Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7415–7419, August 1991
Immunology

Autoantigen recognition by thyroid-infiltrating T cells in Graves disease

(T-cell clone/thyroid peroxidase/autoimmune thyroiditis)

COLIN M. DAYAN*, MARCO LONDEI*, ANNE E. CORCORAN*, BEATRIX GRUBECK-LOEBENSTEIN†, ROGER F. L. JAMES‡, BASIL RAPOPORT§, AND MARC FELDMANN*¶

*Charing Cross Sunley Research Centre, 1 Lurgan Avenue, London W6 8LW, United Kingdom; †Department of Experimental Pathology, University of Vienna, Vienna, Austria; ‡Department of Surgery, Leicester Royal Infirmary, Leicester, United Kingdom; and §Thyroid Molecular Biology Laboratory, University of California, San Francisco, CA 94121

Communicated by J. F. A. P. Miller, May 8, 1991 (received for review March 18, 1991)

ABSTRACT **Graves disease is a common form of human autoimmune thyroiditis. It shares many pathological features and HLA associations with other, less easily studied, organ-specific autoimmune conditions such as insulin-dependent diabetes mellitus, and hence it is also a useful model for understanding these other diseases. We have previously shown that thyroid-infiltrating T cells in Graves disease that have been recently activated *in vivo* specifically recognize autologous thyroid epithelial cells. However, the autoantigens involved were not defined. In this study, we have made use of antigen-independent T-cell cloning techniques to show that at least three different thyroid antigens, three different epitopes on a single antigen, and two HLA class II elements are involved in this recognition process in a single individual. This demonstrates that T cells that are present and activated at the site of a human autoimmune disease may show considerable heterogeneity in their recognition of autoantigen on the target tissue. This contrasts with the limited heterogeneity recently reported in some animal models and has potentially important implications for both our understanding of the autoimmune process in humans and the design of immunotherapies to reverse it.**

The thyroid is the commonest target for organ-specific autoimmunity in humans. Graves disease is a form of thyroid autoimmunity characterized by lymphocytic infiltration of the thyroid and stimulation of the gland by anti-thyrotropin (TSH) receptor autoantibodies resulting in thyrotoxicosis (1). Frequently, high titers of autoantibodies to other thyroid-specific proteins, including thyroglobulin (Tg) and the thyroid microsomal antigen (TMA; ref. 2), are seen as well. The cDNAs for all three autoantigens have now been cloned (3–5), and the TMA has been shown to be identical with the tissue-specific enzyme thyroid peroxidase (TPO), which is required for the biosynthesis of thyroid hormones (6).

Thyroidectomy is often performed as part of treatment for the overproduction of thyroid hormones in Graves disease. This provides large quantities of tissue for *in vitro* study. The lymphocytic infiltrate of the thyroid has been shown to contain a high proportion of T cells (7). Also, the autoantibodies to the TSH receptor, Tg, and TMA seen in patients' sera, the majority of which are produced in the gland itself (8), are of the IgG isotype and often of high titer. Their production is therefore likely to be dependent on T-cell help (9). Furthermore, the target epithelium in the disease has been shown to aberrantly express HLA class II antigens (10), as well as adhesion molecules (11) and cytokines (12) required for antigen presentation to T cells. As a result, studies of autoantigen recognition by T cells in the thyroid in this disease may be expected to contribute key elements to our understanding of the autoimmune process involved.

We (13), and subsequently others (14, 15), have studied T cells from thyroidectomy specimens of multiple patients with autoimmune thyroiditis. A high proportion of the *in vivo* activated [interleukin 2 (IL-2) receptor-expressing] T cells present in these specimens show specific reactivity to autologous thyroid epithelial cells (TECs) in the absence of additional antigen-presenting cells (APCs) (13–15). In contrast, T cells derived from the peripheral blood of the same patients show no such response (16). This recognition of TECs is known to be HLA class II-restricted (13, 16), but the antigens involved have not been defined.

In the current study, we selected a single patient with "typical" HLA type (B8, DR3) and active disease at the time of thyroidectomy for more detailed analysis. A large number of thyroid-infiltrating T-cell clones and lines were generated from this individual by our antigen-independent protocol (17). This panel of *in vivo* selected T cells was then screened, without prior exposure to antigen *in vitro*, for reactivity with a broad collection of thyroid antigens and peptide epitopes. We have thus been able to confirm at the molecular level that thyroid-infiltrating T cells are very highly selected for recognition of antigens expressed exclusively in this tissue. More important, we have been able to study the range of T-cell specificities involved in this recognition process. Such information is not only likely to contribute to our understanding of the pathogenesis of autoimmune disease but is also needed to assess whether the highly selective anti-T-cell immunotherapies developed in animal models (18, 19) will be applicable in humans.

MATERIALS AND METHODS

Patient. Infiltrating mononuclear cells were extracted from the thyroidectomy specimen of a 26-year-old woman (CX81) with persistently relapsing Graves disease. Her HLA type was A1,2; B8,37; DR2,3; DRw52; DQ2,6; DP2,4. She had high titers of both anti-TMA (1:640[2]) and anti-TSH receptor (66.8% inhibition of TSH binding) antibodies. Anti-Tg antibodies were not detected.

Antigens. Microsomes of Chinese hamster ovary (CHO) cells expressing whole recombinant human TPO were prepared as described (20) and used at 25 μg/ml (total protein) in proliferation assays in the presence of autologous APCs. Untransfected CHO cell microsomes at the same protein

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: APC, antigen-presenting cell; EBV, Epstein–Barr virus; IL-2, interleukin 2; PBL, peripheral blood leukocyte; SI, stimulation index; TEC, thyroid epithelial cell; Tg, thyroglobulin; TMA, thyroid microsomal antigen; TPO, thyroid peroxidase (≡ TMA); TSH, thyrotropin.

¶To whom reprint requests should be addressed.

concentration were included as a control in all cases. Pooled tryptic fragments of highly purified human Tg were a gift of I. Roitt (Middlesex Hospital, London) and were used at 50 μg/ml.

Synthetic peptides of TPO were synthesized manually by solid-phase coupling of fluoren-9-ylmethoxycarbonyl amino acid residues with the RaMPS multiple peptide synthesis system (DuPont/NEN). Full-length TPO is 933 amino acids. Twenty-eight sequences containing T-cell motifs according to the algorithms of Rothbard and Taylor (21) and/or DeLisi and Berzofsky (22) were selected and synthesized [residues are numbered from the amino terminus (4)]: 62–75, 84–99, 111–131, 116–131, 188–204, 203–216, 212–228, 234–250, 313–327, 403–417, 414–428, 425–440, 437–450, 461–477, 473–484, 488–504, 514–529, 535–551 (NP-7), 556–572, 572–583, 591–607, 616–631, 632–645 (B6), 670–686, 694–716, 712–727, 724–734, 756–772. Peptides were used at 10 μg/ml.

Epstein–Barr Virus (EBV)-Transformed B Cells. B cells from the patient's peripheral blood were transformed with EBV (EBV81 cells) according to the protocol of Neitzel (23). EBV-transformed B cells of known haplotype were kindly provided by J. Bodmer (Imperial Cancer Research Fund) for use in HLA restriction analysis.

Culture and Cloning of T Cells. Thyroid-infiltrating mononuclear cells were obtained from the nonadherent population released by enzymatic digestion of the surgical specimen (13, 16). After 1 week of culture (RPMI 1640/10% human serum) in the presence of IL-2 (20 ng/ml, Ajinomoto), cells were expanded for a further week with irradiated (4500 rads; 1 rad = 0.01 Gy) autologous peripheral blood leukocytes (PBLs), anti-CD3 monoclonal antibody OKT3 (30 ng/ml), and IL-2 as described (17). Cells were then cloned by limiting dilution (0.3 cell per well; ref. 17). Further expansion and maintenance of all clones was achieved by restimulation with OKT3, IL-2, and HLA-unmatched irradiated PBLs every 1–2 weeks. At no time were clones exposed to specific antigen, except in proliferation assays. Assays were performed at the end of the cycle and a minimum of 5 days after the last exposure to IL-2.

Where indicated (see below), thyroid-infiltrating T-cell lines initially expanded independently of antigen (OKT3/IL-2) were later submitted to selection using TPO-derived peptides. Peptide (10 μg/ml) was presented by irradiated (16,000 rads) EBV81 cells at a T cell/APC ratio of 1:1. After 7 days, IL-2 was added. Cells were assayed 7 days later and then maintained by weekly addition of IL-2 and restimulation with peptide every 2 weeks.

Proliferation Assays. Assays of antigen recognition were performed over 3 days in triplicate in round-bottomed microtitration wells. Ten thousand T cells (clone or line) were washed once and added to 2–5 × 10^4 irradiated autologous PBLs or EBV81 cells together with the appropriate antigen. [^{3}H]Thymidine [Amersham; 1 μCi (37 kBq)] was added for the final 6 hr before harvesting and scintillation counting (16). For proliferation assays with peripheral blood, 10^5 mononuclear cells isolated from heparinized whole blood by buoyant density centrifugation (Lymphoprep, Nycomed) were incubated in triplicate in round-bottomed microtitration wells with appropriate antigen for 5 days before incubation with [^{3}H]thymidine and harvesting as for thyroid-derived cells. Autologous TECs were obtained following enzymatic digestion of the thyroid and used in proliferation assays as described (13, 16). The stimulation index (SI) was calculated as (total cpm with T cells, APCs and antigen − cpm with T cells and APCs alone)/cpm with T cells and APCs alone.

RESULTS

TEC Recognition by T-Cell Lines from the Thyroid Infiltrate. Uncloned T-cell lines were derived from this patient's thyroid tissue on multiple occasions by using OKT3 and IL-2. Such lines showed reactivity to intact autologous TECs (Fig. 1), as we have seen previously with other patients (13, 16). These lines also showed reactivity to a microsomal preparation from CHO cells expressing recombinant human TPO but showed no response to microsomes from control, untransfected CHO cells (Fig. 1) or to tetanus toxoid (data not shown). This provided an initial indication that at least some of the T cells present in the infiltrate that responded to TECs might be specific for TPO.

Recognition of Thyroid Proteins by T-Cell Clones. To analyze the response of the thyroid infiltrate at the level of individual T cells, the expanded infiltrate was cloned at limiting dilution (0.3 cell per well) by our antigen-independent protocol. A panel of 33 clones derived in this way was simultaneously tested for reactivity to TEC, TPO, and pooled tryptic fragments of human Tg (Table 1). The clones separated into four groups: those responding to TPO (e.g., c2.12 and c2.27; 46% of clones), those responding to Tg (c2.7; 3%), those responding to TECs but not TPO or Tg (e.g., c2.1 and c2.5; 36%), and those showing no significant proliferation in response to any of these antigen preparations (e.g., c2.11; 15%) (Table 1). This suggests that T cells recognizing at least three different thyroid-specific antigens were simultaneously present in the thyroid of this individual. Two of the antigens involved have been identified (TPO and Tg); the remainder are present on TECs but not on autologous PBLs (Table 1).

Twelve out of 15 of the TPO-reactive clones also showed significant proliferation ([^{3}H]thymidine incorporation) in response to autologous TECs (SI >2.0 (e.g., c2.23; Table 1). This provides direct evidence that TECs can present an endogenous autoantigen, in this case TPO, to T cells. The

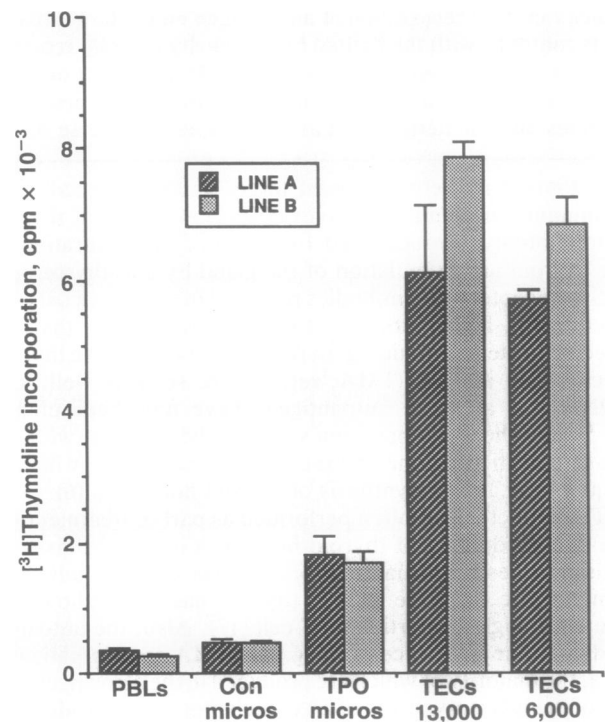

FIG. 1. Proliferation of uncloned thyroid-infiltrating T cells, expanded in the absence of antigen, to TECs and TPO. Lines A and B were derived on separate occasions with OKT3 plus IL-2. PBLs, irradiated autologous PBLs (APCs) plus T cells; Con micros, untransfected CHO microsomes added; TPO micros, microsomes of TPO-transfected CHO cells added; TECs, autologous TECs, either 1.3 × 10^4 (TECs 13,000) or 6 × 10^3 (TECs 6000) per well, plus T cells in the absence of added antigen or APCs. Data are the means of triplicate cultures, and error bars show the standard errors of the mean.

Table 1. Response of thyroid-infiltrating T-cell clones to thyroid antigens

Clone	[^{3}H]Thymidine incorporation, cpm					Specificity
	APCs	TECs	Con micros	TPO micros	Tg	
2.8	128	3,622	155	2,913	17	TPO
2.9	125	1,538	67	1,412	87	TPO
2.12	432	3,142	214	6,852	163	TPO
2.23	90	14,558	62	2,414	156	TPO
2.25	97	2,271	59	1,210	88	TPO
2.26	127	1,257	72	2,649	111	TPO
2.27	91	1,926	84	11,189	95	TPO
2.28	95	3,444	95	2,901	131	TPO
2.30	94	2,554	74	2,541	86	TPO
2.33	86	7,538	87	1,914	86	TPO
2.34	159	3,386	73	6,038	143	TPO
2.35	87	2,018	64	1,184	83	TPO
2.36	127	1,973	69	5,893	99	TPO
2.40	280	2,308	61	4,762	142	TPO
2.14	87	1,773	147	263	146	(B6)*
2.7	139	1,343	91	510	89,003	Tg
2.1	112	32,667	118	83	96	TECs
2.5	171	26,398	85	100	126	TECs
2.10	115	2,093	62	228	100	TECs
2.22	162	20,724	84	115	113	TECs
2.24	116	45,918	51	100	103	TECs
2.32	85	2,065	75	96	119	TECs
2.37	85	3,204	65	532	191	TECs
2.38	78	2,149	64	190	86	TECs
2.39	67	3,816	79	290	149	TECs
2.41	169	57,961	94	167	148	TECs
2.41	131	45,992	232	133	110	TECs
2.11	137	945	71	105	113	None
2.13	251	1,193	148	722	134	None
2.17	97	1,272	70	95	103	None
2.29	108	817	60	103	79	None
2.31	111	1,257	86	115	131	None

Clones were derived without exposure to antigen prior to screening. Results are expressed as mean [^{3}H]thymidine incorporation (cpm) in triplicate cultures. Standard errors were <15% of the mean. Similar results were obtained in two or more experiments. Specificity: clones were considered reactive if the response to a particular antigen was >1000 cpm and represented a SI of >2.0. APCs were irradiated autologous PBLs. TECs were used at 10^4 per well. Control and TPO microsomes (micros) were as in Fig. 1. [^{3}H]Thymidine incorporation of TECs alone was 562 ± 136 cpm.
*Clone c2.14 reacted to a peptide epitope of TPO (see Table 3).

remaining three clones (c2.9, c2.14, and c2.26) and the Tg-reactive clone (c2.7) did show some proliferation above background in response to TECs, although this did not reach a SI of >2.0 in the *in vitro* system used. Eighty-five percent of the clones showed significant proliferation in response to thyroid-derived antigens and/or TECs, demonstrating the highly thyroid-specific nature of the *in vivo* activated T cells present at the disease site in this individual.

Recognition of Two Separate Epitopes of TPO. The response of thyroid-infiltrating T cells to a single thyroid antigen, TPO, was studied in greater detail. Twenty-eight synthetic peptides corresponding to putative T-cell motifs in the TPO sequence were prepared. These were used to screen uncloned, thyroid-derived T cells expanded in the absence of antigen (OKT3/IL-2). Low-level reactivity to two epitopes—NP-7, TPO residues 535–551 (SI = 4.8), and B6, residues 632–645 (SI = 1.4)—was seen (Fig. 2*A*). The responses to the two peptides could be separated and amplified by growth for 4 weeks in the presence of either NP-7 or B6 (SI 208.6 and 22.7, respectively, Fig. 2*B*). This confirms that these regions do indeed contain two distinct, noncrossreactive epitopes of TPO recognized by thyroid-infiltrating T cells.

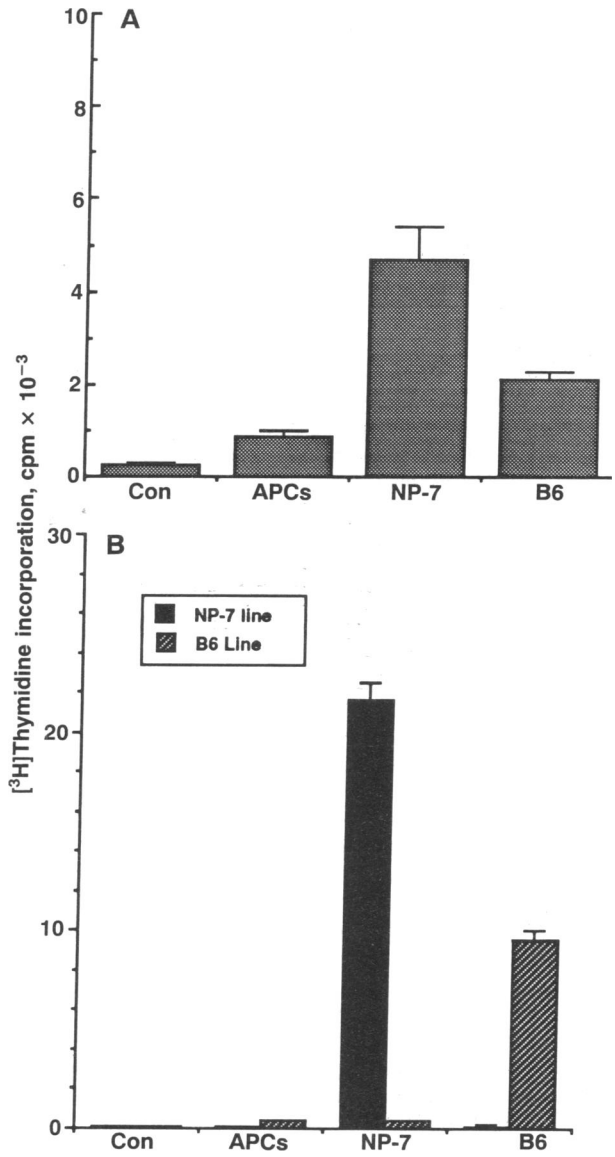

FIG. 2. Response of uncloned thyroid-infiltrating T cells to two peptide epitopes [NP-7 (residues 535–551) and B6 (residues 632–645)] of TPO before and after antigen selection. Additions to proliferation assay wells are shown below the bars. Con, T cells alone; APCs, EBV81 cells were added; NP-7 or B6, peptide was added to T cells plus APCs. (*A*) Infiltrating T cells expanded with OKT3 and IL-2 only. (*B*) Infiltrating T cells after selection with peptide NP-7 (solid bars) or peptide B6 (striped bars). Results are expressed as in Fig. 1.

HLA Class II Restriction of TPO Epitopes. The peptide-specific T-cell lines described above (Fig. 2*B*) were used to examine the HLA restriction elements involved in the response of thyroid-infiltrating T cells to TPO-based peptides NP-7 and B6. A panel of homozygous EBV-transformed B cells of known haplotype was employed for this purpose. Table 2 shows that the two epitopes were restricted by different HLA class II elements: B6 was restricted by DQ2, while NP-7 was recognized in the context of DP2 or DQ6.

Clonal Frequencies of NP-7 and B6 Recognition: Evidence for Further TPO Epitopes. To obtain an estimate of the relative frequency of reactivity to the TPO epitopes NP-7 and B6 in the *in vivo* activated thyroid-infiltrating population, a large panel of clones derived independently of antigen (OKT3/IL-2) was studied. Results of positive clones are shown in Table 3. Six out of 81 clones showed reactivity to NP-7, accounting for 18% of the TPO reactivity in this panel,

Table 2. HLA restriction of T-cell lines specific for TPO peptide epitopes NP-7 and B6

	HLA type						Response, SI	
EBV line	A	B	DR	DRw	DQ	DP	NP-7 line	B6 line
EBV81	1,2	8,37	2,3*	52	2.6	2,4	*67*	*22*
AHB	1	8	17*	52	2	4	0.2	*6.4*
STEINLIN	1	8	17*	52	2	3	0	*30*
RSH-D	30,68	8,38	18*	52	4	1	0	0
IBW9	33	65	7	53	2	1	0.2	*40*
FPF	1	35	11	52	6	2	*148*	0
ARNT	2	38,39	13a	52	6	2,3	*18*	NT
WT46	32	44	13b	52	6	2	*11*	NT
WT52	11	22	14b	52	5	4	0	NT
EFI-ND	1,2	7,37	10		5	NT	0.5	NT

TPO peptide-selected T-cell lines are as in Fig. 2*B*. SI values show the response of T cells to the peptide used for their selection. HLA types of EBV-transformed B cells used as APCs are shown. EBV81 was derived from the patient's own PBLs. Significant responses are shown in italic. NT, not tested.
*DR3 has been subdivided into DR17 and DR18. The patient has not been DR17/18-subtyped.

and 1 of 57 clones responded specifically to B6, corresponding to 4% of the total TPO-specific reactivity (see Table 3 and legend). However, 22 clones showed reactivity to TPO but not to either of these two epitopes (e.g., c2.33 and c2.91, Table 3). This indicates that thyroid-infiltrating T cells recognize at least three and probably more separate TPO epitopes in this patient. We have identified two of these (residues 535–551 and 632–645), accounting for around a quarter of the response to TPO. No response to either of these two epitopes was seen with peripheral blood-derived T cells from the same patient (Table 3).

Some of the less highly reactive peptide-specific clones (c1.75, c1.104, c2.14) did not show a significant response to the whole TPO preparation (Table 3), and in all cases the response to TPO microsomes was considerably lower than the response to the optimal concentration of peptide (10 μg/ml, Table 3). This suggests that the TPO content of this preparation was suboptimal for the stimulation of these T cells. Unfortunately, higher concentrations could not be used because of nonspecific inhibition by CHO cell proteins (data not shown). Peripheral blood T cells responded to both transfected and untransfected microsomes, indicating a response to CHO-derived proteins and masking any specific response to TPO. No response to control (untransfected) microsomes was seen at any time with thyroid-derived T cells, a further indication of their selected specificity (Fig. 1, Tables 1 and 3).

Table 3. Response of thyroid-derived T-cell clones and PBLs to two peptide epitopes of TPO and TPO microsomes

	[^{3}H]Thymidine incorporation, cpm					
T cells	APCs	NP-7	B6	Control micros	TPO micros	Specificity
c1.43	82	17,173	NT	654	2,121	NP-7
c1.75	346	2,544	NT	84	151	NP-7
c1.104	68	5,015	NT	44	260	NP-7
c1.105	599	4,455	NT	172	1,028	NP-7
c2.72	364	61,298	212	254	3,336	NP-7
c2.73	605	54,116	362	360	2,976	NP-7
c2.14	157	125	14,560	133	213	B6
c2.33	148	161	181	202	6,183	TPO
c2.91	183	193	207	573	4,415	TPO
PBLs	753*	797	619	14,824	9,534	None

Clones were derived without prior exposure to antigen. Responses of all peptide-reactive clones are shown with those of two representative clones responding to TPO microsomes but not NP-7 or B6. Results and criteria for a significant response are as in Table 1. Thirty-four of 81 clones screened against NP-7 and 25 of 57 clones screened against B6 responded to TPO microsomes (data not shown). APCs were irradiated autologous PBLs or fixed EBV81 cells. NT, not tested.
*PBLs alone, with no added APCs.

DISCUSSION

T cells present in the thyroid of the patient CX81 were shown to recognize at least three separate autoantigens expressed in this tissue. Two of these antigens have been identified (Tg and TPO) and are known to be exclusive to the thyroid. The frequency of *in vivo* activated clones specific for tissue antigens was striking (85%), confirming at a molecular level that infiltrating T cells are very highly selected for recognition of these antigens. The low frequency of Tg-specific cells identified (3%) compared with TPO reactivity (46%) is consistent with the undetectable level of anti-Tg autoantibodies but very high titers of anti-TMA antibodies in this patient's serum. In view of the central role of anti-TSH receptor (agonist) antibodies in Graves disease (1) and the high level of these antibodies in this patient, the most likely candidate for at least part of the large non-Tg/non-TPO reactivity to TECs seen in this individual (36% of clones) is the TSH receptor itself.

We have also examined the response of thyroid-infiltrating T cells to a single thyroid antigen, TPO, at the peptide epitope level. Infiltrating T cells were shown to recognize at least three distinct epitopes of TPO presented in the context of at least two separate HLA class II elements. The location of two of these epitopes has been identified (residues 535–551 and 632–645). No response to these epitopes was seen with the patient's peripheral blood T cells (Table 3), further emphasizing the degree of antigenic selection in the thyroid. It is of interest that none of the HLA restriction elements involved with the presentation of the TPO epitopes defined here was DR3, the HLA class II element that has been most frequently associated in epidemiological studies with increased susceptibility to Graves disease (1) and that was also present in this individual. This contrasts with the view that the basis for genetic associations between HLA elements and autoimmune disease is the requirement of these elements in presentation of autoantigen. Our observations may indicate (*i*) that the epidemiologically associated HLA element is more concerned with repertoire selection in the thymus than with antigen presentation in the target organ or (*ii*) that epitopes in addition to those identified here play a greater role in disease pathogenesis.

Marked heterogeneity of autoantigen recognition by T cells at the site of disease was observed in this study. This was manifest at three levels: the number of proteins recognized, the number of epitopes on a single protein involved, and the number of HLA restriction elements used. Although no general conclusions can be made from analysis of a single individual, such heterogeneity is consistent with the range of autoantibody specificities known to be present in this disease (24, 25) and the lack of oligoclonality observed in T-cell receptor usage in this (26) and other (27) autoimmune diseases. On the other hand, it contrasts sharply with the limited repertoire of pathogenic T cells reported in some induced models of autoimmunity—most notably, murine experimental allergic encephalomyelitis (18). In addition, it is not immediately reconcilable with the concept that autoimmunity is due to a crossreactive response to a single epitope present on an infectious agent (28). Heterogeneous recognition of autoantigen also poses a challenge to the development of highly targeted anti-T-cell immunotherapies in humans. Such therapies have been possible in induced animal models of autoimmune disease, because of the limited heterogeneity of

pathogenic cells involved (18, 19). Clearly, further studies of the kind seen here are urgently needed to resolve these important issues.

We thank Pui Seto, Anne Hales, and Gina Knyba for expert assistance; Dr. Julie Clayton for her suggestions and collaboration; Dr. G. Goldstein and Dr. E. Golub for OKT3; Dr. Peter Smyth for anti-TSH receptor antibody-binding studies; Drs. J. Markwick, J. Bodmer, and M. Simons for HLA typing our patient; and Prof. P. Bell for his interest in this work.

1. Gossage, A. A. R. & Munro, D. S. (1985) *Clin. Endocrinol. Metab.* **14**, 299–330.
2. Doniach, D., Bottazzo, G. F. & Drexhage, H. A. (1982) in *Clinical Aspects of Immunology*, eds. Lachmann, P. J. & Peters, D. K. (Blackwell, Boston), Vol. 2, pp. 903–937.
3. Malthiery, Y. & Lissitzky, S. (1987) *Eur. J. Biochem.* **165**, 491–498.
4. Kimura, S., Kotani, T., McBride, O. W., Umeki, K., Hirai, J. M., Nakayama, T. & Ohtaki, S. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5555–5559.
5. Nagayama, Y., Kaufman, K. D., Seto, P. & Rapoport, B. (1989) *Biochem. Biophys. Res. Commun.* **165**, 1184–1190.
6. Seto, P., Hirayu, H., Magnusson, R. P., Gestautas, J., Portmann, L., DeGroot, L. J. & Rapoport, B. (1987) *J. Clin. Invest.* **80**, 1205–1208.
7. Warford, A., McLachlan, S. M., Malcolm, A. J., Young, E. T., Farndon, J. R. & Rees Smith, B. (1984) *Clin. Exp. Immunol.* **57**, 626–632.
8. Weetman, A. P., McGregor, A. M., Lazarus, J. H. & Hall, R. (1982) *Clin. Exp. Immunol.* **48**, 196–200.
9. Miller, J. F. A. P. (1972) *Int. Rev. Cytol.* **33**, 77–130.
10. Hanafusa, T., Chiovato, L., Doniach, D., Pujol-Borrell, R., Russell, C. G. & Bottazzo, G. F. (1983) *Lancet* **ii**, 1111–1115.
11. Zheng, R. Q. H., Abney, E., Grubeck-Loebenstein, B., Dayan, C. M., Maini, R. N. & Feldmann, M. (1991) *J. Autoimmun.* **3**, 727–736.
12. Grubeck-Loebenstein, B., Buchan, G., Chantry, D., Kassal, K., Londei, M., Pirich, K., Barrett, K., Turner, M., Waldhausl, W. & Feldmann, M. (1989) *Clin. Exp. Immunol.* **77**, 324–330.
13. Londei, M., Bottazzo, G. F. & Feldmann, M. (1985) *Science* **228**, 85–89.
14. Weetman, A. P., Volkman, D. J., Burman, K. D., Margolick, J. B., Petrick, P., Weintraub, B. D. & Fauci, A. S. (1986) *Clin. Immunol. Immunopathol.* **39**, 139–150.
15. Del Prete, G. F., Mariotti, S., Tiri, A., Ricci, M., Pinchera, A. & Romagnani, S. (1987) *Acta Endocrinol. Suppl.* **281**, 111–114.
16. Grubeck-Loebenstein, B., Londei, M., Greenall, C., Pirich, K., Kassal, K., Waldhausl, W. & Feldmann, M. (1988) *J. Clin. Invest.* **81**, 1608–1614.
17. Grubeck-Loebenstein, B., Turner, M., Pirich, K., Kassal, H., Londei, M., Waldhäusl, W. & Feldmann, M. (1990) *Scand. J. Immunol.* **32**, 433–440.
18. Urban, J. L., Kumar, V., Kono, D. H., Gomez, C., Horvath, S. J., Clayton, J., Ando, D. G., Sercarz, E. E. & Hood, L. (1988) *Cell* **54**, 577–592.
19. Wraith, D. C., Smilek, D. E., Mitchell, D. J., Steinman, L. & McDevitt, H. O. (1989) *Cell* **59**, 247–255.
20. Kaufman, K. D., Rapoport, B., Seto, P., Chazenbalk, G. D. & Magnusson, R. P. (1989) *J. Clin. Invest.* **84**, 394–403.
21. Rothbard, J. B. & Taylor, W. R. (1988) *EMBO J.* **7**, 93–100.
22. DeLisi, C. & Berzofsky, J. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7048–7052.
23. Neitzel, H. (1986) *Hum. Genet.* **73**, 320–326.
24. Bresler, H. S., Burek, C. L., Hoffman, W. H. & Rose, N. R. (1990) *Clin. Immunol. Immunopathol.* **54**, 76–86.
25. Banga, J. P., Barnett, P. S., Ewins, D. L., Page, M. J. & McGregor, A. M. (1990) *Autoimmunity* **6**, 257–268.
26. Lipoldova, M., Londei, M., Grubeck-Loebenstein, B., Feldmann, M. & Owen, M. J. (1989) *J. Autoimmun.* **2**, 1–13.
27. Cooke, A. (1991) *Clin. Exp. Immunol.* **83**, 345–346.
28. Oldstone, M. B. (1987) *Cell* **50**, 819–820.