	0.9
C	Absorbed with anti-kappa	0.917	0.02	892
	Absorbed with anti-lambda	0.917	612	0.07
	Control absorption	0.917	245	880
	Eluate from anti-kappa	0.372	155	0.58
	Eluate from anti-lambda	0.372	0.2	278
	Eluate from control column	0.100	0.7	0.8
D	Absorbed with anti-kappa	0.590	0.9	370
	Absorbed with anti-lambda	0.590	300	3.2
	Control absorption	0.590	215	280
	Eluate from anti-kappa	0.150	56	4.9
	Eluate from anti-lambda	0.150	4.2	70
	Eluate from control column	0.05	0.2	4.2

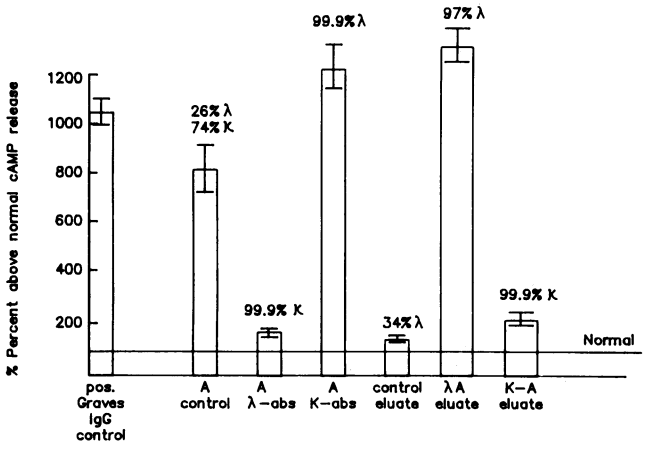

Figure 1. Results in FRTL-5 cell cAMP bioassay using γ-globulin from Graves' patient A prepared by polyethylene glycol precipitation and immunodepleted for kappa or lambda molecules using insoluble anti-kappa or anti-lambda immunoabsorbents. To the left are shown the results with the immunoabsorbed preparation and to the right results using eluates obtained by dissociating lambda or kappa molecules and adjusting eluates to equivalent protein concentration. Control absorption was performed with a nonspecific mouse monoclonal linked to sepharose and control elution prepared in the same manner as those from specific anti-kappa or lambda immunoabsorbents.

Table II. Kappa/Lambda Ig Distribution of FRTL-5 Cell Line cAMP-releasing Activity among γ-Globulins from 11 Graves' Patients

Patient studied	By anti-kappa or anti-lambda immunoabsorption		
	λ	λ + κ	κ
A	+++		
B	++		
C			+++
D		++	
F	+++		
G	+++		
H	++		
I			++
J		++	
K		++	
L		++	

Since in the three sera where anti-lambda depletion resulted in almost complete elimination of cAMP-releasing activity, it seemed possible that some additional oligoclonality might be present in lambda-enriched fractions, these latter materials were examined using both high-resolution agarose electrophoresis and isoelectric focusing procedures. A number of experiments were performed using lambda-enriched (kappa-depleted) fractions from patients showing predominant lambda cAMP-releasing activity. These showed a broad electrophoretic distribution of residual Ig lambda molecules and no clear evidence for oligoclonal or monoclonal lambda Ig components. Therefore, these results provided no clear evidence for concomitant H-chain subgroup or further detectable oligoclonal Ig restriction in the immunodepleted Graves' samples studied.

In two other Graves' sera, immunodepletion of kappa or lambda Ig also confirmed lambda Ig predominance for cAMP releasing activity (Graves' patients B and H, Tables I and II). Both anti-kappa and anti-lambda immunodepletion produced residual fractions which were 95–96% enriched for the opposite Ig class. Almost complete depletion of lambda resulted in disappearance of significant cAMP release by the absorbed sample whereas depletion of kappa Ig molecules resulted in apparent relative increase in cAMP release.

In two Graves' sera, immunodepletion studies indicated a predominant kappa Ig TsAb activity. An example of this is shown in Fig. 2 and Table I (Graves' serum C). It can be seen that virtual complete immunodepletion of lambda Ig molecules increased TsAb activity, whereas anti-kappa immunodepletion which again was virtually complete (99% lambda) resulted in complete loss of activity. Results with eluates from the specific immunoabsorbents once again reflected the same kappa predominance, however, there was moderate but significant cAMP release in the anti-lambda eluate that contained 99% lambda Ig. A second Graves' serum (data not shown) gave almost identical results, which were reproduced on two different occasions.

Four Graves' sera showed clearcut TsAb activity in both kappa and lambda enriched fractions. Representative data are shown in Fig. 3 and Table I (Graves' patient D). Neither anti-

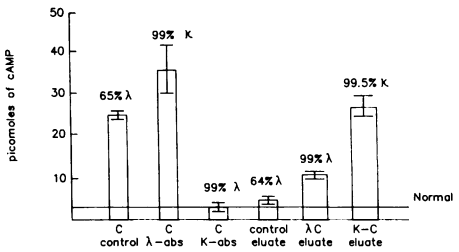

Figure 2. FRTL-5 cell line cAMP results using γ-globulins from Graves' patient C. Depletion of lambda Ig molecules increased the TsAb potency whereas depletion of kappa abolished the response. The result and mean±SD of triplicate determinations are shown. Studies with eluates reflected clearcut kappa predominance, however, moderate cAMP release was also recorded with the anti-lambda eluate.

lambda nor anti-kappa absorptions produced dramatic falls in cAMP release (Fig. 3). Nor was there the increased potency above the control values observed in the data presented in Figs. 1 and 2 for either the lambda or kappa absorbed fractions. Due to the theoretical significance of these results, the experiment was repeated using a fresh serum sample from one of these patients and the same results were obtained. In addition both anti-kappa and anti-lambda eluates showed moderately high levels of cAMP-releasing activity. These same patterns of approximately equal division of cAMP-releasing activity between Ig kappa and lambda fractions were reproducible when experiments were completely repeated from the beginning.

A summary of relative profiles of kappa and lambda Ig class distribution in TsAb established by the immunoabsorption and elution studies can be seen in Table II. It was clear that TsAb activity as measured by cAMP release in the FRTL-5 cell in vitro bioassay was relatively restricted in five sera to lambda Ig class and to kappa Ig light chain class in two sera. In four other sera no such restriction was observed.

RFLP analysis. Studies of Cλ gene Eco RI RFLP in Graves' patients and normal controls provided some interesting comparisons. Taub et al. (20) described four allelic Eco RI fragments at the Cλ locus of 8 kb (two Cλ genes); 13 kb (three Cλ genes), 18 kb (four Cλ genes), and 23 kb (five Cλ genes). Fig. 4*A* shows a representative autoradiogram of one of our Southern blots, with examples of the three most common genotypes (8/8, 8/18, 18/18). The allelic frequencies of the polymorphic fragments in the various populations studied are shown in Table III. A number of points arise from these data. First, we observed no examples of the 13-kb allele, although we would have expected to see 28 such fragments within the population studied, based on the allelic frequency of 0.05 determined by Taub et al. (20). Further analysis of populations is required to explain this difference. Secondly, our studies identified two examples of the 23-kb allele in 298 subjects, indicating a much lower allelic frequency (0.0035) than that suggested by Taub et al. (20) on the basis of the one example seen by them. Interestingly, both 23-kb alleles were found in the group of patients with autoimmune hypothyroidism. Thirdly, we have identified one normal individual with alleles of 18 and ~4 kb. This latter allele has not been previously described and may represent the absence of either the $C\lambda_2$ or the $C\lambda_3$ gene (20). Finally, whereas the allelic frequencies of the 8- and 18-kb fragments in our normal population and in patients with non-Graves' autoimmune hypothyroidism are close to those reported by Taub et al. (20) for a normal population (0.75 and 0.196, respectively), the Graves' patients show an excess of the 8 kb allele over the 18 kb allele (0.86 and 0.14, respectively), which is statistically significant ($P < 0.05$) as determined by applying a χ^2 test to the values obtained from the total Graves' and non-Graves' populations.

The increased expression in the 8-kb allele in Graves' disease patients as well as Graves' patients with severe ophthalmopathy (Table III) was further explored by an analysis of clinical data from the 119 patients with Graves' disease. This analysis showed that 92.3% of these latter patients with eye findings were 8/8 genotype. Moreover, of the 21% of Graves' patients with a family history of thyroid disease, 89.4% showed a 8/8 genotype for Cλ.

In two of the three samples in which both peripheral blood leukocyte DNA and thyroid gland lymphocyte DNA were compared in this manner, a good yield of highly purified thyroid lymphocytes was obtained. When genomic DNA obtained from peripheral blood samples was compared with DNA prepared from lymphocytes actually isolated from the same Graves' disease patients' thyroid glands removed at surgery, no evidence for lambda gene rearrangement within thyroid lymphocytes was obtained. The germ-line DNA from peripheral blood showed the same genotypic pattern as did that of the thyroid lymphocytes (Fig. 4*B*).

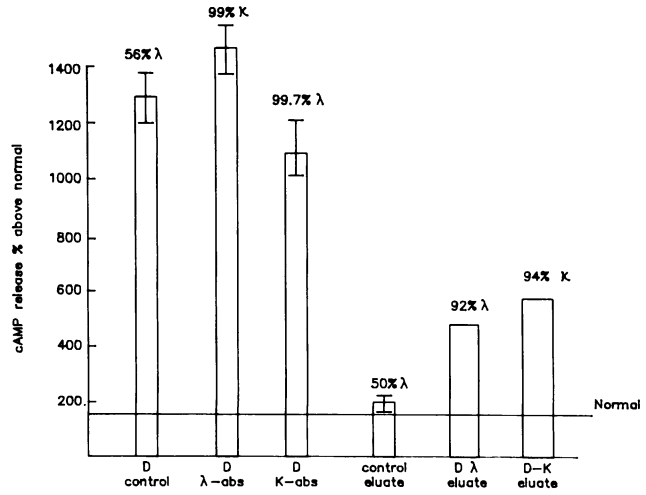

Figure 3. cAMP release using a Graves' serum (patient D) γ-globulin with activity in both kappa and lambda fractions. In this experiment neither anti-lambda nor anti-kappa absorptions produced major falls in cAMP release. In parallel specific anti-kappa and anti-lambda eluates showed moderately strong relative levels of cAMP-releasing activity.

Discussion

The present study was prompted by the initial report by Knight and co-workers (11) indicating that TsAb measured in the LATS functional bioassay usually contained only lambda light chains. Our immunoabsorption results using the FRTL-5 cell line assay do not favor a uniform restriction of TsAb to a single lambda light chain type as can be seen in Table II. However, the results do show that in 5 of the 11 Graves' patients studied, a remarkable apparent lambda light chain predominance was present. Thus we can partially confirm the

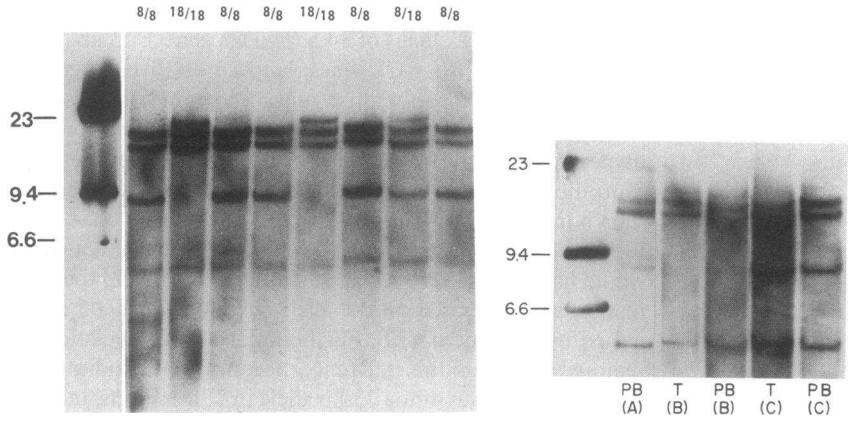

Figure 4. (*A*) Southern blot patterns of the three most common Cλ genotypes noted with the 0.8-kb Cλ probe hybridized to Eco RI digested genomic DNA from normal subjects in parallel with Graves' patients. Relative positions of bacteriophage λ Hind III fragments of 23, 9.4, and 6.6 kb are shown to the left. All Graves' and normal Eco RI digests showed a 5-kb pseudogene. (*B*) Southern blot, analysis of DNA Cλ genotypes in Graves' thyroid lymphocyte DNA and peripheral blood DNA in the same patient. Molecular mass markers at 23, 9.4, and 6.6 kD shown at left. Peripheral blood DNA from Graves' patient A lane *1*, thyroid DNA from Graves' patient B (lane *2* marked *T*) and peripheral blood DNA from same Graves' patient B (lane *3* marked *PB*). Comparison of lanes *2* and *3* shows same 8/8 genotype and no rearrangement. Thyroid (*T*) and peripheral blood DNA (*PB*) from third patient (*C*) in lanes *4* and *5* also show the same 8/8 genotype, without evidence of rearrangement. Studies of thyroid DNA freed of lymphocytes showed the same genotype as that noted in peripheral blood leukocytes and isolated thyroidal lymphocyte populations.

initial report by Knight et al. (11) that a significant proportion of individual patients with TsAb appears to be restricted to Ig molecules of the lambda light chain class. Of equal interest was the finding of apparent relative kappa preponderance in only 2 of 11 Graves' patients (C and I, Table II). This also confirms the results of Knight et al. (11) who found only 1 of 11 patients with predominant kappa Ig LATS activity. 4 of our 11 Graves' patients studied showed approximately equal TsAb activity in both kappa and lambda Ig classes. Our finding of relative lambda restriction in a significant proportion of TsAb from Graves' patients suggests that in some patients a unique predilection for lambda light chain involvement may somehow relate to the underlying disorder. Precisely how this occurs cannot at present be defined. Despite the apparent lambda restriction in five patients and kappa restriction in two, isoelectric focusing of absorbed Ig fractions produced no evidence for oligoclonal bands. It is conceivable that such Ig fractions still contain H chain subgroup restriction which would only be demonstrable by comparative serial absorptions with insoluble anti-IgG 1, 2, 3, or 4 immunoabsorbents. However, the finding that four sera showed neither kappa nor lambda Ig restriction is a strong argument against oligoclonal restriction occurring within all TsAb.

It is not surprising that TsAb as measured in the current study are not uniformly or predominantly of lambda light chain class. The patients selected by Knight et al. (11) for study of LATS activity before and after similar immunodepletion of Ig lambda or kappa molecules may have represented a relatively homogeneous group since they all showed marked elevations of LATS by biologic assay and several had pretibial myxedema. None of our patients showed the latter finding. The patients with Graves' disease that we studied showed marked elevations of cAMP-releasing activity in their serum γ-globulin fractions but were not preselected as one subgroup within active thyrotoxicosis. Hyperthyroidism may represent a microheterogeneity of subsets of one underlying disorder. We were fully aware of this problem from the outset and in collecting samples for study, intentionally selected patients with unequivocal clinical and laboratory support for a definitive diagnosis. All Graves' patients included had diffuse goiters,

Table III. Distribution of λ-Genotypes and Allelic Frequencies among Graves' Disease Patients, Normal Controls, and Patients with Autoimmune Hypothyroidism

Cλ genotypes	Graves' disease (n = 119)		Graves' disease severe ophthalmopathy (n = 39)		Normal controls (n = 112)		Autoimmune hypothyroidism (n = 29)		Taub *et al.* (20) normals
%		%		%		%		%	
8/8	90/119	75.6	31/39	79.5	76/112	67.9	17/29	58.6	NR
8/18	24/119	20.2	6/39	15.4	23/112	20.5	8/29	27.5	NR
18/18	5/119	4.2	2/39	5.1	13/112	11.6	2/29	6.8	NR
8/23	0	0	0	0	0	0	2/29	6.8	NR
18/4	0	0	0	0	1/112	0.9	0	0	0
				Allelic frequencies					
8	6.1:1*	0.86		0.87	3.5:1*	0.78		0.76	0.75
18		0.14		0.13		0.22		0.21	0.196
13		0		0		0		0	0.05
23		0		0		0		0.04	0.01

NR, not reported. * Difference $P < 0.05$ by χ^2 test.

elevated T4 by radioimmunoassay and many had confirmation of hyperthyroid Graves' with prior FRTL-5 assays. No patients with single toxic nodules or an equivocal clinical picture were included. Despite the intentional effort to study a relatively uniform group of Graves' patients, it is certain that some degree of clinical heterogeneity was present among our patients.

TSH receptor antibodies in Graves' disease may represent a spectrum of distinct specificities. This is, for instance, illustrated by the fact that in our own hands Graves' serum samples giving high levels of cAMP release in the FRTL-5 cell assay did not always show parallel marked elevations in other test systems such as the radioimmunoassay for anti-TSH receptor antibodies (26, 27) which may also measure blocking TSH receptor activity. The problem of antibodies to the TSH receptor complex is one which has intrigued investigators for more than three decades. Initially attention was directed to LATS as an important primary criterion of Graves' disease activity (28, 29). Later with the studies of Kohn et al. (30–32) and others (33, 34), it became apparent that there probably exists a large repertoire of distinct antigenic epitopes actually making up TSH receptors (31, 32). For this reason alone it would be difficult to expect that a lambda class restriction would universally apply to antibodies reacting with a spectrum of related epitopes. On the contrary, actual epitopes involved as antigens reacting with a subpopulation of autoantibodies producing positive results in the bioassay for LATS may well be much more restricted and hence induce a more clonally restricted autoimmune response in patients selected for high LATS activity. This could explain the apparent high frequency of biologically active lambda IgG molecules found by Knight et al. (10) in serum from Graves' patients with high levels of LATS activity.

Results presented here indicate that a significantly higher proportion of Graves' disease patients have the 8-kb Eco RI Cλ allele than is found in the normal population. This was even more pronounced in the subset of Graves' patients with severe opthalmopathy. It is not clear how the differences in genotype described here are related to shifts in kappa/lambda ratios within autoantibodies centrally involved in the fundamental driving immunologic mechanisms of Graves' disease itself. Possibly the finding of marked lambda light chain restriction in 5 of 11 Graves' patients studied by immunoabsorption and elution assays suggests that lambda light chains perhaps with an 8/8 phenotype may be involved in the immunologic genesis of at least certain cases of thyrotoxiosis. Of interest in this regard are results recently reported by Sidebottom et al. (35) who found a marked increase in Cλ 8/8 phenotype and 8-kb allele among 108 unrelated patients with classical or definite rheumatoid arthritis when compared to a similar number of normal controls. These latter findings in parallel with ours suggest that several autoimmune disorders share a similar increase in expression of the 8/8 genotype and 8-kb Cλ allele.

The precise mechanisms actually involved in selecting out both constant and variable regions of a wide range of autoantibodies have recently been examined by a number of workers (36–38). If indeed there is some interesting predilection for Graves' patients of a certain Cλ phenotype to produce autoantibodies with self-reactive specificity, such a finding (if confirmed) would be of considerable interest. The most direct way to examine this question in Graves' disease would involve definition of lambda gene arrangements from human cell lines producing monoclonal immunoglobulin G with TsAb specificity. Such an approach is presently in progress in our laboratory. A repertoire of human monoclonals with well-characterized anti-TSH receptor antibody activity has previously been extensively studied by Kohn et al. (30–32). Thus far among these antibodies there does not appear to be any striking kappa or lambda class restriction.

The association of the 8-kb Eco RI Cλ allele with Graves' disease described here is of considerable interest and probably should now be analyzed with respect to HLA-DR or immunoglobulin Gm or Km allelic distributions similar to those which have been performed in the past (39–43). On the basis of the findings presented here, it would now appear appropriate to again examine additional markers on lambda Ig light chains such as those recently described (44) for a possible correlation to the Cλ genotypic profiles described in the present study.

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