Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 12461–12466, October 1996
Immunology

Expansion of a recurrent Vβ5.3$^+$ T-cell population in newly diagnosed and untreated HLA-DR2 multiple sclerosis patients

(T-cell receptor/positive selection/autoimmunity)

P. MUSETTE*†, D. BEQUET‡, C. DELARBRE*, G. GACHELIN*§, P. KOURILSKY*, AND D. DORMONT†

*Unité de Biologie Moléculaire du Gène, Institut National de la Santé et de la Recherche Médicale U277, Institut Pasteur, 75015 Paris, France; ‡Service de Neurologie, Hôpital du Val de Grâce, 74 Boulevard de Port-Royal, 75005 Paris, France; and †Laboratoire de Neurovirologie du Commissariat à l'Energie Atomique, 92265 Fontenay aux Roses, France

Communicated by Avrion Mitchison, Deutsches Rheuma-Forschungszentrum Berlin, Berlin, Germany, August 19, 1996 (received for review March 1, 1996)

ABSTRACT **We have used a PCR-based technology to study the Vβ5 and Vβ17 repertoire of T-cell populations in HLA-DR2 multiple sclerosis (MS) patients. We have found that the five MS DR2 patients studied present, at the moment of diagnosis and prior to any treatment, a marked expansion of a CD4$^+$ T-cell population bearing Vβ5-Jβ1.4 β chains. The sequences of the complementarity-determining region 3 of the expanded T cells are highly homologous. One shares structural features with that of the T cells infiltrating the central nervous system and of myelin basic protein-reactive T cells found in HLA-DR2 MS patients. An homologous sequence was not detectable in MS patients expressing DR alleles other than DR2. However, it is detectable but not expanded in healthy DR2 individuals. The possible mechanisms leading to its *in vivo* proliferation at the onset of MS are discussed.**

Multiple sclerosis (MS) is characterized by chronic demyelination of the central nervous system (CNS). The causes of the disease are still unknown, but MS appears to be the consequence of immunological processes involving T cells. In addition to probable environmental factors (discussed in ref. 1), two groups of genetic factors causing a predisposition for MS have been defined: a factor accounting for familial aggregation (2) and a higher risk associated with HLA-DR2 in Caucasian populations (3).

The characterization of T cells involved in MS is crucial to the description of the disease. Several strategies have been used to identify possible biases in the T-cell repertoire of MS patients. They include flow cytometry analysis, extensive cell cloning, and determination of the nucleotide sequences of the T-cell receptor (TcR) of T cells either present at the site of the brain lesions or proliferating on brain autoantigens. T cells reacting against myelin basic protein (MBP) are found both in the CNS and in the peripheral blood lymphocytes (PBLs). A recent review of the sequences of the TcR of MBP-reactive cells reported the absence of an association of specific Vβ and Jβ with MS (4). However, restriction at the major histocompatibility complex was not taken into account in this review. In view of the association of MS with HLA-DR2, Vβ usage and complementarity-determining region 3 (CDR3) diversity of the TcR could be considered in their relationship with the DR type of the patients (5, 6).

The techniques mentioned above tell which Vβ and which CDR3 sequence is used in the sample studied. However, they cannot tell if a T-cell clone characterized by the distinctive β chain of its TcR is expanded *in vivo*. To determine if a T-cell population is expanded in an MS patient, we have used a new PCR-based technology that generates a "snapshot" of the T-cell populations at the clonal or oligoclonal level and that tells, on the basis of Vβ, Jβ, and CDR3 length, which population of T cells is expanded at the moment the sample is taken (7).

We have restricted our study of the T-cell repertoire in MS to genetically defined MS patients (patients and controls were all HLA-DR2). Also, to avoid the consequences of treatment on the diversity of the T-cell repertoire, we have recruited HLA-DR2 MS patients at the moment of diagnosis and prior to any treatment. In view of the large number of signals that could be generated from a unique blood sample (7) not all Vβ–Jβ combinations could be studied. Several Vβ segments other than Vβ5 and Vβ17 are used by autoreactive peripheral T cells in MS DR2 patients (8). However, the T cells infiltrating the CNS in DR2 MS patients have been found to be enriched in Vβ5.2/5.3$^+$ T cells (5) and their main targets are CNS self proteins: MBP, proteolipid protein, and myelin oligodendrocyte glycoprotein (9, 10). Also, some authors have reported that T-cell clones responding to MBP use predominantly Vβ5.2/5.3 (6). Finally, DR2-restricted T-cell clones that proliferate with the peptide 84–102 of MBP are predominantly Vβ17$^+$ (11). We have therefore restricted the present study to the Vβ5$^+$ and Vβ17$^+$ repertoires.

We have found that a distinctive subpopulation of Vβ5.3$^+$ T cells is expanded in the blood of DR2 MS patients but not in non-DR2 MS patients. It uses TcR β chains sharing features with the Vβ5$^+$ T cells infiltrating the CNS and with MBP-reactive clones in the context of DR2. Some similar T cells are present in healthy HLA-DR2 subjects.

METHODS

Patients. The lymphocytes of 10 Caucasian patients at the onset of the disease were collected from the Neurology Unit, Hôpital d'Instruction des Armées du Val de Grâce, Paris. Five were HLA-DR2. The diagnosis of MS was confirmed by clinical history and by clinical examination, evoked response testing, brain and spinal chord magnetic resonance imaging, and cerebrospinal fluid analysis. All the patients fulfilled all criteria for the diagnosis of MS (12). The patients were 40 years old on the average and included six females and four males. The average delay between the moment of inclusion in the study and the occurrence of the first symptoms was 1 year. At the time of the study, no patients had received any immunosuppressive treatment. Three chronic HLA-DR2 MS patients were also analyzed. Out of the 5 HLA-DR2 patients, 1 patient

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Abbreviations: MS, multiple sclerosis; MBP, myelin basic protein; TcR, T-cell receptor; CDR, complementarity-determining region; CNS, central nervous system; PBL, peripheral blood lymphocyte.

Data deposition: The sequence reported in this paper has been deposited in the GenBank data base (accession no. Y08295).

§To whom reprint requests should be addressed at: Unité de Biologie Moléculaire du Gène, Institut Pasteur, Département d'Immunologie, 75015 Paris, France. e-mail: ggachel@pasteur.fr.

was studied 12 months after the initial treatment (five doses of 500 mg of methyl prednisolone) and showed no signs of relapse. In addition, members of a family at risk (six individuals, all HLA-DR2) with two chronic MS patients (a brother and a sister) with a record of 30 years of disease were studied. HLA-DR2 Caucasian control individuals were obtained from the Blood Bank, Hopital Saint Louis, Paris. Three HLA-DR2 patients with non-MS inflammatory disease of the nervous system, including optic neuritis with no signs of MS, were included as controls in the study. All individuals were negative for venereal diseases, human immunodeficiency virus, and human T-cell leukemia virus. The DR2 serotype of the MS patients and healthy controls was confirmed by examining DR2a and DR2b expression by reverse PCR using specific primers for the two isotypes of DR2: TTCTTGCAGCAGGATAAGTAT and ACCGCGGCGCGCCTGTCT for DR2a and TTCCTGTGGCAGCCTAAGAGG and ACCGCGGCCCGCGCCTGC for DR2b (13). They all expressed DR2a,b mRNA, with the exception of three healthy controls in whom the expression of DR2b was hardly detectable.

RNA Extraction, cDNA Preparation, and TcR β-Chain Analysis. Total RNA was extracted from PBLs by using a technique described elsewhere (14). The strategy used to measure TcR diversity has been described (15). Briefly, 10 μg of total RNA was reverse-transcribed using avian myeloblastosis virus reverse transcriptase. The resulting cDNAs were PCR-amplified through 40 cycles (94°C, 1 min; 60°C, 1 min; 72°C, 4 min) using a probe specific for Cβ and each Vβ as described (15). The Vβ probes used here were AGCCTCTGAGCTGAATGTGAACGCC, which hybridizes with the Vβ5 sequence, and TCCTCTCACTGTGACATCGGCCCA, which hybridizes with Vβ17. The Cβ-specific probe was CGGGCTGCTCCTTGAGGGGCTGCG. Each Vβ/Cβ-amplified product was submitted to 5 cycles of primer extension using as probes each of the 13 fluorescent Jβ-specific probes (1.1 to 2.7) with the same experimental protocol (94°C, 1 min; 60°C, 1 min; 72°C, 4 min). Starting from a single sample of RNA, 13 samples per each Vβ were loaded onto a sequencing gel and analyzed using an automatic sequencer (Applied Biosystems) equipped with a computer program allowing the determination of the intensity of fluorescence of each band as well as its actual size (Immunoscope, Applied Biosystems). The results are depicted as peaks corresponding to the intensity of fluorescence. To minimize the risks of contamination of the samples by PCR products, the different steps of the analysis were carried out at separate places. Blood was taken at the hospital. RNA and cDNA were prepared in a room separate from the room in which the reagents for PCR were mixed. PCR amplification and analysis of PCR products were carried out each in separate rooms.

Given the Vβ–Dβ–Jβ sequence of the clone characterized in patient 2, we designed more specific probes to replace the Jβ1.4-specific probe, which allowed the detection of the Vβ5.3$^+$ clones with a highly homologous CDR3 region with an increased sensitivity. The following probes denoted 1 through 3, are depicted in Fig. 2: Oligonucleotide primers 1 and 2 penetrate into the CDR3 region and were labeled with the fluorescent dye FAM (Perkin–Elmer and Applied Biosystems) and used instead of the generic Jβ1.4-specific probe to detect and quantify the β chain of the expanded T cells. The clonotypic probe 3, which encompasses the entire CDR3 region, was not labeled and was used in PCR along with the Vβ5 primer.

Direct Sequencing. Direct sequencing of PCR products was carried out according to published procedures (16). Briefly, the amplification products were loaded on an agarose gel. The fragment to be sequenced was excised and DNA was electroeluted. Direct sequencing was carried out using the Sequenase kit (Amersham).

Cloning. Cloning of PCR products was carried out as described elsewhere (17). Cloned PCR products were sequenced using the Sequenase kit (Amersham).

Cell Sorting. CD4 and CD8 single-positive T cells were isolated from PBLs by using magnetic beads (Dynabeads; Dynal, Oslo).

Quantification of PCR Products. Quantification of PCR products was carried out using competitive PCR as described (18). Briefly, a known number of copies of a Vβ5.3–Jβ1.4–Cβ PCR product with a 2-bp deletion was mixed with the cDNA solution and PCR-amplified in the same tube. CD3 and actin mRNA were quantified in the same sample in a similar way. PCR was run at saturation (40 cycles–94°C, 1 min; 60°C, 1 min; 72°C, 4 min). All samples were subjected to 5 cycles (94°C, 1 min; 60°C, 1 min; 72°C, 4 min) of primer extension using fluorescent primers specific for each of the constructs and experimental couples and analyzed on an automatic sequencer allowing the detection of the two products and the measurement of their relative intensity. The number of copies of TcR β chain is expressed relative to a fixed number of CD3 or actin mRNA copies.

RESULTS

A T-Cell Population Characterized by a Distinctive β Chain Is Expanded in HLA-DR2 MS Patients. Five HLA-DR2 patients and five patients expressing HLA-DR alleles other than DR2, at the onset of MS and prior to any treatment, were selected. Peripheral blood from five HLA-DR2 healthy individuals was included as five independently studied controls.

The first series of experiments was aimed at defining expanded populations of Vβ5$^+$and Vβ17$^+$ T cells that could be potentially associated with MS in HLA-DR2 MS patients and thus could be further analyzed. Thus, total RNA was extracted from PBLs and reverse-transcribed. The cDNAs were PCR-amplified using a Vβ5-specific primer, a Vβ17-specific primer, and a unique Cβ-specific primer. Vβ5 and Vβ17 Cβ PCR-amplified products were further analyzed through five cycles of primer extension using each of the 13 fluorescently labeled Jβ-specific probes. The 13 samples obtained from each Vβ were loaded onto a sequence gel and analyzed using an automatic sequencer equipped with a computer program allowing the determination of the fluorescence intensity of each band as well as its actual size. Thus, the repertoires of Vβ5$^+$ and Vβ17$^+$ T cells were now split each into 80–100 independent signals. The absence of specific stimulation appears as a gaussian-like distribution of the CDR3 sizes for a given Vβ–Jβ couple. Specific antigenic stimulation leads to the expansion of single peaks.

All Vβ17–Jβ combinations gave gaussian distributions superimposable with those obtained from HLA-DR2 healthy controls. The analysis of the Vβ5 T-cell repertoires showed that the T lymphocytes bearing a β chain composed of Vβ5–Jβ1.4 with a 9-amino acid CDR3 region were obviously and markedly expanded in three out of the five HLA-DR2 MS patients (patient 1 in Fig. 1 *Left*, for whom all Vβ–Jβ combinations are shown, and patients 1, 2, and 3 in Fig. 1 *Right* in which only Vβ5–Jβ1.4 PCR amplifications are shown). No obvious expansion was seen for patients 4 and 5 (Fig. 1 *Right*).

The other Vβ5–Jβ combinations gave a gaussian signal superimposable with that of the HLA-DR2 healthy individuals used as controls and characteristic of unstimulated T cells. The expansion of some Vβ5–Jβ combinations was occasionnally observed in a patient but was not found in the other patients, such as Vβ5–Jβ2.2 (Fig. 1 *Left*). These "scattered" expansions were not further studied since they may reflect individual responses to antigens irrelevant to MS although their role in MS is not excluded. Out of the five non-HLA-DR2 (including DR1, DR4, and DR7) MS patients, none presented either the Vβ5–Jβ1.4 bias or any other systematic bias, in the Vβ5$^+$ and

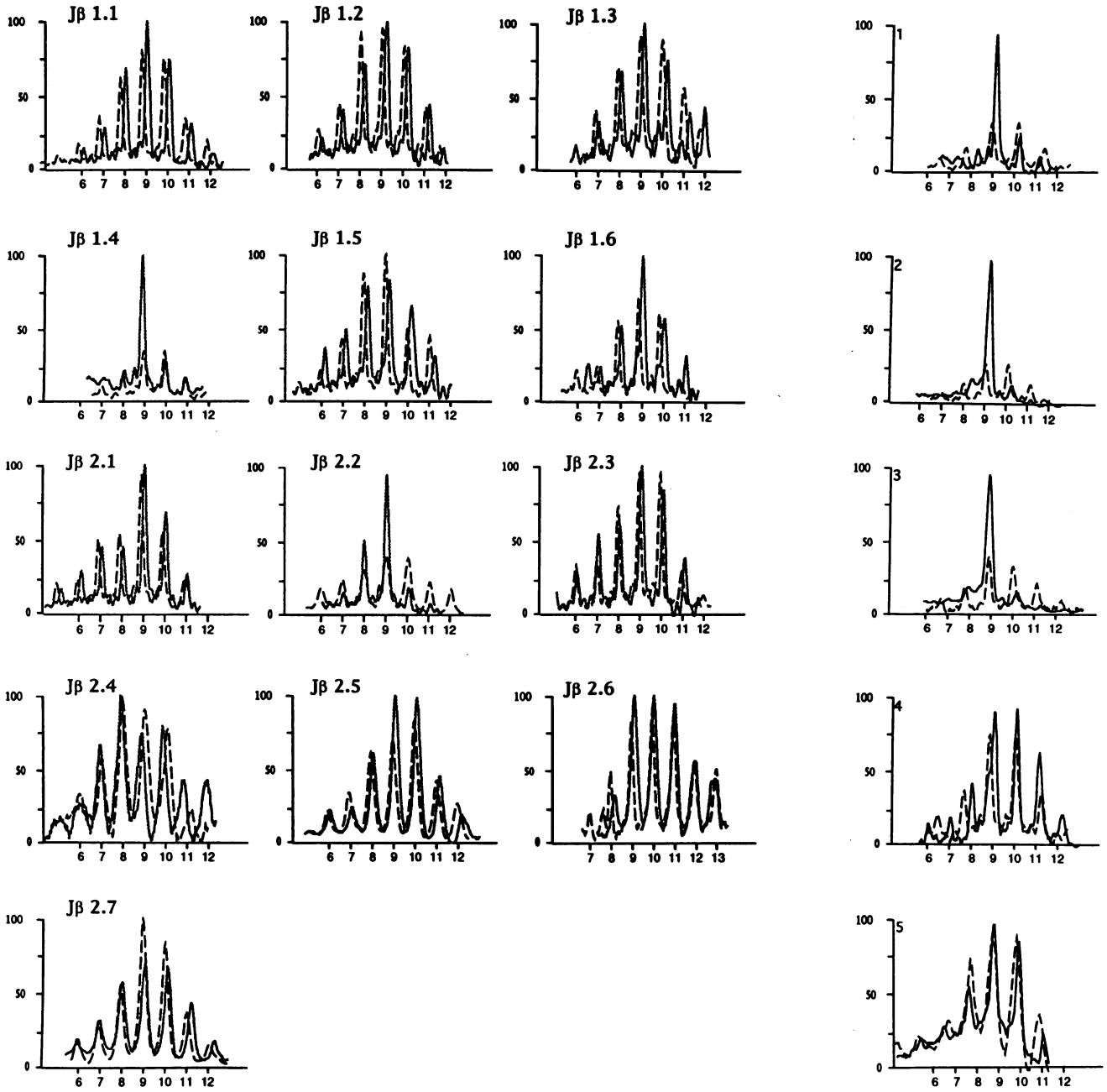

FIG. 1. (*Left*) Vβ5 repertoire in an HLA-DR2 MS patient (patient 1) prior to any treatment. Twenty milliliters of blood was taken, lymphocytes were isolated, and mRNA was extracted and reverse-transcribed. The resulting cDNA was PCR-amplified with Vβ5- and Cβ-specific primers. The PCR products were subjected to primer extension using fluorescent primers specific for each of the 13 Jβ. Extension products were analyzed on an automatic sequencer. Results are expressed in relative fluorescence units (ordinate) as a function of the β-chain CDR3 length (abscissa, in amino acids). Dotted line, healthy HLA-DR2 individuals; solid line, HLA-DR2 MS patient. An expansion not found in the control is observed with Jβ1.4 and Jβ 2.2. (*Right*) Results of Vβ5–Jβ1.4 peripheral T-cell repertoire of patients 1–5 and of healthy HLA-DR2 controls 1–5.

$V\beta17^+$ T-cell populations. Since inflammation of the CNS is a key feature of MS, we have also examined the expansion of the clone in the blood of three DR2 patients suffering of non-MS inflammatory diseases of the CNS, including a case of optic neuritis without other signs of MS. The $V\beta5^+$ T-cell clone was not expanded in these patients.

The expanded $V\beta5$–$J\beta1.4^+$ T-cell population with a 9-amino acid CDR3 was selected as a candidate for an MS-associated expansion of a particular population of T cells at the onset of the disease. We first determined by direct sequencing of the Vβ5–Jβ1.4 PCR-amplified products of patients 1, 2, and 3, if the peak indicative of a marked T-cell expansion was due to the contribution of a unique or of several β chains. The nucleotide sequence was found heterogeneous in patients 1 and 3, indicating that several clones contributed to the unique peak observed. In the case of patient 2, a predominant nucleotide sequence was unambiguously identified. The sequence was confirmed by cloning of PCR fragments and sequencing of several clones, which yielded a unique sequence. The deduced amino acid sequence of the β chain is shown in Fig. 2. It is a Vβ5.3–Dβ1.1–Jβ 1.4 chain, with a CDR3 length of 9 amino acids. The expansion of the Vβ5.3–Dβ1.1–Jβ 1.4^+ T cells in patient 2 is primarily monoclonal.

The availability of a distinctive expanded sequence allowed an easier search, in other patients and controls, for T cells using Vβ5.3 and Jβ1.4 in combination with CDR3 sequences show-

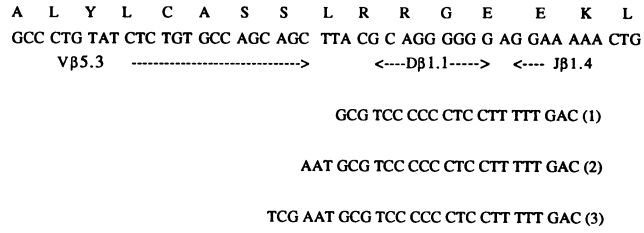

FIG. 2. The Vβ5–Jβ1.4 PCR product (patient 2, see Fig. 1 *Right*) was directly sequenced. The arrows indicate the nucleotide sequences corresponding to Vβ5.3, Dβ1.1, and Jβ1.4. Based on this sequence, nucleotide primers 1–3 (penetrating primers) were designed.

ing a high homology to that of the clone detected in patient 2. From the CDR3 sequence, we designed nucleotide probes covering 9 nt of the Jβ1.4 region and extending into the CDR3 region of the expanded β chain, probes 1, 2 and 3 (Fig. 2). Under the conditions used, the primers should be able to accommodate a few mismatches and, therefore, are expected to anneal to a family of closely related, but not identical, nucleotide sequences. At least some of these highly homologous nucleotide sequences should encode related CDR3 amino acid sequences. Probes 1 and 2 were labeled with a fluorescent dye and were used instead of Jβ1.4 in a primer-extension reaction, which increased the sensitivity and the specificity of detection. In a previous work, this approach had provided a 10-fold increase in the resolution power of the technique (19). Using this probe, we reexamined patients 1, 3, 4, and 5 for the presence of β-chain-encoding mRNAs using the Vβ5.3 segment in combination with CDR3 sequence showing homology with that of patient 2 and the Jβ1.4 segment. The thus defined sequences were found to contribute predominantly to the expansion observed in patients 1 and 3. An expansion was also observed in patients 4 and 5 (Fig. 3) whereas healthy DR2 controls remained negative in this test. Thus, a T-cell subset defined by the usage of Vβ5.3 and Jβ1.4 and a family of sequence-related CDR3 regions are found to be expanded in all five DR2 MS patients studied and not in controls. Throughout the remaining text, this particular population of T cells will be named "the T-cell subset".

Study of a HLA-DR2 Chronic Patients and of an HLA-DR2 Family at Risk of MS. We studied a family of six HLA-DR2 persons among whom two cases of MS had been diagnosed during the past 30 years, and also one HLA-DR2 patient with chronic MS. No systematic bias could be detected in the $V\beta17^+$ T cell repertoire of the members of the family. One out of the two HLA-DR2 MS patients (patient A, with a record of 30 years of chronic MS) of the family showed the expansion of the Vβ5–Jβ1.4 chain with a 9-amino acid CDR3, reacting also with primers 1 and 2. However, there was no specific expansion in the second MS patient (patient B) in the family that had been monitored clinically during 30 years of chronic MS. A similar finding was obtained on an unrelated chronic HLA-DR2 MS patient. The absence of expansion of the T-cell subset in chronic patients may reflect the decline of proliferation associated with the absence of MS evolution, as also noted in treated patient 3 whose initial expansion has disappeared after 1 year of remission after treatment (Fig. 4).

The T-Cell Subset Is Expanded up to 400-Fold in DR2 MS Patients. Quantitative competitive PCR (18) was used to determine the number of copies of the TcR β chain of the T-cell subset in healthy HLA-DR2 individuals and in HLA-DR2 MS patients. In healthy individuals the value was found to be lower than $1/10^8$ CD3 mRNA copies, thus less than one copy per 10^5 T cells. In patients in the acute phase, the number of copies varied from 14 (patient 5) to 326 (patient 2) per 10^8 CD3 mRNA, thus per 10^5 T cells. Chronic patient A had 280 copies per 10^8 CD3 copies; whereas chronic patient B had less

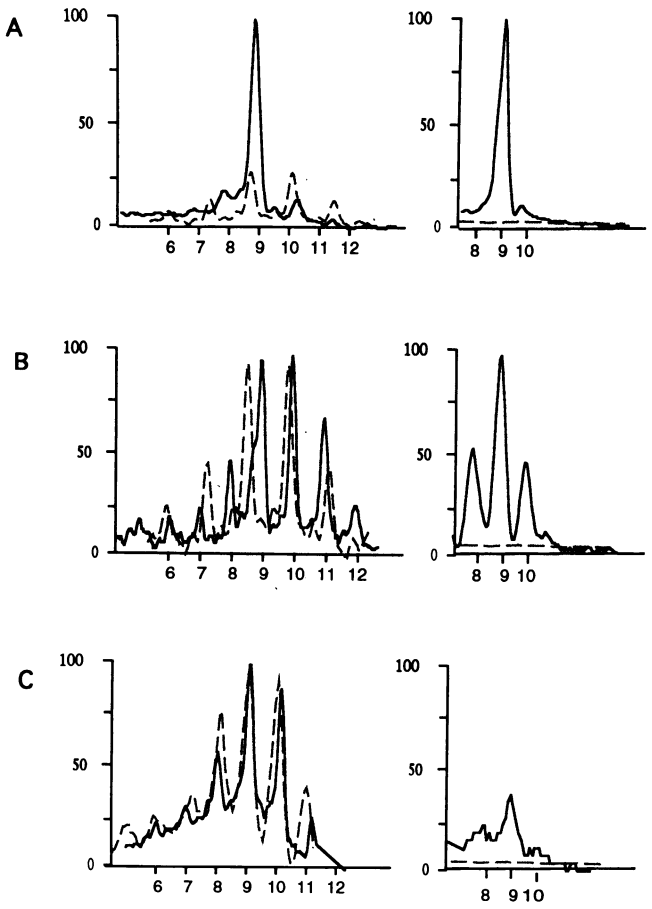

FIG. 3. Use of penetrating probes to detect the T-cell subset in HLA-DR2 MS patients. (*Left*) The result of primer extension using Vβ5–Jβ1.4 primers on cDNA from patients 2 (*A*), 4 (*B*), and 5 (*C*). (*Right*) The results of primer extension using Vβ5 primer and the penetrating primer 1 on cDNA from the same patients. Solid line, results obtained on patients 2, 4, and 5; dotted line, results obtained on healthy HLA-DR2 individuals.

than $1/10^8$ CD3 copies, similar to the levels in the unrelated chronic patients and the healthy HLA-DR2 controls (Fig. 5).

The T-Cell Subset Is $CD4^+$. We performed cell sorting with anti-CD4- and anti-CD8-coated magnetic beads on the PBLs of HLA-DR2 MS patient 3. RNA was extracted from the $CD4^+$ and from the $CD8^+$ populations and reverse-transcribed. A run off with the CDR3-specific fluorescent primer 1 and 2 was performed on Vβ5–Cβ PCR products. Results showed that the subset is detected in $CD4^+$ T cells but not in the $CD8^+$ population (Fig. 6).

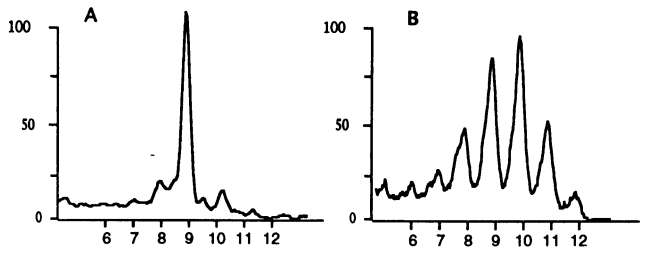

FIG. 4. Effect of therapy on the expansion of the recurrent T-cell subset. An HLA-DR2 MS patient (patient 3) displayed a marked bias in the Vβ5.3–Jβ1.4 T-cell population (*A*). The patient was treated for 5 days with steroids; clinical signs disappeared and no relapses were observed over a 1-year period. A blood sample was taken a year after treatment and analyzed for the presence of the unique β chain. No expansion was detected (*B*).

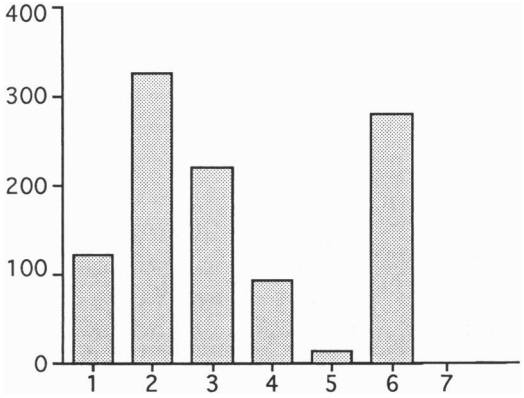

FIG. 5. Quantitative method used to determine the number of mRNA copies coding for the TcR β chains used by the T-cell subset. Ordinate, number of β-chain mRNA copies per 10^8 copies of CD3-encoding mRNA. Bars: 1–5, HLA-DR2 MS patients 1–5 at the onset of the disease; 6, HLA-DR2 chronic MS patient A; 7, mixed healthy HLA-DR2 individual. In the last case, the number of β-chain mRNA copies was undetectable, thus less than 1 out of 10^8 CD3-encoding mRNAs.

The Population Is Present but not Expanded in Healthy DR2 Individuals. Probe 3 was used to determine if the T-cell subset was present, although in much lower amounts, in HLA-DR2 healthy individuals. Thus, we carried out a two-step PCR. We PCR-amplified already amplified Vβ5–Cβ DNA, using the Vβ5 primer and the primer 3. Under these conditions, all HLA-DR2 healthy individuals gave a positive signal, while none was not detected in non-HLA-DR2 MS patients (Fig. 7). Using the same double-PCR strategy, the T-cell subset was found in all the healthy HLA-DR2 members of the family at risk of MS, as well as in the two chronic patients that did not show an expansion of it in the direct approach. In all cases the size distribution of the amplified products was found to be gaussian.

DISCUSSION

The technology we used herein allows a direct analysis of the T-cell repertoire at any precise moment and can, therefore, be used to identify the repertoire of the T-cell receptor β chain of T cells in a variety of human and murine diseases (7, 15, 20). The pattern obtained corresponds to a "snapshot" of the T-cell populations at the moment that the blood sample is taken (in the present case, during the acute phase that has led to diagnosis of MS).

Since the expansion of a T-cell population is indicative of specific antigenic stimulation, we searched for T-cell subsets expanded in all patients, thus corresponding to a recurrent [or public (21)] response. The populations expanded only in one individual, which might be the result of the exposure to non-MS-related antigens, were not considered in the present study.

Among the two β-chain families surveyed in DR2 MS patients, namely, Vβ5 and Vβ17, no expansion could be detected in the Vβ17$^+$ T-cell population either at the onset of the disease or in chronic MS. By contrast, a subset of CD4$^+$ T cells in the Vβ5 population, characterized by Vβ5.3 and Jβ1.4 usage in association with a family of related 9-amino acid CDR3 sequences, was found to be expanded in the blood of five out of five beginning patients and in one out of three chronic patients. Since the presence of this family of TcRs is associated with the expression of HLA-DR2 (and also possibly of DQ6, because of the linkage disequilibrium between DR and DQ genes), it is conceivable that it could recognize a peptide presented by the HLA-DR2 molecule. The nature of the peptide, and thus the specificity of the T-cell clone, is

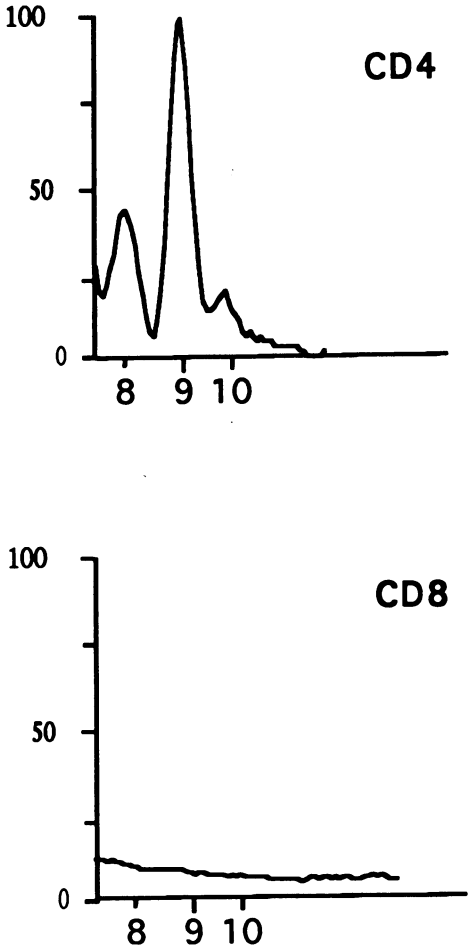

FIG. 6. CD4/CD8 cell sorting and distribution of the T-cell subset in patient 3. T cells were sorted according to their CD4$^+$ and CD8$^+$ phenotypes by using magnetic beads. The presence or absence of the T-cell subset was ascertained by primer extension of Vβ5–Cβ PCR products, using penetrating oligonucleotide 2. The T-cell subset was found exclusively in the CD4$^+$ T-cell population (*Upper*) and not in the CD8$^+$ T-cell population (*Lower*).

unknown. However, the β chain of the expanded T cells found in patient 2 uses the Vβ5.3 segment preferentially used by MBP-reactive clones in HLA-DR2 patients (6). Also, it displays an LR motif in its VDJ region that has been found in some of the T cells infiltrating the demyelinating plaques in the CNS of HLA-DR2 MS patients (5). Moreover, the LR motif has been found associated with the reactivity of a T-cell clone to the MBP peptide of residues 88–100 (22). It is thus tempting to speculate that at least some members of the T-cell subset recognize a peptide derived from MBP.

Whatever its possible reactivity toward a brain autoantigen may be, a distinctive T-cell subset is found expanded in association with the first acute clinical signs of MS. The expansion is detected in the five "newly diagnosed" HLA-DR2 MS patients studied here. The expansion may disappear in the case of a chronic pathology or after immunosuppressive treatment. Although the number of cases studied is small, these results are in favor of the association of this particular Vβ5$^+$ population with part of the T-cell response in DR2 MS patients. The T-cell subset may thus play some role at the onset of the disease. The extent of its expansion does not correlate directly with the severity of the disease. Such a discrepancy is not unexpected since circulating peripheral T cells may not reflect the T-cell levels in the CNS infiltrates and since the severity of the disease markedly depends on the localization of the lesions in the brain. The technique that we used thus

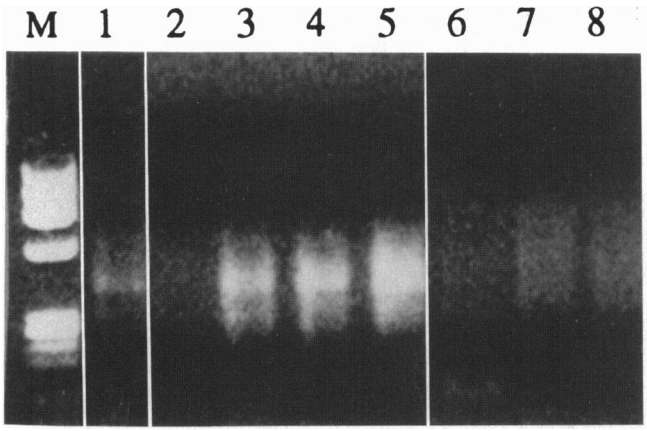

FIG. 7. T-cell subset is detectable in HLA-DR2 healthy individuals. mRNA was extracted from the following sources. Lanes: 1, HLA-DR2 MS patient 1; 3–5, HLA-DR2 healthy individuals; 6–8, non-HLA-DR2 MS patients. Lane 2 is the negative control for PCR amplification. cDNA was first amplified with a Vβ5 and Cβ. PCR products were PCR-amplified with a Vβ5-specific primer and the primer 3. They were analyzed by electrophoresis in a 2% agarose gel. Size marker is φX174 digested by *Hae*III. PCR products of the expected size were obtained for all HLA-DR2 individuals but not for non-HLA-DR2 MS patients.

defines T cells of potential interest for further investigation on the role of T cells in DR2 MS patients.

Finally, this T-cell subset is expanded in DR2 MS patients but not in non-DR2 MS patients and is present in healthy HLA-DR2 individuals, whereas it is not detectable in non-HLA-DR2 individuals. Hence, the subset was probably positively selected in the HLA-DR2 restriction context without deleterious effects. Its activation and expansion in HLA-DR2 MS patients most likely results from the stimulation of the small number of resting T cells positively selected in healthy individuals by some unknown stimulus (23). In this respect, we have found that interleukin 10 expression is reduced 100-fold in the same MS patients and is also reduced, but less so, in the family at risk of MS (unpublished data). If the T-cell subset we have described is reactive against brain autoantigens, we propose that genetic and/or environmental factors alter the interleukin 10-associated balance, which prevents the expansion of autoreactive T cells. Activated autoreactive T cells could then cross the brain–barrier and contribute to the formation of the lesions.

We thank A. Lim for quantification experiments and D. Ojcius for critical reading of the manuscript.

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