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T Cell Repertoire Diversity Is Required for Relapses in Myelin Oligodendrocyte Glycoprotein-Induced Experimental Autoimmune Encephalomyelitis

Nicolas Fazilleau,2,3∗ Cécile Delarasse,2† Iris Motta,† Simon Fillatreau,‡ Marie-Lise Gougeon,§ Philippe Kourilsky,*§ Danielle Pham-Dinh,∥ and Jean M. Kanellopoulos4†

Comparison of TCRβ repertoires of myelin oligodendrocyte glycoprotein (MOG)-specific T lymphocytes in C57BL/6 and TdT-deficient littermates (TdT+/−) generated during experimental autoimmune encephalomyelitis (EAE) highlights a link between a diversified TCRβ repertoire and EAE relapses. At the onset of the disease, the EAE-severity is identical in TdT+/− and TdT−/− mice and the neuropathologic public MOG-specific T cell repertoires express closely similar public Vα-Jα and Vβ-Jβ rearrangements in both strains. However, whereas TdT+/− and TdT−/− mice undergo successive EAE relapses, TdT−/− mice recover definitively and the lack of relapses does not stem from dominant regulatory mechanisms. During the first relapse of the disease in TdT+/− mice, new public Vα-Jα and Vβ-Jβ rearrangements emerge that are distinct from those detected at the onset of the disease. Most of these rearrangements contain N additions and are found in CNS-infiltrating T lymphocytes. Furthermore, CD4+ T splenocytes bearing these rearrangements proliferate to the immunodominant epitope of MOG and not to other immunodominant epitopes of proteolipid protein and myelin basic protein autoantigens, excluding epitope spreading to these myelin proteins. Thus, in addition to epitope spreading, a novel mechanism involving TCRβ repertoire diversification contributes to autoimmune progression. The Journal of Immunology, 2007, 178: 4865–4875.
In the CNS, these public MOG-specific T cells have CD3 TdT cells during the disease and in MOG-stimulated lymph nodes. Results from one representative experiment of two are shown for A and one of 10 for B. The same experiment was performed on TdT-deficient littermates and the results are similar to those observed with TdT+/− mice (data not shown).

α-chain and V, D, and J for the β-chain, that yields the CDR3 of the TCR (17). During this process, TdT catalyzes the addition of template-independent nucleotides at the coding ends of V, (D), and J gene segments (18, 19). The repertoire of TdT-deficient mice (TdT−/−) is characterized by shorter CDR3s (20), and the αβ TCR diversity is diminished by a factor of 10–20 with no compensatory mechanism counterbalancing this decrease (21). Nonetheless, TdT−/− mice are not immunodeficient (22) and respond to viruses and protein Ags (22, 23). Moreover, epitope immunodominance is unaltered in TdT−/− animals (22).

To delineate the potential role of TCR diversity in autoimmune diseases, we compared the T cell repertoires of C57BL/6 TdT−/− and TdT+/− littermates in the MOG-induced EAE model for several reasons: 1) this Th17-mediated disease is reproducible in C57BL/6 mice (1, 2, 24); 2) the autoantigen is well-defined compared with those involved in autoimmune-prone animals; 3) there is one ID peptide in C57BL/6 mice (MOG 35–55); and 4) a single MHC class II molecule, I-Ak, presents MOG 35–55.

We have previously established that MOG 35–55-specific T lymphocytes express public rearrangements (25), i.e., common to all mice of the same MHC haplotype (26). These rearrangements, Vε9-Jε23, Vε9-Jε31, and Vβ8.2-Jβ2.1, have characteristic CDR3α and CDR3β sequences and are found in CNS-infiltrating cells during the disease and in MOG-stimulated lymph nodes cells (LNC) (25). In the CNS, these public MOG-specific T cells have an inflammatory phenotype, i.e., secreting IFN-γ and TNF-α (25).

In this work, we show that TdT−/− and wild-type (wt) mice reproducibly develop EAE after immunization with the recombinant MOG protein or MOG 35–55 peptide. However, whereas TdT−/− animals recover definitively after the first peak of EAE, TdT+ mice recover definitively after the first peak of EAE, TdT+ littermates undergo successive EAE relapses. The lack of EAE relapses in TdT−/− is not due to regulatory mechanisms peculiar to this strain. T cell repertoire analyses at the onset of the disease show that the Vβ repertoire of MOG-specific T lymphocytes in TdT−/− mice is identical with its wt counterpart while Vα rearrangements are closely similar. Interestingly, in the first EAE relapse the public Vα and Vβ rearrangements identified at the first peak of the disease disappear in wt mice and new public MOG-specific Vα and Vβ rearrangements, the majority of which containing N additions, emerge. Thus, in the absence of obvious epitope spreading, MOG EAE relapse is linked to the highly diversified T cell repertoire of wt mice and results from the emergence of MOG-specific T cells bearing novel TCR rearrangements.

Materials and Methods

Mice

All mice used in this study were 8 wk old and were obtained from Pasteur Institute (Paris, France) housing facilities. Mice were housed under conventional conditions. TdT−/−, TdT+/−, and TdT+/− littermates were used in EAE and immunological experiments. Mice were used in accordance to the Pasteur Institute’s guidelines.
FIGURE 3. Encephalomyelitis in TdT+/+ and TdT−/− mice after adoptive transfer. EAE was induced in both TdT+/− and TdT−/− littermates. Total splenocytes of some of these animals were recovered 30 days after the induction of EAE. These cells were depleted of CD11c+ and B220+ cells. Then, 15 × 10⁶ B220−CD11c− splenocytes were injected in non-irradiated animals suffering form EAE (day 30). The mean of three different experiments (at least five mice per group) is presented.

Peptide and protein
The MOG 35–55 (MEVGYWRSFSPRVHLVRGNK), PLP 178–191 (NTWTTGSIAPFSK), PLP 54–72 (SIHHAARTTHGSLPOKSRQ) and MBP 120–134 (YWVSPGVLTLIALVP), MOG 183–197 (FIVVPGVGLVALIH) have been described as potential SD peptides of MOG (6). All peptides were produced by NEOSYSTEM. Their purity was tested by HPLC. Murine recombinant MOG 1–116 was prepared as described in (6).

Induction and assessment of EAE
TdT+/+, TdT+/−, and TdT−/− littermates were immunized subcutaneously with 200 μg of rMOG 1–118 or 100 μg of murine MOG 35–55 in CFA containing 600 μg of Mycobacterium tuberculosis H37RA (Difco Laboratories) according to a previously described protocol (6). For EAE induction, animals received additional i.v. injections of 200 ng of pertussis toxin (List Biological Laboratories) on days 0 and 2. Disease severity was monitored daily according to the following scale: 0, no disease; 1, flaccid tail; 2, hind limb weakness; 3, hind limb paralysis; 4, forelimb weakness; and 5, moribund.

Immunization and cell cultures
Mice were immunized in the hind footpads with 10 nmol of MOG 35–55 peptide in CFA. Nine days later, LNC were cultured with increasing concentrations (μM) of peptide or with 10 μg/ml PPD for 4 days. The cultures were then pulsed with 1 μCi of [3H]thymidine for the last 8 h. Data represent the mean of proliferative responses for five mice of each group (A). TdT−/− and wt mice were immunized in the hind footpads with 10 nmol of MOG 35–55 peptide in CFA. Nine days later, LNC were cultured with either 30 μM peptide or 20 μg/ml PPD for 4 days. In addition, TdT−/− mice were immunized in the hind footpads with 10 nmol of MOG 35–55 peptide in CFA and pertussis toxin. Fifteen days later, mice were killed and CNS-infiltrating cells were recovered by a Percoll gradient as described in Materials and Methods. cDNA from MOG stimulated-LNC (B), PPD-recalled LNC (C), and CNS-infiltrating cells (O) were subjected to immunoscope analyses. Data represent CDR3a and β size distributions for two representative TdT−/− mice. Rearrangements shown are Vβ8.2-Jβ2.1 and Vε9-εα.

CDR3 size distribution among MOG 35–55-stimulated LNC and CNS-infiltrating T cells during EAE in TdT-deficient mice. A, TdT−/− and wt mice were immunized in the hind footpads with 10 nmol of MOG 35–55 peptide in CFA. Nine days later, LNC were cultured with increasing concentrations (μM) of peptide or with 10 μg/ml PPD for 4 days. The cultures were then pulsed with 1 μCi of [3H]thymidine for the last 8 h. Data represent the mean of proliferative responses for five mice of each group (A).

Immunoscope analysis, cloning, and sequencing
Total RNA from splenocytes, LNC, or CNS-infiltrating cells was extracted using an RNasea mini kit from Qiagen and reverse transcribed into cDNA using oligo(dT) and SuperScript II (Invitrogen Life Technologies). Immunoscope analyses were performed as described elsewhere (29). Sequencing of CDR3 sequences was done using a TOPO Blunt cloning kit (Invitrogen Life Technologies). Briefly, a PCR amplification was performed on cloned bacteria followed by a second step of elongation using an ABI PRISM BigDye Terminator kit (Applied Biosystems). Reaction mixtures were then analyzed on a 48-capillary 3730 DNA Analyzer (Applied Biosystems).
could reflect holes in the T cell repertoires of TdT regulatory mechanisms such as regulatory T cells. Alternatively, it the lack of relapse in TdT animals (data not shown). Similar EAE-profiles have been animals, respectively. Importantly, whereas all mice suffered from EAE, relapses occurred only in TdT mice. Thus, we collected the splenocytes from animals (Fig. 2). Therefore, the lack of relapse in TdT mice did not inhibit the relapses in either TdT mice did not inhibit the relapses in either TdT mice. To inhibit the relapses. Thus, we collected the splenocytes from animals suffering from EAE (day 30). The cells from animals did not inhibit the relapses in either TdT mice did not inhibit the relapses in either TdT mice. The lack of relapse in TdT animals may be due to dominant regulatory mechanisms such as regulatory T cells. Alternatively, it could reflect holes in the T cell repertoires of TdT mice. To determine whether dominant regulatory mechanisms prevent relapse in TdT mice, we transferred CD4+ T lymphocytes from naive C57BL/6 animals bearing CD45.1 as a marker for grafted cells into TdT mice. After 2 wk, EAE was induced in these animals. As shown in Fig. 2, all mice developed the disease and presented a relapse. At the peak of the relapse, five animals were sacrificed and CNS-infiltrating cells were analyzed by flow cytometry. The vast majority of CNS-infiltrating CD4+ cells bear CD45.1 (mean of 80.71 ± 3.14%), indicating that they are of donor origin (Fig. 2). Therefore, the lack of relapse in TdT mice is recessive and can be complemented by the transfer of wt CD4+ T cells.

However, one could argue that regulatory T cells need to be diminished T cell repertoire of TdT mice (Vβ8.2-Jβ2.1 rearrangement)
challenge with MOG 35–55 or with a purified protein derivative (PPD) (Fig. 4A).

We have previously determined that C57BL/6 mice express Va and Vβ public repertoires in response to the MOG 35–55 (25). We then analyzed the repertoire of TdT−/− MOG-specific T lymphocytes using the immunoscope method (29). LNC from TdT−/− mice immunized with MOG 35–55 were recalled in vitro with MOG 35–55 or PPD and collected. Their RNAs were extracted and reverse transcribed into cDNA. Aliquots were amplified by PCR with the 24 Va- and Vβ-specific primers. A single peak corresponding to a CDR3 sequence from MOG 35–55-specific T LNC (1–5) and CNS-infiltrating lymphocytes during EAE (6–10) in TdT−/− animals (Fig. 4A). Immunoscope analyses performed on CFA-stimulated LNC recalled in vitro with PPD revealed bell-shaped curves (Fig. 4B). Immunoscope analyses of CNS-infiltrating cells from five different mice immunized with MOG 35–55 were recalled in vitro with MOG 35–55 or with a purified protein derivative (29). LNC from TdT−/− mice nos. 1, 4, 7, and 10) while the SDYNQGKL sequence is found in 7 animals of 10 (Table II, boldface letters) and the SAYNQGKL sequence is found in 4 mice (Fig. 4B). For the Vβ, only the Vβ8.2 segment was amplified in all mice and MOG 35–55-stimulated T cells used preferentially the Vβ8.2-Jβ2.1 rearrangement with CDR3βs of 8 and 10 aa (Fig. 4B). Immunoscope analyses performed on CFA-stimulated LNC recalled in vitro with PPD revealed bell-shaped curves for Vβ8.2-Jβ2.1 and Vα9-Cα rearrangements characteristic of polyclonal repertoires without oligoclonal expansions (Fig. 4C). Therefore, the Vβ8.2-Jβ2.1 and Vα9-Cα amplifications reflect the expansion of MOG-specific T cells rather than cells reactive to Ags of the CFA. These findings were corroborated by immunoscope analyses of CNS-infiltrating cells from five different TdT−/− mice on day 18 after immunization with MOG 35–55. Oligoclonal expansions are observed for Vα9-Cα and Vβ8.2-Jβ2.1 rearrangements with CDR3 lengths identical with those found in LNC (Fig. 4D).

To further define the recurrent CDR3 identified in TdT−/− mice, the Vα9-Cα and Vβ8.2-Jβ2.1 PCR products were cloned and sequenced. A CDR3β of 10 aa with the GETGGNYAEQ sequence is found in all TdT−/− animals (Fig. 4A). In C57BL/6, eight different nucleotide sequences encode this public CDR3β among which seven contain N diversity (25). One sequence lacking N addition was found in both strains. Shorter CDR3β sequences are also found in LNC and cells infiltrating the CNS of TdT−/− mice: GGTGDAEQ (two of five for LNC and two of five for CNS-infiltrating cells), GDAGDAEQ (two of five and one of five) and AGTGDAEQ (two of five and two of five). These shorter CDR3β sequences are characteristic of TdT−/− rearrangements as reported for naïve T lymphocytes (21, 30) and T cells specific for various protein epitopes (22, 23, 31).

As previously observed for wt mice, two rearrangements, Vα9-Jα23 (10 animals of 10) and Vα9-Jα31 (7 of 10 animals), are preferentially used in TdT−/− animals (Table II). Three different CDR3α sequences are encoded by the Vα9-Jα23. The SNYNQGKL sequence is found in 7 animals of 10 (Table II, boldface letters) and the SAYNQGKL sequence is found in four mice (TdT−/− mice nos. 1, 4, 7, and 10) while the SDYNQGKL sequence is observed in mice nos. 1, 2, and 5. Thus, SxYNQGKL is the public CDR3α consensus sequence. For the Vα9-Jα31 combination, two sequences are observed: SRNSNNRI in four mice (Table II, italic letters) and SANSNNRI in TdT−/− mice nos. 1 and 3.

In summary, MOG-reactive T cells from C57BL/6 and TdT−/− mice carry the same public CDR3β sequence (GETGGNYAEQ) and their repertoires for the TCRα-chain are very similar. Indeed, the Vα9-Jα31 CDR3α sequence SRNSNNRI, which is public in...
EAE-RELAPSES AS A CONSEQUENCE OF TCRαβ DIVERSITY

### Table III. CDR3β sequences from CNS-infiltrating cells during EAE-relapse in C57BL/6 TdT^−/− mice (Vβ8.3-β rearrangement)

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<th>CDR3 Length</th>
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<th>N/P/Dβ</th>
<th>5' Jβ Beginning</th>
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* Underlined nucleotides are due by TdT activity.

### Table IV. CDR3α sequences of CNS-infiltrating T cells from EAE-induced C57BL/6 TdT^−/− mice during EAE relapse (Vα4-Cα rearrangement)

<table>
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<th>Jα</th>
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<th>5' Jα Beginning</th>
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<td>ggttcacgccttagggaggtgccatcttttggg</td>
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</table>

* Underlined nucleotides are due to TdT activity.

### T cell repertoires in first EAE relapse in wt mice

We have excluded the possibility that the lack of relapses in TdT^−/− mice was due to an increase in T-regulatory cells or to a distinctive first phase of the disease. Altogether, our results suggest that it may reflect the limited T cell repertoire of TdT^−/− mice, resulting in an inability to activate an encephalitogenic TCR repertoire specifically responsible for the relapses. Should that be

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wt mice, was found in 4 of 10 TdT^−/− animals. The public Vα9-Jα23 rearrangement generating the CDR3α (RSYNNQGKL) in wt mice is replaced in TdT^−/− animals by CDR3α sharing the consensus sequence SXYNQGKL. Altogether, these data show that TdT-deficiency does not impair the development of the encephalitogenic MOG-specific public T cell repertoires, consistent with the similar disease course observed in the two strains. Thus, TdT deficiency does not impact on the first stage of the disease.
FIGURE 5. Ag specificity of splenocytes from EAE-induced C57BL/6 TdT<sup>−/−</sup> mice during EAE relapse. A. Forty-five days after the induction of EAE in TdT<sup>−/−</sup> mice, the animals were killed and cells were collected from either the spleen or the CNS. Splenocytes were labeled with CFSE, cultured for 4 days with various epitopes of the myelin, and their proliferation was tested. Data represent SI of TdT incorporation (cpm) from triplicate cultures of three different mice. SI (SI = [1]Hthymidine cpm incorporated in lymphocytes stimulated by MOG, MBP, or PLP peptides/[1]Hthymidine cpm incorporated in unstimulated cells). B. Stimulated CFSE-labeled CD4<sup>+</sup> splenocytes were sorted out and immunoscope analyses were performed.

Table V. CDR3<sup>β</sup> sequences of CD4<sup>+</sup> CFSE-labeled MOG35–55-stimulated T splenocytes from EAE-induced C57BL/6 TdT<sup>−/−</sup> mice during EAE relapse (Vβ8.3-Cβ rearrangement)

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<thead>
<tr>
<th>Jβ</th>
<th>CDR3 Length</th>
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<th>N/PDP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>5′ Jβ Beginning</th>
<th>Deduced Amino Acid Sequences</th>
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<td>SDAWGGAETL</td>
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</table>

<sup>a</sup> Underlined nucleotides are due to TdT activity.
Vβ8.3-Jβ1.1, and Vβ8.3-Jβ2.3 oligoclonal expansions were found in the sorted CD4+ population of the three mice, whereas Vβ8.2-β, Vβ8.2-Jβ2.1, and Vε9-ε PCR products were undetectable. All CDR3α and β sequences found in the CNS were also identified in CFSE<sup>a</sup>CD4<sup>+</sup> splenocytes (Tables V and VI). The consensus CDR3β sequence SDGXGETV, found in the brain of mice nos. 11, 12, and 13, was also present in MOG 35–55-proliferating T cells (Table V). Three sequences, SDAWGGAETL, SDGWGVAETL, and SDALGGAETL, identified in CNS, were also found in spleens from at least two mice. However, 9 of 24 CDR3<sup>α</sup> sequences were absent in the CNS. Importantly, unique CDR3β sequences found in the CNS of mice nos. 11 (SDRRGTEV) and 12 (SFRENTEV) were present in the splenocytes of animals nos. 13 and 12, respectively. Concerning the three public CDR3<sup>α</sup> sequences observed in CNS-infiltrating T cells, they were also found in CFSE<sup>a</sup>CD4<sup>+</sup> T splenocytes from at least two of three animals (Table VI). These results strongly suggest that the CNS-public rearrangements are borne by MOG 35–55-specific T lymphocytes.

We also determined which repertoire was expressed by CNS-infiltrating lymphocytes during the first relapse occurring in TdT<sup>−</sup> mice (Fig. 2) that have received CD4<sup>+</sup> T lymphocytes from naive C57BL/6 animals before EAE-induction. Public Vβ8.3-Jβ1.1 and Vβ8.3-Jβ2.3 rearrangements, previously found

### Table VI. CDR3α sequences of CD4<sup>+</sup> CFSE-labeled MOG 35–55-stimulated T splenocytes from EAE-induced C57BL/6 TdT<sup>−/−</sup> mice during EAE relapse (Vε4-ε rearrangement)

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<td>cgggg</td>
<td>acaggcaatcaggagcaacgctcatttctttggga</td>
<td>EAGGSNAL</td>
</tr>
</tbody>
</table>

<sup>a</sup> Underlined nucleotides are due to TdT activity.

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### Table VII. CDR3β sequences of CNS-infiltrating cells and of CD4<sup>+</sup> CFSE-labeled, MOG35–55-stimulated T splenocytes during EAE relapse from two various TdT<sup>−/−</sup> mice in which CD4<sup>+</sup> T cells from wt animals were previously transferred (Vβ8.3-Cβ rearrangement)

<table>
<thead>
<tr>
<th>β</th>
<th>Length</th>
<th>3' Vβ End</th>
<th>N/P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>5' β Beginning</th>
<th>Deduced Amino Acid Sequences&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS 16</td>
<td>1.1</td>
<td>8</td>
<td>tgtgcgcagctgtagtgg</td>
<td>gggggggg</td>
<td>acagaacttccttttgggt</td>
</tr>
<tr>
<td>CNS 16</td>
<td>2.3</td>
<td>10</td>
<td>tgtgcgcagctgtagtggagagggggggg</td>
<td>gttcagcttcttttggt</td>
<td>SDAWGGAETL</td>
</tr>
<tr>
<td>CNS 17</td>
<td>1.1</td>
<td>8</td>
<td>tgtgcgcagctgtagtggagaggggggg</td>
<td>ctcagggggg</td>
<td>acagaacttccttttgggt</td>
</tr>
<tr>
<td>CNS 17</td>
<td>2.3</td>
<td>10</td>
<td>tgtgcgcagctgtagtggagagggggggg</td>
<td>gttcagcttcttttggt</td>
<td>SDAWGGAETL</td>
</tr>
<tr>
<td>Spleen 16</td>
<td>1.1</td>
<td>8</td>
<td>tgtgcgcagctgtagtggagaggggggggg</td>
<td>gttcagcttcttttggt</td>
<td>SDAWGGAETL</td>
</tr>
<tr>
<td>Spleen 16</td>
<td>2.3</td>
<td>10</td>
<td>tgtgcgcagctgtagtggagagggggggg</td>
<td>gttcagcttcttttggt</td>
<td>SDAWGGAETL</td>
</tr>
<tr>
<td>Spleen 17</td>
<td>1.1</td>
<td>8</td>
<td>tgtgcgcagctgtagtggagagggggggggg</td>
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<td>SDAWGGAETL</td>
</tr>
<tr>
<td>Spleen 17</td>
<td>2.3</td>
<td>10</td>
<td>tgtgcgcagctgtagtggagaggggggggg</td>
<td>gttcagcttcttttggt</td>
<td>SDAWGGAETL</td>
</tr>
</tbody>
</table>

<sup>a</sup> Underlined nucleotides are due to TdT activity.
in the relapsing C57BL/6 mice, were strongly expressed in the brains of individual recipient animals (Table VII) and were similar to those described in Table III. To ascertain that in this experimental setting EAE relapse is not due to epitope spreading, the splenocytes from these mice were stimulated with the same peptides as those used in Fig. 5A. T cells responded to MOG 35–55 only (data not shown). Additionally, splenocytes from two mice (nos. 16 and 17) were labeled with CFSE before stimulation and the CFSE+/CD4+ T lymphocytes were isolated after 4 days in culture. TCR rearrangements were sequenced and revealed CDR3β sequences identical with those found previously (Table VII). Taken together, our results indicate that the first and second disease peaks involve two independent waves of MOG-reactive T cells. This suggests that the lack of relapse in TdT−/− mice is primarily caused by an inability of these animals to mount the second autoreactive wave and that no regulatory mechanisms preclude the appearance of MOG-specific T cells expressing the Vβ8.3-Jβ1.1 and Vβ8.3-Jβ2.3 public rearrangements.

Do T lymphocytes found in the brain of relapsing mice expand during the first peak of the disease?

We determined whether the public rearrangements (Vβ8.3-Jβ1.1 and Vβ8.3-Jβ2.3) found in the first relapse were generated early in the immune response against MOG 35–55. LNC from wt mice were collected 9 days after immunization and recalled in vitro for 4 days with MOG 35–55. The public Vβ8.2-Jβ2.1 CDR3β sequence GETGGNYAEQ was found in all mice, whereas the Vβ8.3-Cβ amplification was productive in five of nine mice (data not shown). After sequencing, the public CDR3β sequences SDAWGGAETL and SDGGGTEV, characteristic of the first relapse, were observed in two mice only (data not shown). Similar amplifications were performed on cDNA from CNS-infiltrating cells during the first peak of the disease in eight wt mice. We found that one mouse expressed the public CDR3β SDGGGTEV only and another one expressed SDGGGTEV and the private SDAGQ GAETL, also found in the splenocytes of two mice (nos. 11 and 12) (Table V and data not shown). This suggests that the public rearrangements found in the first relapse are expressed by T lymphocytes that emerge progressively during the initiation of the disease and become predominant while T cells bearing the Vβ8.2-Jβ2.1 rearrangement disappear.

Discussion

In the present study, we have used TdT−/− and TdT−/− littermates to investigate the impact of TCRαβ diversity on the occurrence of MOG-induced EAE relapse. At the onset of the disease, the severity of MOG-induced EAE is identical in both strains and their public T cell repertoire is highly homologous. However, the course of the disease is dramatically different depending on the strain because EAE relapses occur in TdT−/− mice only. The lack of EAE relapses in TdT−/− mice is in agreement with the decrease in the incidence of clinical symptoms in TdT-deficient, autoimmune-prone animals. Indeed, when the TdT-deficiency was backcrossed onto autoimmune disease-prone backgrounds, these mice were less susceptible to autoimmune nephritis (32), insulin and diabetes (20, 33), and lupus disease (33, 34). It was suggested that the lack of N additions and/or the decrease in diversity of the T and B cell repertoires due to TdT deficiency might be responsible for the delayed onsets of different autoimmune diseases and for the less severe clinical and biological symptoms at the end stages of these diseases. In addition, it was recently shown that in C57BL/6 mice immunization with recombinant murine MOG does not generate pathogenic B cells or demyelinating Abs (35). Thus, anti-MOG B cells do not play a major pathogenic role in this EAE model. In this report, we show that failure to relapse in TdT−/− mice does not stem from dominant regulatory mechanisms. Importantly, relapse in wt animals is not due to epitope spreading but to MOG-specific T lymphocytes expressing new public Vα and Vβ rearrangements containing N additions.

As reported for various ID epitopes (22, 23), MOG 35–55–specific public T cell repertoires of TdT−/− and TdT−/− littermates are closely similar. Wt and TdT−/− mice share the public CDR3β sequence GETGGNYAEQ (Ref. 25) and this work, suggesting a strong bias for this amino acid sequence encoded by eight different nucleotide sequences. Concerning the Vα-chain, the public Vα9-Jα23 rearrangement generating the CDR3α (RSYNQGL) in wt mice is replaced in TdT−/− mice by CDR3α sharing the consensus sequence SxYNQGL. Moreover, the Vα9-Jα31 CDR3α SRNSSNNRI is public in wt mice and is found in four of 10 TdT−/− animals. It is worth noticing that MOG-specific public T cell repertoires from CNS-infiltrating cells and stimulated LNC in TdT−/− mice are identical. Thus, in the two strains the first peak of EAE involves encephalitogenic MOG-specific CD4+ T cells that express highly homologous public TCR rearrangements.

In contrast, new Vα and Vβ public rearrangements, most of which contain N additions, are found in CNS-infiltrating T cells of wt mice undergoing the first EAE relapse. CD4+ T splenocytes bearing these rearrangements and derived from these mice proliferate to MOG 35–55 and not to other ID peptides of PLP and MBP autoantigens. ID epitopes of MBP and PLP are immunogenic in TdT−/− and TdT−/− animals and the ID epitope of PLP is encephalitogenic in both strains (data not shown), whereas C57BL/6 mice are known to be resistant to MBP-induced EAE. Thus, the absence of relapse in TdT−/− mice is not related to holes in their T cell repertoires, and in wt mice the first EAE relapse is not due to epitope spreading of the T cell response even though we cannot rule out the implication of other myelin autoantigens. Thus, EAE relapse predominantly involves MOG-specific T cells bearing public N diversified rearrangements.

Strikingly, public T lymphocytes emerging during the first peak of the disease are undetectable by PCR in wt mice during the relapse and thus do not participate in it. This suggests that these public T lymphocytes are not anergic or do not acquire a protective Th2 phenotype and are probably eliminated. Many hypotheses could explain this phenomenon: 1) autoreactive T cells can be deleted through activation induced cell death; 2) Fas-FasL and/or TNF-TNF-R1 interactions are responsible for EAE remissions as deleted through activation induced cell death; 2) Fas-FasL and/or TNF-TNF-R1 interactions are responsible for EAE remissions; 3) various regulatory T cells specifically inhibit EAE (41–43). These regulatory T cells interact through their αβ TCR with their MHC class I and/or MHC class II peptides complexes present on autoimmune CD4+ T cells (44–46). Recently, the importance of this regulatory pathway was evidenced in Qa-1-deficient mice (47). In the absence of Qa-1-restricted CD8+ T cells capable of inhibiting PLP-specific CD4+ T cells, these Qa-1-deficient mice were susceptible to EAE relapses after secondary and tertiary immunization with PLP while wt animals became resistant (47).

Transfer experiments using CD4+ T splenocytes from naïve wt mice as donor cells and naïve TdT−/− mice as recipients clearly establish that no regulatory mechanisms in TdT−/− animals inhibit...
the expansion of autoreactive T cells. Indeed, wt CD4+ T cells induce EAE relapse in TdT−/− mice and express the characteristic public Vβ8.3-Jβ1.1/Vβ8.3-Jβ2.3 rearrangements. Nevertheless, regulatory T cells could emerge in TdT−/− mice during the course of the disease and inhibit relapses. Because various subsets of T cells endowed with regulatory properties have been described, we transferred purified T splenocytes from EAE-recovered TdT−/− and TdT+ +/− littersmates into EAE-recovered TdT−/− and TdT+/+ mice. As shown in Fig. 3, no suppressive activity peculiar to the TdT−/− strain could explain the absence of relapses.

The emergence of MOG-specific T lymphocytes bearing the new public Vβ rearrangements during the first EAE relapse led us to investigate whether these T cells were present at the onset of the disease. The public CDR3 sequences were only present in stimulated-LNC from two of nine mice and in CNS-infiltrating cells from two of eight mice. This suggests that T cells bearing these CDR3β sequences expand progressively and become predominant as the public Vβ8.2-Jβ2.1 T lymphocytes from the initial EAE peak disappear. The sequential emergence of these public repertoire-bearing autoreactive T cells could emerge in TdT−/− and TdT−/− mutant-LNC from two of nine mice and in CNS-infiltrating cells from two of eight mice. This suggests that T cells bearing these CDR3β sequences expand progressively and become predominant as the public Vβ8.2-Jβ2.1 T lymphocytes from the initial EAE peak disappear. The sequential emergence of these public repertoire-bearing autoreactive T cells could emerge in TdT−/− and TdT−/− mutant-LNC from two of nine mice and in CNS-infiltrating cells from two of eight mice. This suggests that T cells bearing these CDR3β sequences expand progressively and become predominant as the public Vβ8.2-Jβ2.1 T lymphocytes from the initial EAE peak disappear.

The first relapse is due to intramolecular epitope spreading of the immune response characterized by the expansion of PLP 78–191-specific T lymphocytes (48). In this model, the preferential expansion of PLP 78–191 CD4 T cells in the CNS during the first EAE relapse may be explained by a repertoire of PLP 139–151-specific T lymphocytes less diversified than the MOG 35–55 T cell repertoire or to higher affinity of the TCRs specific for PLP 78–191 when compared to the low-affinity PLP 139–151-specific T lymphocytes remaining after remission.

Determinant spreading occurring during ongoing autoimmune diseases involves the sequential emergence(s) of autoreactive T cells recognizing self-epitopes different from the disease-inducing self-determinant (7). The public repertoire-bearing encephalitogenic T lymphocytes from the second peak of the disease are specific for the MOG 35–55 peptide like those of the first peak, indicating that no epitope spreading happens in our MOG-induced EAE model. Thus, the absence of spreading to the ID determinant of MBP and PLP could be due to the following nonexclusive reasons: 1) predominant Ag processing and presentation of MOG 35–55 vs ID epitopes of MBP and PLP; 2) persistence of a large T cell repertoire against MOG 35–55 (indeed, comparing MOG−/− and MOG+/+ mice, we have previously shown (25) that MOG is unable to impact on the public MOG-specific T cell repertoire); and 3) lower avidity and/or lower frequencies of PLP- and MBP-specific T cells remaining after tolerance induction.

Our studies in TdT−/− and TdT+ +/− mice have unraveled major modifications of the T cell repertoire that impact upon the evolution of MOG-induced EAE. If these observations can be generalized to other organ-specific autoimmune diseases, one can speculate that successive waves of pathogenic T lymphocytes expressing different repertoires lead to progressive destruction of tissues. Thus, one wave of autoimmune T lymphocytes that arises is eventually eliminated or down-regulated but, in the highly diversified T cell repertoire of TdT−/− individual, another subset of specific T cells expands and leads to relapse. Our works show that during the natural course of an organ-specific autoimmune disease a highly adaptable T cell repertoire is able to induce relapses against a single autoantigen without obvious epitope spreading. Thus, it would be of high interest to determine the relative role played by autoantigen spreading vs the fine tuning of pathogenic T cell repertoires in organ-specific autoimmune diseases.

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Disclosures

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References


