Identification of Autoimmune T Cells Among In Vivo Expanded CD25⁺ T Cells in Multiple Sclerosis

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Identification of Autoimmune T Cells Among In Vivo Expanded CD25+ T Cells in Multiple Sclerosis

Zsolt Illés, Takayuki Kondo, Kazumasa Yokoyama, Takashi Ohashi, Takeshi Tabira, and Takashi Yamamura

Although clonal expansion of autoimmune T cells has been reported in multiple sclerosis (MS), very limited information is available on specificities, clonal size, or activation state of the expanded clones. Here we address the issue of clonal expansion by using a novel technique demonstrating clonotypes defined by single-strand conformation polymorphism of TCR β-chain cDNAs. Examination of activated T cells (CD3^+CD25^+) isolated from the peripheral blood of MS revealed limited numbers (20–82) of expanded clones defined by single-strand conformation polymorphism clonotype. To estimate the Ag specificities of dominant clonotypes in the activated T cells, these samples were examined in parallel with Th1 T cell clones specific for myelin basic protein or proteolipid protein (PLP) derived from the same patients. Analysis of two patients demonstrated that the dominant clonotypes would contain those specific for myelin basic protein or PLP. Although the majority of the clonotypes could be detected only transiently, a PLP95–116-specific clonotype was found to persist for over 1 yr. Thus, single-strand conformation polymorphism clonotype analysis allows us to monitor the kinetics of given T cell clones in vivo and could provide useful information for designing clonotype (Id)-specific manipulation of human diseases such as MS. The Journal of Immunology, 1999, 162: 1811–1817.

M

ultiple sclerosis (MS) is a presumed autoimmune disease in which T cells reactive to central nervous system autoantigens such as myelin basic protein (MBP) and proteolipid protein (PLP) may play a central role (Reviewed in Refs. 1 and 2). This postulate is based on substantial evidence, such as increased frequencies of MBP- or PLP-specific T cells in IL-2-stimulated culture of blood T cells from MS vs healthy individuals (3) or significant homologies in TCR CDR3 sequences between MBP- or PLP-specific T cell clones and T cell infiltrates in MS brain lesions (4, 5). Repetitive acquisition of identical T cell clones specific for MBP at different time points also supports the role of MBP-reactive T cells in MS and has been regarded as evidence for persistent expansion of MBP-reactive T cells in MS (6). Furthermore, a PCR-based study measuring TCR mRNAs indicates that the frequencies of MBP-reactive T cells in MS could be much higher than previously estimated (7). These findings suggest the outstanding role of limited numbers of autoimmune T cell clones in the pathogenesis of MS and that specific deletion of these clones by means of T cell vaccination may lead to amelioration of clinical manifestations (8). However, it was not clear in the previous studies (6, 7) whether the T cell clone, estimated to be expanded, was one of a few largest clones in the repertoire or represented the numerous subdominant clones. The advantage of anti-clonotype therapy could be expected only in the former case.

In the present study, we addressed the questions for autoimmune T cell expansion in MS by using a novel technique relying on the single-strand conformation polymorphism (SSCP) of TCR messenger signals. The SSCP-based clonality analysis (9, 10), referred to also as SSCP clonotype analysis, depends on the fact that a single strand of the nucleotide chains would form a unique conformation according to its sequence and migrate to its own position during electrophoresis in the SSCP gel (11). As such, when cDNAs of TCR β-chains from a given sample are amplified by RT-PCR, denatured, and electrophoresed, the TCR message of clonally expanded T cells (clonotype) can be visualized as a distinct band, while those from minor clones are erased in the background smear (Reviewed in Ref. 10). While SSCP clonotype analysis of freshly isolated PBMC or biopsy samples provides basic information for T cell clonality in vivo (9, 10, 12–15), comparison of accumulated T cell clones in different samples is also informative because of the reproducible mobility of each clonotype. As reported in a recent study (16), parallel examination of T cell clones with defined Ag specificities could lead to identification of Ag specificities of accumulated clonotypes in vivo. However, because of limited availability of T cell clones specific for target autoantigens, the feasibility of this strategy has not been formally proven in the field of autoimmune disease research. Owing to a panel of T cell clones specific for putative encephalitogenic epitopes, the present study could provide definitive evidence for expansion and activation of autoimmune T cells reactive to defined epitopes of MBP or PLP in MS. Furthermore, we obtained insights into the dynamics of the autoimmune T cell clones and its relative dominance during clinical course.
Materials and Methods

Subjects

All patients except for one (patient TN with acute MS) had definite MS fulfilling the diagnostic criteria proposed by Poser et al. (17). The diagnosis was further assisted by magnetic resonance imaging in all. Clonal expansion of MBP or PLP peptide-specific T cells was investigated in two relapsing/remitting MS patients (patient OK: 54-yr-old female, DRB1*1501/1502 and patient SK: 45-yr-old male, DRB1*1502/1403) and one secondary progressive MS (patient IS: 30-yr-old female, DR1/DRB1*0410). HLA-DR types were determined by a hybridization protection assay using acridinium-ester-labeled DNA probes (18) or by a standard serological typing. None of the patients received immunosuppressive agents during the period of study.

T cell clones and lines (TCCL)

A panel of CD4\(^+\) TCR\(\beta\)^+ T cell clones and lines (TCCL) (Table I) had previously been established from PBMC by our modification of the “split-well” technique (19, 20). All the TCCLs (here we use “TCCL” as an operational term representing an independently established long-term culture composed of mono- or oligoclonal T cells) were restricted by HLA-DR in the recognition of MBP or PLP peptide as revealed by proliferation assays with DR-specific blocking Abs or with DR-transfected L cells as APCs (20). They were characterized as Th1-type T cells based on their ability to produce IFN-\(\gamma\) but not IL-4 in response to Ag (our unpublished data). Although there was no particular bias in the use of either MBP82–102, PLP95-116, 105–124, 118–139, or 139–155 peptide for generation of TCCL, our TCCL panel was biased for PLP95-116-specific TCCLs, a majority of which were derived from OK bearing DR2 haplotype. This is consistent with our previous work showing that PLP95-116 is an HLA-DR2-associated epitope in MS (20). SSCP analysis had revealed monoclonal or oligoclonal compositions of the TCCLs and characterized the \(\beta\) chain usage of each clonotype in TCCL (Table I).

Cell sorting

For analysis of activated T cells, PBMC were doubly stained with anti-CD25-FITC (anti-IL-2 receptor \(\alpha\)-chain) and anti-CD3-phycocerythrin (PE) mAbs (Becton Dickinson, Mountain View, CA). The CD3\(^+\) CD25\(^+\) fraction was collected by a standard sorting method using a FACSort flow cytometer (Becton Dickinson). CD4\(^+\) cells were isolated by a magnetic cell sorter MACS (Miltenyi Biotec, Auburn, CA) after labeling with anti-CD4 mAb (PharMingen, San Diego, CA).

SSCP clonotype analysis

SSCP clonotype analysis was performed in essentially the same condition as described by Yamamoto et al. (9, 10). In brief, mRNA was isolated from PBMC, CD3\(^+\) CD25\(^-\) cells, cerebrospinal fluid (CSF), or TCCL with QuickPrep Micro mRNA Purification Kit (Pharmacia Biotech, Uppsala, Sweden) and converted to cDNA by using First-Strand cDNA Synthesis Kit (Pharmacia Biotech). One microliter of the diluted cDNA reaction was amplified by a set of a \(\beta\) chain and the C\(\beta\) primers (from left to right: VB1–5.1, 5.2, and 6–20). A, SSCP profile of a MS showing a smear pattern. B, SSCP profile of an MS in relapse demarcating multiple distinctive bands.

Table I. The list of TCCL used as probes for SSCP clonotype analysis

| Patient | TCCL Code | Specificity | SSCP Clonotypes
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<tbody>
<tr>
<td>IS</td>
<td>4SC5</td>
<td>MBP82–102</td>
<td>3, 6a, 6b, 6c, 8, 14</td>
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<tr>
<td></td>
<td>65C6</td>
<td>MBP82–102</td>
<td>6a, 6b, 14</td>
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<td>OK</td>
<td>2A7</td>
<td>PLP95–116</td>
<td>5.1, 6, 15</td>
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<td></td>
<td>3C11</td>
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<td></td>
<td>2BA205</td>
<td>PLP95–116</td>
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<td>2.5</td>
<td>PLP95–116</td>
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<td></td>
<td>4.1</td>
<td>PLP95–116</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4.12</td>
<td>PLP95–116</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>118.19</td>
<td>PLP118–139</td>
<td>3, 6, 8, 14, 20</td>
</tr>
<tr>
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<td>PLP118–139</td>
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<td>139</td>
<td>PLP139–155</td>
<td>3, 6, 13a, 13b</td>
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<td>SK</td>
<td>95.2</td>
<td>PLP95–116</td>
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</tr>
<tr>
<td></td>
<td>105</td>
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<td>105.18</td>
<td>PLP105–124</td>
<td>1a, 1b, 10, 20</td>
</tr>
</tbody>
</table>

\(a\) cDNAs from these TCCLs were used as probes for exploring the possible existence of MBP- or PLP-reactive cells among in vivo expanded clones. 

\(b\) SSCP analysis of cDNAs revealed that each TCCL contains from 1 to 6 clonotypes. Each clonotype is designated by the number of \(\beta\) chain gene number. Clonotypes that were detected in blood or CSF samples are in bold.
for electrophoresis. Only when the migration positions of the corresponding clonotypes were identical in the additional experiments (80–90% in the cases), the two clonotypes were regarded as being identical.

TCR DNA sequencing

In brief, we cut out a small area of the SSCP gel corresponding to the band and then extracted the TCR clonotype message as previously described (14). The extract was submitted to a second amplification by PCR with a corresponding V\textsubscript{\text{b}} (21) and an internal C\textsubscript{\text{b}} primer (GCGACCTCGGGTG GGGAC). The PCR products were ligated to M13 mp19 vector arms obtained through Sma\textsubscript{I} digestion. Phages were grown on XL-1 Blue \textit{Escherichia coli} cells (Stratagene, La Jolla, CA), and recombinants were selected by hybridization with a C\textsubscript{\text{b}} probe (21). The single-strand DNA was isolated from the positive plaques, and sequencing reactions were induced with BucabEST Dideoxy Sequencing Kit (Takara) after priming with M13-47 primer.

Results and Discussion

SSCP analysis of unseparated PBMC vs CD3\textsuperscript{1}CD25\textsuperscript{1} T cells

In preparatory experiments, eleven PBMC samples from healthy subjects (HS) and 21 from MS were examined by SSCP clonotype analysis. In accordance with previous studies (9, 10, 12, 13), the samples from HS showed a smear pattern or demarcated variable numbers of bands on the smear backgrounds (Fig. 1A). While samples from MS in remission were not significantly different from HS samples, MS in relapse tended to demarcate higher numbers of bands than HS or MS in remission (Fig. 1B). However, there were remarkable individual differences (data not shown), and the SSCP profile in a given subject correlated poorly with the clinical state. Then we examined three PBMC samples from patient OK and two PBMC from patient SK in parallel with all the PLP-reactive TCCLs derived from the patients shown in Tables I and II. This preliminary analysis showed the presence of a V\textsubscript{\text{b}}14 clonotype of TCCL 118.19 in one of the three samples from patient OK and a V\textsubscript{\text{b}}20 clonotype in TCCL 105.18 in one of the two samples from patient SK (data not shown), indicating that Ag specificities of clonally expanded T cells could be determined by this method. However, seeing that distinct clonotypes could hardly be demonstrated in samples obtained from MS in remission, we speculated

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**Table II.** TCR amino acid sequences of in vivo expanded clonotypes\textsuperscript{a}

<table>
<thead>
<tr>
<th>Patient</th>
<th>Specificity</th>
<th>TCCL</th>
<th>V\textsubscript{\text{b}}</th>
<th>n-D-n</th>
<th>J\textsubscript{\text{b}}</th>
<th>C\textsubscript{\text{b}} Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS</td>
<td>MBP82–102</td>
<td>4SC5</td>
<td>V\textsubscript{\text{b}}6-LCASSL</td>
<td>NVNSY</td>
<td>NEQFFG-J\textsubscript{\text{b}}2.1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>MBP82–102</td>
<td>65C6</td>
<td>V\textsubscript{\text{b}}6-ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V\textsubscript{\text{b}}14-LYFCAS</td>
<td>SGLGN</td>
<td>NEQFFG-J\textsubscript{\text{b}}2.1</td>
<td>2</td>
</tr>
<tr>
<td>OK</td>
<td>PLP95–116</td>
<td>2BA205</td>
<td>V\textsubscript{\text{b}}16-YVFCAS</td>
<td>GH</td>
<td>NYGYTF-J\textsubscript{\text{b}}1.2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>PLP118–139</td>
<td>118.19</td>
<td>V\textsubscript{\text{b}}3-YLCAS</td>
<td>TKGH</td>
<td>VLTFG-J\textsubscript{\text{b}}2.6</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V\textsubscript{\text{b}}8-ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>V\textsubscript{\text{b}}14-LYFCAS</td>
<td>LQGAR</td>
<td>YEQFFG-J\textsubscript{\text{b}}2.7</td>
<td>1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} This Table shows the TCR sequences of MBP- or PLP-specific clonotypes whose expansion in vivo were confirmed by the SSCP technique. Each TCCL was run on the gel together with CD25\textsuperscript{1} T cell population sorted from PBMC or unseparated CSF. Identical bands shared by TCCL and the blood or CSF samples were cut out, and the TCR genes were amplified and sequenced as described in Materials and Methods. ND, Not determined. These sequence data are available from DDBJ/EMBL/GenBank under accession numbers from AB011247 to AB011254.

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**FIGURE 2.** Flow cytometric identification of CD3\textsuperscript{1}CD25\textsuperscript{1} population. PBMC from HS and MS were stained with anti-CD25-FITC and anti-CD3-PE and analyzed by flow cytometer. This is a representative profile of the sample of an MS. The CD3\textsuperscript{1}CD25\textsuperscript{1} population was sorted and used for SSCP clonotype analysis.

**FIGURE 3.** SSCP-based comparison of CD3\textsuperscript{1}CD25\textsuperscript{1} population with unseparated PBMC. The unseparated PBMC of an HS (upper panel) was compared with the CD3\textsuperscript{1}CD25\textsuperscript{1} sorted from the same PBMC (lower panel) by SSCP clonotype analysis.
that only a small proportion of expanded clones in blood may be visualized by this method. Next we examined the activated T cell population expressing both CD3 and CD25 (IL-2Rα-chain) (Fig. 2). Notably, analysis of this population was found to demarcate much higher numbers of distinct clonotypes on the SSCP gels as compared with unseparated PBMC (Fig. 3). Based on this interesting observation, we presume that the mixture of heterogeneous CD25 population may lower the efficiency in detecting clonal accumulations of CD25 T cells. Because of technical merits to detect higher number of bands and as activated T cells should play critical roles in vivo, we decided to use the CD3 CD25 population in following studies.

Limited heterogeneity of in vivo-activated T cells in MS

Collectively, five CD3 CD25 samples from HS and 10 samples from MS were investigated by the SSCP technique (Table III). The results showed a tendency that the samples from MS demarcate a lower number of clonotypes that are characterized by more limited use of Vβ genes as compared with HS. However, the proportion of CD3 CD25 cells among total lymphocytes was not significantly different between MS and HS (Table III). An extreme end of the spectrum was seen in the first sample obtained from patient IS with secondary progressive MS, demarcating only 20 bands with biased TCR usage for Vβ6 and Vβ14 (Fig. 4A) and in patient TN, who has recently developed an acute episode of probable MS. Although less remarkable, samples from relapsing/remitting MS (patient OK) also showed similar profiles characterized by fewer bands and biased Vβ use (Fig. 5, A and B). These results suggest a narrowing of activated T cell repertoire in association with development of MS. Although the underlying mechanism is unclear, we may speculate that T cell recognition targets in MS may tend to be shifted from those for regulatory cells (such as TCR peptides) to a limited epitopes for self-destructive T cells.

The patients’ SSCP profiles were not stable during the clinical courses. For example, in the second sample of patient IS (June 1997), the number of SSCP bands had increased up to a total of 50, and the Vβ usage had become more widely distributed (Fig. 4B). Similarly, the number and distribution of the SSCP bands varied among samples from patient OK (Table III).

![SSCP profiles of CD3 CD25 population from patient IS with secondary progressive MS. A, January 1997. Note the reduced number of bands and the marked bias for Vβ6 and Vβ14 usage as compared with HS (Fig. 3). Among these bands, two corresponded to clonotypes of MBP-specific TCCL (arrow a indicates a Vβ6 clonotype of 4SC5, while b corresponds to Vβ14 clonotype of 65C6). B, June 1997. Biased Vβ usage has become less remarkable than A. See also Table II.](http://www.jimmunol.org/)

### Table III. Distribution of SSCP clonotypes in the CD3 CD25 population

| Vβ Family | 1 | 2 | 3 | 4 | 5.1 | 5.2 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | Total | % of CD25 |
|-----------|---|---|---|---|-----|-----|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|------|
| HS-1      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    | 178  |    | 8.17 |
| HS-2      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |  76  |    | 4.14 |
| HS-3      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    | 131  |    | 1.17 |
| HS-4      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    | 102  |    | 4.46 |
| HS-5      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    | 138  |    | 4.83 |
| MS-1      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  8.17 |
| MS-2      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  4.03 |
| MS-3      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  4.07 |
| MS-4      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  3.56 |
| MS-5      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  1.87 |
| MS-6      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  1.62 |
| MS-7      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  4.11 |
| MS-8      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  5.62 |
| MS-9      |   |   |   |   |     |     |   |   |   |   |    |    |    |    |    |    |    |    |    |    |    |     |    |  4.22 |

*The CD3 CD25 population was isolated from PBMC of five healthy individuals (HS-1, -2, -3, -4, -5) and five MS patients (IS, OK, SA, MS, TN) by flow cytometry and then processed for SSCP analysis. The number of clonotypes in each Vβ lane and the summation of all bands (total) are shown. Patients IS and OK were repeatedly examined and month/year of the sampling is shown.

The percentage of CD3 CD25 T cells among total lymphocyte fraction is shown.

sm, Indicates smear pattern without distinct bands.
Identification of MBP- or PLP-reactive T cells among CD3$^+$CD25$^+$ population

We next analyzed 7 of the 10 samples from MS (2 from patient IS and 5 from patient OK) together with the patient-derived TCCLs listed in Table I. The results showed evidence for transient or persistent expansion of MBP- or PLP-specific clonotypes in MS (Table IV and Fig. 6). A most remarkable observation was that two of the dominant clonotypes in the first sample of patient IS (Jan 97) would correspond to those specific for MBP82–102 (indicated by arrow a and b in Fig. 4A), implying that a few MBP-specific T cells could play overwhelming roles in certain phases of MS. Together with the fact that the expanded clones are activated in vivo and the corresponding clones belong to Th1 T cells, we speculate that, if a few pathogenic clones can be deleted by anti-clonotype vaccine, it may greatly reduce the clinical activity of this patient in the active phase. It was also striking that a most prominent clonotype in a sample of patient OK corresponded to that of PLP95–116-specific T cell clone 2BA205 (Fig. 5A). SSCP analysis and TCR sequencing (Fig. 7) revealed the presence of the 2BA205 clonotype in all the samples, indicating that 2BA205 clone is probably in a continuously activated state in the peripheral circulation (Table IV).

Although previous studies suggested the presence of persistent MBP-specific clones (6, 7), it remained elusive whether they were continuously or periodically activated, since the interval of sampling was quite long (1 yr). This is the most convincing proof that myelin-specific T cell clones could exist in a persistently activated state. In contrast, the V$\beta$15$^+$ clonotype of 118.23 was found only in a single relapse, and the V$\beta$8$^+$ clonotype of 118.19 was found in a remission phase.

Table IV. Temporal profiles for the appearance of autoimmune T cells in Patients IS and OK

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sampling Time</th>
<th>Clinical State</th>
<th>Sample</th>
<th>MBP82–102</th>
<th>PLP95–116</th>
<th>PLP118–139</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>45C5</td>
<td>65C6</td>
<td>2BA205</td>
<td>118.23</td>
</tr>
<tr>
<td>IS</td>
<td>Jan 1997</td>
<td>Progressive</td>
<td>AT</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td></td>
<td>June 1997</td>
<td>Progressive</td>
<td>AT</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>June 1997</td>
<td>Progressive</td>
<td>CSF</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>OK</td>
<td>June 1996</td>
<td>Remission</td>
<td>AT</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>July 1996</td>
<td>Remission</td>
<td>AT</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Nov 1996</td>
<td>Relapse</td>
<td>AT</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>June 1997</td>
<td>Intermediate$^a$</td>
<td>AT</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>June 1997</td>
<td>Intermediate$^a$</td>
<td>CSF</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

$^a$This table shows the temporal profile for clonal expansion of MBP- or PLP-specific T cells in patients IS and OK. As described in the text, identities of SSCP bands in the collected samples (CD3$^+$CD25$^+$-activated T cells (AT) and CSF) were individually compared with the TCCL listed in Table I. Among 31 clonotypes from the 11 TCCLs examined, eight clonotypes shown in the table were detected at least once in the AT and/or CSF samples.

$^b$Intermediate; A recovery state from relapse (not clearly classified as relapse or remission).
The PBMC into CD4 by another T cell clone expressing CD8. We therefore separated 1
result: the 2BA205 TCCL (corresponding band are identical. Shown are a representative sequencing
binder and possibly infiltrate the site of inflammation.
the MBP- or PLP-reactive clones could cross the blood-brain bar-
Figure the MBP- or PLP-reactive clones could cross the blood-brain bar-
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Surface phenotypes of the persistent clone
A previous study by Masuko et al. reported that clonal persistence
is specific for the same PLP epitope. This does not only imply
that the kinetics of autoimmune T cell clones are quite heteroge-
Figure 7. Confirmation for TCR sequence identity of corresponding
SSCP bands. The TCR sequences of an SSCP band corresponding to the
2BA205 clonotype and of the 2BA205 TCCL were determined as de-
scribed in Materials and Methods. We examined five subclones from the
2BA205 TCCL and five from the corresponding band within the
CD3⁺ CD25⁺ T cells and found that five from the TCCL and four from the
corresponding band are identical. Shown are a representative sequencing
right panel)
only once during remission (Table IV), although these clonotypes
are specific for the same PLP epitope. This does not only imply
that the kinetics of autoimmune T cell clones are quite heteroge-
Figure 8. Analysis of CSF T cells. After PCR amplification for 38
cycles, the cDNAs derived from CSF were processed and displayed on the
SSCP gel. The result of the November 1996 sample from patient OK is
shown. Arrow a corresponds to 2BA205, and b and c to clonotypes from
TCL118.19.
Concluding remarks
Clonal expansion of autoimmune T cells was previously estimated
in MS by using limiting dilution analysis (6) or the RT-PCR assay
measuring mRNA transcripts encoding TCR chains of MBP-reactive
clones (7). These techniques are useful for exquisite analysis of
limited populations defined by either Ag specificity (6) or by T
cell clonotype (7). However, they do not estimate the significance of
examined populations in the total repertoire. In addition, while
limiting dilution analysis assays are influenced by multiple factors
inherent in in vitro manipulation (7), the PCR assay could handle
only a limited number of clones. In contrast, the SSCP analysis (9,
10) figures out the overall profile of dominant clonotypes without
depending on in vitro culture. Owing to this technical advantage
and the availability of T cell clones specific for putative enceph-
halitogenic peptide, we were able to identify the presence of auto-
immune T cells in the activated T cell population of blood and CSF
in two patients with MS. Although care should be taken in its use
for quantitative estimation, the SSCP analysis is a most reliable
and efficient tool for identifying and tracking Ag-specific T cells in
human diseases such as MS. Our results revealed that only a small
proportion of clonotypes from in vitro established clones can be
detected in a series of activated T cell populations in vivo. We
assume that the in vivo detected clones might have been involved
in the pathology of MS at the time of sampling, while the other
clones had played a role previously or may be unrelated to disease
as being derived from the naive T cell pool. Such information on
in vivo expanded and activated clones might prove truly useful for
designing “individually tailored” clonotype-specific treatment for
autoimmune diseases in the future.
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References


