Analysis of T-cell Receptor Usage in Activated T-cell Clones from Hashimoto’s Thyroiditis and Graves’ Disease

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Rearrangements to the T-cell receptor (TcR) β and γ gene loci were studied in T cells derived from the thyroid glands of a patient with Hashimoto’s (HT) and another with Graves’ (GD) autoimmune thyroiditis. The cells studied were freshly isolated mononuclear cells, T-cell lines grown in the presence of anti-CD3 and IL-2 and T-cell clones. Numerous different rearrangements to the constant regions of TcRβ and TcRγ and in the variable gene region of TcRβ were observed. These findings indicate that the T-cell response in autoimmune thyroiditis is multiclonal and may have implications for the epitopes recognized by autoreactive T cells and for the mechanisms of the disease.

To function effectively, the immune system must be able to distinguish self from non-self. The mechanism(s) that mediate this process are not yet fully understood. Various ones have been implicated, such as the involvement of suppressor cells, tolerance induction and idiotypic regulation (reviewed in [1]).

The major structure which discriminates between T cells of various specificities is the T-cell receptor (TcR) for antigen. Studies over the past 3 years have identified the TcR on the great majority of T cells as a disulphide-linked αβ heterodimeric glycoprotein, in close association on the membrane with the signal transducing CD3 complex [2]. It would be predicted that T-cell receptor usage should be related to the mechanisms of discrimination of self from non-self, and recently Kappler and colleagues have produced evidence that this is so. Thus, in certain mouse strains,
whilst there are immature thymocytes expressing the Vβ 17a gene segment, these cells do not mature into T cells. A clear correlation was found with the expression of I-E suggesting that Vβ 17a was involved in I-E recognition and that Vβ 17a suppression was related to the process of self recognition [3].

In autoimmune disease, there is a failure of the mechanisms preventing self recognition. This has been demonstrated at the T-cell level in Graves' disease, where there is a high frequency of T cells recognizing thyroid cell surface antigens in association with HLA Class II antigens [4]. Graves' disease and Hashimoto's thyroiditis are two autoimmune diseases of the thyroid which have distinct endocrine sequelae, with the former being associated with hyperthyroidism whilst the latter, due to destruction of the thyroid follicular cells, leads to hypothyroidism. In both conditions, the thyroid is infiltrated by T lymphocytes many of which are activated, as judged by the expression of the IL-2 receptor and Class II antigens. The majority of the cells, including the thyroid follicular cells, express Class II antigens. Based on these observations, it was proposed that the pathogenesis of these diseases involved the chronic restimulation of activated T cells by epithelial antigen-presenting cells whose Class II expression was maintained by lymphokines produced by the activated T cells [5]. Several aspects of this hypothesis have been substantiated, with IFNγ being shown to induce Class II antigen expression on thyroid epithelial cells [6], which can then act as antigen-presenting cells in several systems [7], including autoreactive T-cell clones [4].

However, little is known about why these diseases occur in certain individuals. Both diseases are HLA-D region associated, as are most autoimmune diseases [8], but the remaining components of the aetiology are not understood (reviewed in [9]).

We have investigated the nature of the T-cell receptors on the activated T cells in Graves' and Hashimoto's thyroiditis in order to determine whether the TcR may contribute to causing these diseases. In this study TcRβ gene rearrangement was investigated using a variety of probes. These experiments failed to show a simple predominant pattern of rearrangement in T cells from Hashimoto's or Graves' thyroids and indicate significant heterogeneity in these cells. These results have implications both for the epitopes recognized on the thyroid membrane and for TcR repertoire usage.

Materials and methods

Patients

Intrathyroidal T lymphocytes from a patient with Hashimoto's thyroiditis (HT) (female, aged 39 years) and from another with Graves' disease (GD) (female, aged 46 years) were used. Purification and culture of thyroid-infiltrating lymphocytes were performed as follows: surgically removed thyroid tissue was dispersed with collagenase (Cooper Biomedical Collagenase, Type IV, 5 µg/ml, in RPMI 1640 medium containing 15% fetal calf serum, Gibco) for 3 h and pipetted through a 200 µ mesh. The red blood cells were then lysed with an ammonium chloride buffer. Infiltrating mononuclear cells were separated from thyroid follicular cells on the basis of 16 h adherence and purified over Ficoll gradient centrifugation (Lymphoprep, Nyegard, Oslo, Norway). In vivo activated T cells were selected from
this population by the expression of functional IL-2 receptors, by one week's culture in RPMI supplemented with 10% human serum and recombinant interleukin-2 (IL-2, 0.025 μg/ml; Sandoz, Vienna, Austria, or Ajinomoto, Japan). The T-cell lines were established by culturing these cells in the presence of CD3 antibody (OKT3, 0.03 μg/ml; American Type Culture Collection, ATCC, Rockville, Maryland, USA) together with irradiated (3,000 rad) autologous peripheral blood mononuclear cells as feeders and IL-2 (0.025 μg/ml). This technique supplies non-specific stimuli and allows the growth of T cells while retaining their specificity [10, 11]. After 3 weeks of culture, T-cell clones were prepared from the lines. Cloning and screening of the clones for phenotype (T4/T8) and thyroid autoantigen recognition (specific proliferation in response to autologous thyroid follicular cells and thyroglobulin) was performed as previously described [12, 13]. A detailed description of the clones is given elsewhere [12, 14].

**Southern blot analysis**

DNA from 5 x 10⁵ cells was prepared in agarose blocks as described by Williams [15]. Briefly, the cells were immobilized in low gelling temperature agarose, protein was digested by incubation for 2 h at 50°C with proteinase K, and the products of digestion were removed by washing with 10 mM Tris-Cl pH 8.0 and 1 mM EDTA at 4°C. DNA was then digested in agarose with one of the restriction enzymes BamHI, EcoRI or HindIII. The fragments were separated by electrophoresis through 0.8% agarose, transferred to Amersham Hybond N nylon membrane and hybridized for 16 h at 65°C with 3²P labelled probes (specific activity 2–5 x 10⁶ dpm/μg) in 0.45 M NaCl, 0.045 M sodium citrate, 10 x Denhardt’s, 0.1% SDS, 5% dextran sulphate and 50 μg/ml salmon sperm DNA. Blots were washed twice for 30 min at 65°C with 0.3 M NaCl, 0.03 M sodium citrate, 0.1% SDS and once for 15 min at 65°C with 0.015 M NaCl, 0.0015 M sodium citrate, 0.1% SDS and exposed at -70°C on Kodak XAR-5 film [16].

The probes used detected Cβ1 and Cβ2 sequences [17], TcRγ sequences (a gift from Dr T. Mak), Vβ8 (a gift from Dr T. Rabbitts) [18], Vβ2 and Vβ11 (gifts from Dr D. Loh) [19], and Vβ5 which was subcloned from a TcR cDNA from the cell line HPB-ALL [20].

**Northern blot analysis**

Total RNA from 1–2 x 10⁶ cells prepared by the guanidinium CsCl method [21] was electrophoresed on 1% agarose [22], transferred to Hybond N and hybridized for 16 h at 42°C with 3²P labelled probes (specific activity 2–5 x 10⁶ dpm/μg) in 0.75 M NaCl, 0.075 M sodium citrate, 50% formamide, 0.05 M sodium phosphate pH 6.4, 1 x Denhardt’s, 0.1% SDS, 50 μg/ml salmon sperm DNA. Blots were washed twice for 30 min at 50°C with 0.075 M NaCl, 0.0075 M sodium citrate, 0.1% SDS.

**Results**

**TcRβ gene rearrangements in Hashimoto's thyroiditis**

In order to estimate whether one T-cell clone dominates the immune response to Hashimoto’s thyroiditis or whether the response is polyclonal, T-cell receptor rearrangements were examined in freshly isolated thyroid mononuclear cells and in the
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Figure 1. Rearrangements to the Cβ 1 and Cβ 2 loci in T-cell clones from HT. DNA from the B-cell line BR18 and from clones HTCX25-24, HTCX25-41, HTCX25-42 and HTCX25-54 was digested with the restriction enzyme EcoRI (RI) or Hind III (H3) and examined by Southern blot analysis using a Cβ probe.

corresponding T-cell lines grown for one month in the presence of IL-2 and subsequently CD3 antibody. Several restriction enzymes were used in these experiments. Thus, EcoRI and Hind III were used to detect rearrangements to the Cβ 1 and Cβ 2 loci respectively, whereas Bam H1 was used to detect allelic rearrangements. Only the 23 kb germline band was detected by Southern blotting analysis of Bam H1-digested DNA from freshly isolated thyroid mononuclear cells, whereas a smear of rearrangements was detected in Bam H1-digested DNA from the T-cell line grown for one month in the presence of IL-2 and anti-CD3 (data not shown). An additional diffuse band of about 20 kb was also detected. This band was not, however, exclusive to Hashimoto's thyroiditis since it was also observed in other T-cell populations grown in IL-2, such as phytohemaglutinin stimulated T blasts and T cells stimulated with an anti-clonotypic T-cell receptor antibody 3D6, and may reflect an 'average' of many rearrangement events detected with Bam H1.

These experiments using Bam H1 failed to provide evidence for a predominant population of T cells with rearrangements characteristic for Hashimoto's thyroiditis. Similar experiments, in which rearrangements to Cβ 1 and Cβ 2 were studied separately using EcoRI and Hind III, gave similar results (data not shown).

In order to study further the TcRβ rearrangements in Hashimoto's thyroiditis, 12 T-cell clones were studied. A representative blot from these experiments is shown in Figure 1 and the results from the entire set of clones are summarized in Table 1. Each clone exhibited a different rearrangement pattern. For example, as shown in Figure 1, in clone HTCX25-24 one Cβ 1 gene was deleted and the second rearranged, whereas in clone HTCX25-41 both Cβ 1 genes were deleted. In clone HTCX25-42 both Cβ 1 genes were rearranged and in clone HTCX25-54 one Cβ 1 gene was in the germline configuration and the second was rearranged. The Cβ 2 locus also showed a different pattern of rearrangement in these clones. Thus, in clone HTCX25-24 the
Table 1. Cβ gene arrangements in thyroid-derived T-cell clones from a patient with Hashimoto's thyroiditis

<table>
<thead>
<tr>
<th>BR18</th>
<th>T4</th>
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<tr>
<td>Cβ 1</td>
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<td>R</td>
<td>R</td>
<td>D</td>
<td>D</td>
<td>R</td>
</tr>
<tr>
<td>Cβ 2</td>
<td>G</td>
<td>R</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>D</td>
<td>G</td>
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</table>

The phenotypes (T4+ or T8+) of the clones used are presented under their names. Rearrangements (G = germline, D = deleted, R = rearranged) to the Cβ 1 and Cβ 2 loci are shown.

Cβ 2 gene(s) were in a germline configuration, whereas in clones HTCX25-41 both Cβ 2 genes were rearranged. Clone HTCX25-42 exhibited one Cβ 2 gene in the germline configuration and a deleted allele. In clone HTCX25-54 Cβ 2 gene(s) were in the germline configuration. There was no correlation between the phenotype (T4+ or T8+) and the rearrangement pattern of the respective clones (Table 1). Taken together, these data indicate that at the clonal level there is no predominant TcRβ gene rearrangement associated with Hashimoto's thyroiditis. The clones used were of independent origin. This conclusion is confirmed by the results of Southern blotting analysis using a TcRγ gene probe in which multiple different rearrangement patterns were also found (Figure 2).

Eighteen Vβ subfamilies have been described for the human TcRβ locus [23]. We have studied the rearrangement and use of four Vβ subfamilies, Vβ 2, Vβ 5, Vβ 8 and Vβ 11 which together constitute at least 25% (as estimated by the number of bands hybridizing on Southern blots) of human Vβ genes and are dispersed over about 400 kb of DNA [23]. Sequences hybridizing with a Vβ probe can in principle be either rearranged in T-cell DNA, indicating non-productive or productive re-arrangement to the TcRβ constant region, or deleted, indicating usage of a more 5’ Vβ gene (Figure 3).

The results of Southern blotting analysis using the Vβ probes are summarized in Table 2. Representative blots are shown in Figure 3. No deletions or rearrangements were observed using a Vβ 5 probe, consistent with this subfamily being the most distant from the Cβ locus of the four subfamilies studied [23]. Co-deletions in parts of the Vβ 8 and Vβ 11 subfamilies in clones HTCX25-19, HTCX25-82, HTCX25-66, HTCX25-76, and HTCX25-15, probably reflect linkage disequilibrium between the two Vβ families. A linkage disequilibrium between a 2 kb fragment of the Vβ 8 subfamily and a 25 kb fragment of the Vβ 11 subfamily, and a 23 kb band from Vβ 8 and 22 kb band from Vβ 11 has been described [24]. Thus, the patient from whom the present clones have been derived is probably heterozygous for these differently linked fragments.

Rearranged bands which were coincident with bands hybridizing to a Cβ probe were observed in clone HTCX25-82 using Vβ 2 and Vβ 8 probes. To assess whether
Figure 2. Rearrangements to the TcRγ locus in HT T-cell clones. DNA from BR18 and from clones HTCX25-22 and HTCX25-15 was digested with the restriction enzyme Bam HI (B) or Hind III (H3), electrophoresed and hybridized with a TcRγ probe.

one of these rearrangements was productive, total RNA was isolated from the clones HTCX25-15, HTCX25-41, and HTCX25-82, and probed for Vβ expression. The results of this experiment are shown in Figure 4. Using a Vβ2 probe a 1.3 kb transcript was detected in RNA from clone HTCX25-82 but not in the other clones analysed. When this blot was hybridized with a Cβ probe, 1.3 kb transcripts were detected in all samples, although the weakest hybridization was in the HTCX-82 track.

TcRβ rearrangements in Graves' disease

Six clones from a patient with Graves' thyroiditis (GT) were studied. Phenotypes and thyroid autoantigen specificity are listed in Table 3. Three of the six clones proliferated in response to autologous thyroid follicular cells, suggesting specificity to one or several not yet defined membrane-bound autoantigens. None of the six clones reacted to thyroglobulin. Figure 5 shows the allelic TcRβ rearrangements in these clones. As was the case for HT, no predominant rearrangements were detected, demonstrating that all the clones were of independent origin. The rearrangements involving Vβ2 and Vβ5 were also studied. No deletions or rearrangements were found using a Vβ5 probe. After hybridizing with a Vβ2 probe the T-cell clone 14.4 showed rearranged bands and clones B14.9 and B14.12 showed deletions in this subfamily. However no predominant use of these subfamilies was detected. T-cell
Figure 3. Southern blot analysis of four Vβ subfamilies in HT T-cell clones. DNA from clones HT25-82, HT25-76, HT25-66, HT25-19 and BR18 was digested with Bam HI and hybridized in sequence with (A) Vβ 2, (B) Vβ 5, (C) Vβ 8 and (D) Vβ 11 probes. After each hybridization the blot was stripped and checked for absence of a signal before rehybridizing.

receptor rearrangements did not distinguish thyroid autoreactive and non-specific clones and the three autoreactive clones did not show a common rearrangement pattern.

Discussion

There is considerable evidence that T cells are intimately involved in both human autoimmune diseases and animal models. The chronic phase or perpetuation of many human autoimmune diseases, such as thyroiditis or insulin-dependent diabetes, depends on the HLA Class II-expressing target tissues, and autoreactive T-helper cells. These cells in turn produce mediators which further induce tissue Class II expression and initiate the inflammation and destruction of the target tissues [5].

Evidence to support this concept has come from a variety of sources. First, immunohistological analysis has shown that most autoimmune diseases (e.g. Graves' and Hashimoto's thyroiditis, diabetes, rheumatoid arthritis) over-express HLA Class II in the tissues [e.g. 9, 25]. Histological and functional analysis shows that there are many activated T-cells infiltrating the tissues in these diseases [4]. These cells may be cloned, and in Graves' disease it has been shown that many of these are restimulated by HLA Class II-expressing thyrocytes [4]. Products of activated T cells such as IFNγ and tumour necrosis factor or lymphotoxin are involved in the induction of Class II in tissue cells in vitro [6, 26].

In animal models, the role of T-cells has been demonstrated even more directly. T cells and T-cell clones may transfer a variety of diseases, such as experimental allergic
Table 2. Comparison of hybridization of Vβ probes to DNA of B-cell line BR18 and to DNA fragments from thyroid-derived T-cell clones from a patient with Hashimoto’s thyroiditis

| Vβ 2/R1  | 6.2  | G   | G   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 2/H3  | 18   | G   | G   | G   | G   | G   | D   | G   | ?   | |
| Vβ 2/H3  | 13   | G   | G   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 2/B   | 17   | G   | G   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 2/B   | 4.5  | G   | G   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 5/R1  | 11   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 5/R1  | 9.5  | G   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 5/R1  | 6.4  | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 5/H3  | 6.5  | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 5/H3  | 0.117| G   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 6/B   | 12   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 8/B   | 6.5  | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 8/R1  | 6    | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 8/H3  | 13   | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 8/B   | 4.6  | G   | D   | G   | G   | D   | G   | G   | |
| Vβ 8/R1  | 6    | G   | D   | G   | G   | D   | G   | G   | |
| Vβ 8/H3  | 13   | G   | G   | G   | G   | D   | G   | G   | |
| Vβ 8/B   | 8    | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 8/B   | 3.2  | G   | G   | G   | G   | G   | G   | G   | |
| Vβ 8/B   | 2    | G   | G   | G   | G   | D   | G   | G   | |
| Vβ 11/   | 13   | G   | G   | ?   | G   | |
| H3       | 10.4 | G   | D   | G   | ?   | G   | |
| Vβ 11/B  | 9.7  | G   | G   | G   | G   | G   | G   | |

The rearrangements identified after digestion of DNA with restriction enzymes EcoRI (R1), Hind III (H3) and Bam HI (B) and hybridization with V probes Vβ 2, 8, 5 and 11 are shown.

For BR18 (germline control) the DNA fragment length is presented in kb. (G) signifies that in the autoreactive T-cell population (pop), line or clone the germine band is present; (D) indicates that the fragment is deleted, (R) that a new band different in size from that of the germline appears; (?) indicates that we were unable to conclude whether or not the fragment was present. Empty windows: experiment not done.

encephalitis, experimental myasthenia gravis, and adjuvant and collagen arthritis [e.g. 27, 28]. Treatment which blocks T-cell activation, such as anti-Ia antibodies which can block antigen presentation [29] or anti-IL-2 receptor antibodies which can block T-cell growth [28] or anti-CD4 antibodies [29], can all block the progression or even cure experimental autoimmune disorders.

The lines and clones used in this study were derived from the activated T cells infiltrating the thyroid in HT or GD. These activated T cells form part of the pool responsible for the disease process, although currently it is not known what these
Figure 4. Expression of Vβ2 and Cβ transcripts. Total cellular RNA from BR18 (5 × 10⁶ cells), T-cell clones HTC25-15, HTC25-41, HTC25-82 (10⁶ cells), peripheral blood lymphocytes grown in the presence of IL-2 (5 × 10⁶ cells) and 1 μg poly(A)⁺ RNA from T-cell lines HUT78 and HSB2 were used for Northern hybridization analysis. The RNA was hybridized with a Vβ2 probe (A), stripped and rehybridized with a Cβ probe (B). Exposure time in Figure 4A was 10 d at -70°C. In Figure 4B BR18, HTC25-15, HTC25-41 and HTC25-82 tracks were exposed overnight at -70°C and peripheral blood lymphocytes, HUT78 and HSB2 tracks for 2 h at room temperature.

Table 3. Phenotype and thyroid autoantigen reactivity of thyroid-derived T-cell clones from a patient with Graves’ disease

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<td>Phenotype (T4 = helper/T8 = cytotoxic suppressor)</td>
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<td>autologous thyroid follicular cells</td>
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<td>thyroid autoantigen reactivity</td>
<td>thyroglobulin</td>
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cells recognize. Some of the GD clones recognize thyrocytes, but the lack of residual thyrocytes makes it impossible to ascertain if this is the case in HT. However, the expression of IL-2 receptor implies very recent activation (within the last week [30]), and thus these clones are clearly relevant to the disease process. It is conceivable that a single dominant clone may be responsible for the autoimmune disease process.
as proposed by Burnet [31]. However, the heterogeneity of autoantibodies detected in GD and HT would seem to render unlikely the idea that a single helper T-cell clone could be responsible, on the basis of linked recognition of T and B cell epitopes [32]. The results presented here demonstrate that no predominant β gene rearrangement is found in infiltrating cells, autoreactive T-cell lines and clones in either Hashimoto’s or Graves’ thyroiditis. In the latter disease it was possible to ascertain that these were autoreactive T cells (Table 3) and even these were heterogeneous in their rearrangement pattern, with the sizes of the observed rearrangements implying different Vβ usage. Productive TcRβ gene rearrangements are generated in two stages, namely DJβ to Jβ and Vβ to DJβJβ. In principle, different rearrangements, as detected by Southern hybridization analysis, could be generated by the use of different Vβ and/or Jβ segments. Although none of the four Vβ families studied here are preferentially utilized in clones from HT, it cannot be ruled out that another Vβ subfamily is used by these clones without the use of additional Vβ probes or direct DNA cloning. However, the variation in size of the observed rearrangements strongly suggests different Vβ usage in these clones in addition to any differential Jβ usage.

The results presented here provide the first molecular data about T-cell receptors in HT and GD obtained by studying autoimmune clones derived from the activated T-cell pool. Similar experiments in other autoimmune diseases have given conflicting results. Thus, in human multiple sclerosis (MS) IL-2 dependent clones derived from active MS plaques and blood, two of seven clones from blood and one of six clones from brain exhibited the same rearrangement pattern [33]. In thymus and peripheral lymph nodes of lpr/lpr and glrlglrl mice which develop a disease similar to systemic lupus erythematosus, non-clonal rearrangements of TcRβ were found [34]. In T-cell populations grown from synovial specimens from patients with rheumatoid arthritis, distinct β gene rearrangements were detected in 13 of 14 cultures, suggesting a limited number of T-cell specificities [35].
Analysis of a variety of model systems has indicated that clones recognizing a defined antigen and restricted to a defined MHC allele generally use a restricted number of $\alpha$ and/or $\beta$ genes. In the immune response to pigeon cytochrome c, Fink et al. have shown that in five TCrS studied, TCR gene usage correlated with epitope specificity. Thus three different receptors using closely related $\alpha$ and $\beta$ genes were identified with $\beta$ usage, correlating with MHC specificity of the TCR [36]. Consistent with this finding was the transfer of MHC specificity by transfection of a TCR$\beta$ gene from a TCR recognizing moth cytochrome c plus IE$^+$ [37]. In addition Winoto et al. demonstrated that there was predominant use of a $\alpha$ gene in several T-cell clones recognizing pigeon cytochrome c [38]. Also, dominance of one TCR has been found in the response to a simple determinant TNP [39]. However, in another study different specificities of two TCRS with the same $\alpha$ and $\beta$ regions were obtained by usage of separate diversity and joining regions [40]. Taken together, these results indicate that T-cell clones recognizing the same or very similar epitope tend to utilize a severely restricted number of V regions. The epitopes recognized by autoreactive T cells on thyroid tissue are unknown. However, the observed heterogeneity of $\beta$ expression suggests that multiple epitopes are recognized as foreign. Alternatively epitope recognition may indeed be restricted but may be associated primarily with $\alpha$ (or $\alpha$) expression. The extended nature of the $\alpha$ locus makes analysis of gene rearrangement using Southern blotting generally uninformative. Detailed sequence analysis will be required to analyse further both $\alpha$ and $\beta$ usage in these clones.

Recently, such an analysis was described with murine T-cell clones recognizing peptide 1-9 of myelin basic protein in PL/J and (PL/J x SJL) mice [41]. A very restricted usage of TCR $\alpha$, $\beta$, and $\gamma$ segments was noted. Sequence analysis of the HT and GD clones described here will determine whether a difference in the heterogeneity of the T-cell response indeed exists between these two autoimmune diseases.

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References

T-cell receptor usage in autoimmune thyroiditis


