ANNALS of Clinical and Translational Neurology
Open Access
AMERICAN NEUROLOGICAL ASSOCIATION

RESEARCH ARTICLE

A new clustering method identifies multiple sclerosis-specific T-cell receptors

Fumie Hayashi[1], Noriko Isobe[1], Jacob Glanville[2], Takuya Matsushita[1], Guzailiayi Maimaitijiang[1], Shoko Fukumoto[1], Mitsuru Watanabe[1], Katsuhisa Masaki[1] & Jun-ichi Kira[1]

[1]Department of Neurology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
[2]Computational and Systems Immunology Program, Stanford University School of Medicine, Stanford, California

Correspondence
Jun-ichi Kira, Department of Neurology, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. Tel: +81-92-642-5340; Fax: +81-92-642-5352; E-mail: kira@neuro.med.kyushu-u.ac.jp

Funding information
"Japan Society for the Promotion of Science (JSPS) KAKENHI" should be changed to "Japan Society for the Promortion of Science". "Health and Labour Sciences Research" is not needed.
"Ministry of Health, Labour, and Welfare, Japan" should be changed to "Ministry of Health, Labour and Welfare".
In addition, could you add one more grant number and change the funding information in the main text as follows.
"This study was supported by grants from the Japan Society for the Promotion of Science [Grant Nos. 16H02657 (to J.K.), 16K09694 (to K.M), 18K07529 (to N.I.), 19H01045 (to J.K.), 19K07995 (to M.W.), and 19K07997 (to T.M., M.W., and N.I.)], and a Health and Labour Sciences Research Grant on Intractable Diseases [H29-Nanchitou (Nan)-Ippan-043 and 20FC1030 (to J.K.)] from the Ministry of Health, Labour, and Welfare, Japan.

Received: 2 October 2020; Accepted: 6 November 2020

Annals of Clinical and Translational Neurology **2021; 8(1): 163–176**

doi: 10.1002/acn3.51264

Abstract

Objective: To characterize T-cell receptors (TCRs) and identify target epitopes in multiple sclerosis (MS). **Methods**: Peripheral blood mononuclear cells were obtained from 39 MS patients and 19 healthy controls (HCs). TCR repertoires for α/β/δ/γ chains, TCR diversity, and V/J usage were determined by next-generation sequencing. TCR β chain repertoires were compared with affectation status using a novel clustering method, Grouping of Lymphocyte Interactions by Paratope Hotspots (GLIPH). Cytomegalovirus (CMV)-IgG was measured in an additional 113 MS patients and 93 HCs. Regulatory T cells (Tregs) were measured by flow cytometry. **Results**: TCR diversity for all four chains decreased with age. TCRα and TCRβ diversity was higher in MS patients ($P = 0.0015$ and 0.024, respectively), even after age correction. *TRAJ56* and *TRBV4-3* were more prevalent in MS patients than in HCs ($p^{corr} = 0.027$ and 0.040, respectively). GLIPH consolidated 208,674 TCR clones from MS patients into 1,294 clusters, among which two candidate clusters were identified. The *TRBV4-3* cluster was shared by *HLA-DRB1*04:05*-positive patients (87.5%) and predicted to recognize CMV peptides (CMV-TCR). MS Severity Score (MSSS) was lower in patients with CMV-TCR than in those without ($P = 0.037$). CMV-IgG-positivity was associated with lower MSSS in *HLA-DRB1*04:05* carriers ($P = 0.0053$). *HLA-DRB1*04:05*-positive individuals demonstrated higher CMV-IgG titers than *HLA-DRB1*04:05*-negative individuals ($P = 0.017$). CMV-IgG-positive patients had more Tregs than CMV-IgG-negative patients ($P = 0.054$). **Interpretation**: High TCRα/TCRβ diversity, regardless of age, is characteristic of MS. Association of a CMV-recognizing TCR with mild disability indicates CMV's protective role in *HLA-DRB1*04:05*-positive MS.

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disorder in which both genetic and environmental factors play important pathogenic roles.[1] Clinical features of MS differ by ethnicity. For instance, Japanese patients with MS have a milder disease course than patients in the United Kingdom[2] and cerebellar hemispheric lesions are less frequently observed in Japanese patients than in Caucasian patients.[3]

The class II sub-region of *human leukocyte antigen* (*HLA*) has the strongest genetic influence on MS susceptibility. In Caucasian patients, *HLA-DRB1*15:01* is an established risk allele for MS,[1] whereas *DRB1*15:01* and *DRB1*04:05* are the most prevalent *HLA* risk alleles for MS in Japanese patients.[4] Nearly half of European patients with MS carry *DRB1*15:01*, whereas *DRB1*04:05* is rare.[5] In contrast, in Japanese MS patients, phenotype frequencies of *DRB1*04:05* and *DRB1*15:01* have been reported to be 39.6%–44.8% and 26.2%–28.8%, respectively.[4,6] Such differences in *HLA* allele frequencies partly account for differences in the worldwide MS prevalence, and may contribute to the distinct MS manifestations by race. Indeed, Japanese MS patients with *HLA-DRB1*04:05* demonstrate relatively mild disease progression[4,6] involving a lower number of intracortical lesions.[7]

Since the 1980s, multiple studies have searched for target antigens in MS.[8,9] However, even now, unambiguous target antigens have not been identified. Most of these studies were performed by a hypothesis-driven approach; candidate target antigens, such as myelin basic protein, myelin oligodendrocyte glycoprotein, and proteolipid protein, were selected according to the perceived pathogenesis of the disease. Clinical trials for T-cell receptor (TCR) vaccination were undertaken but none showed any reliable efficacy.[8]

Since 2009, high-throughput sequencing of immune repertoires has been developed, generating datasets ranging from 100 million to billions of reads.[10,11] This has driven repertoire studies utilizing advanced bioinformatics methods and computation-driven interpretation and data visualization.[12] A recent study focused on the TCRβ repertoire in MS, reporting higher TCRβ repertoire diversity in MS patients than in control subjects and a characteristic pattern of complementarity-determining region 3 (CDR3) sequences in MS.[13] However, it did not lead to the identification of possible target antigens in association with specific *HLA* alleles.

One of the most recently developed methods is Grouping of Lymphocyte Interactions by Paratope Hotspots (GLIPH), which utilize sequence similarity for clustering to predict antigen specificity, and yields highly accurate predictions of antigen-specific TCRs.[14] Furthermore, GLIPH can integrate many factors included in repertoire data, such as V gene usage, CDR3 length, clonal expansion, and *HLA* alleles. In this study, we aimed to clarify characteristic TCR repertoires for all α/β/δ/γ chains in MS using next-generation sequencing, and to identify TCRs associated with MS pathogenesis by comparing TCR characteristics between Japanese MS patients and healthy controls (HCs) using GLIPH. Furthermore, given that patients with different *HLA* alleles demonstrate distinct disease progression, we also aimed to search for MS-associated TCRs that are enriched in a group of MS patients with particular MS-associated *HLA-DRB1* alleles. Finally, we characterized the clinical and immunological features of patients with certain MS-associated TCRs.

Subjects and methods

Participants

Peripheral blood mononuclear cells were obtained from 39 MS patients and 19 HCs. All patients were recruited at Kyushu University Hospital and all of them fulfilled the 2010 McDonald criteria.[15] Exclusion criteria were: (i) those with collagen diseases, (ii) pregnant women, and (iii) those receiving treatments that can affect the TCR repertoire (i.e., fingolimod, natalizumab, and high-dose corticosteroids). For each patient, clinical and laboratory data [e.g., Kurtzke's Expanded Disability Status Scale (EDSS) score[16] and MS severity score (MSSS)[17]] were collected. HCs were included from the same geographic area as the enrolled patients. For the extended analysis based on the identified disease-associated TCRs, we additionally enrolled 113 MS patients and 93 HCs. The study was approved by the Ethical Committee of Kyushu University.

TCR repertoire analysis

Complementary DNA was synthesized from 1 μg of total RNA extracted from fresh peripheral blood mononuclear cells. Next-generation sequencing combined with an unbiased adaptor-ligation PCR for TCR α/β/δ/γ chains, data processing, and aggregation were conducted by Repertoire Genesis Inc. (Ibaraki, Japan), as described previously.[18] TCR sequences were assigned using reference sequences from the international ImMunoGeneTics information system database (http://www.imgt.org). To estimate TCR repertoire diversity, the Shannon index was calculated using the R package, Vegan. The definition and formula for the index were described previously.[19] A larger index value represents greater diversity.

TCR Sequence Clustering by GLIPH

GLIPH is a method to cluster a large number of TCRs into distinct groups with shared specificity.[14] In the three-step

GLIPH algorithm, first, global similarity (i.e., CDR3 differing by up to one amino acid) and local similarity (that is, shared CDR3 amino acid motifs: >10-fold enriched and < 0.001 probability of occurring at this level of enrichment in the control TCR pools) were searched for. Second, all identified global and local similarities were used to construct clusters of TCR specificity groups. Third, each specificity group was analyzed for enrichment of a CDR3 length distribution, clonal expansions, shared *HLA* alleles in subjects, motif significance, and cluster size, and a total summary score was obtained by combining the above probabilities into a single score by conflation.[14] To handle the huge amounts of raw repertoire data, we divided the repertoire data according to each V gene and applied GLIPH separately to compare MS patients with HCs possessing the same V gene. From the integrated summary scores, we extracted the candidate TCR clusters that were enriched with an MS susceptibility *HLA-DRB1* allele (*HLA* p-value < 0.05) and in which the shared motif had been positionally aligned from the N-terminus. Additionally, the TCR clusters with total summary scores $< 1.0 \times 10^{-17}$, irrespective of *HLA* p-values, were evaluated to determine whether the TCR clusters were positionally well aligned.

Search for target antigens of candidate TCRs using VDJdb

VDJdb is a large curated database that stores and aggregates the results of published T-cell specificity assays coupling antigen specificities with TCR sequences.[20] We used VDJdb to search for the target epitopes to which the candidate TCRβ CDR3 was predicted to bind.

Analysis of humoral immune responses to cytomegalovirus

Serum IgG antibodies against cytomegalovirus (CMV-IgG) levels were analyzed in duplicate wells using a SEIKEN enzyme immunoassay kit (DENKA SEIKEN, Tokyo, Japan). CMV-IgG seropositivity was defined as an enzyme immunoassay titer> 2.0.

Analysis for CMV-DNA

Serum CMV-DNA levels were analyzed by real-time PCR. The cut-off value for CMV-DNA positivity was defined as 2×10^2 copies/ml.

Flow cytometric analysis

Cell surface staining and flow cytometry of peripheral blood mononuclear cells were conducted as previously described.[21] Regulatory T cells (Tregs) were defined as $CD4^+CD25^+CD127^{low}$, based on FoxP3 expression in $CD4^+CD25^+$ T cells, which are tightly linked to the suppressive functions of Tregs.[22]

HLA typing

High-resolution HLA allele typing was conducted; *HLA-DRB1* allele typing was conducted for all study participants.

Statistical analysis

Categorical variables are described as counts and percentages, whereas continuous variables are summarized as medians and interquartile ranges. Dichotomous variables were analyzed using the chi-square test or Fisher's exact test. The Mann–Whitney U test was used to analyze continuous variables. A linear regression model was applied to assess the association of age with TCR diversity. Interaction p-values were calculated to clarify whether differences in affectation status predict different slopes or rates of change for the diversity of each TCR with aging. Bootstrap simulations were performed in the MS patients, randomly selecting 19 MS samples 1000 times to assess the possible impact of different sample sizes between MS patients and HCs on TCR diversity. For analysis of V/J usage, the Bonferroni correction was

Table 1. Demographic and clinical characteristics of the study cohort for the TCR repertoire analysis.

	MS (n = 39)	HCs (n = 19)	P
Sex (M/F)	6/33	4/15	0.71
Age at examination (y)[1]	52 [40–63]	53 [43 – 59]	0.98
Disease type (RR/SP/PP)	27/10/2	–	
Age at onset (y)[1]	29.5 [24–43]	–	
Disease duration (y)[1]	14.4 [10.8–24.5]	–	
EDSS[1]	3.0 [1.0–6.0]	–	
MSSS[1]	2.15 [0.94–6.00]	–	
Carrier frequencies of			
HLA-DRB1 04:05* (%)	19/39 (48.7)	10/19 (52.6)	1.0
HLA-DRB1 15:01* (%)	13/39 (33.3)	7/19 (36.8)	1.0
Allele frequencies of			
HLA-DRB1 04:05* (%)	20/78 (25.6)	11/38 (28.9)	0.82
HLA-DRB1 15:01* (%)	14/78 (17.9)	7/38 (18.4)	1.0
Treatments			
None (%)	26/39 (66.7)	–	
IFN-beta (%)	8/39 (20.5)	–	
PSL 5 mg (%)	4/39 (10.3)	–	
AZP (%)	1/39 (2.6)	–	

AZP, azathioprine; EDSS, expanded disability status scale; F, female; HCs, healthy controls; HLA, human leukocyte antigen; IFN, interferon; M, male; MS, multiple sclerosis; MSSS, multiple sclerosis severity score; n, number; PP, primary progressive; PSL, prednisolone; RR, relapsing remitting; SP, secondary progressive; y, year.
[1]Median [interquartile range].

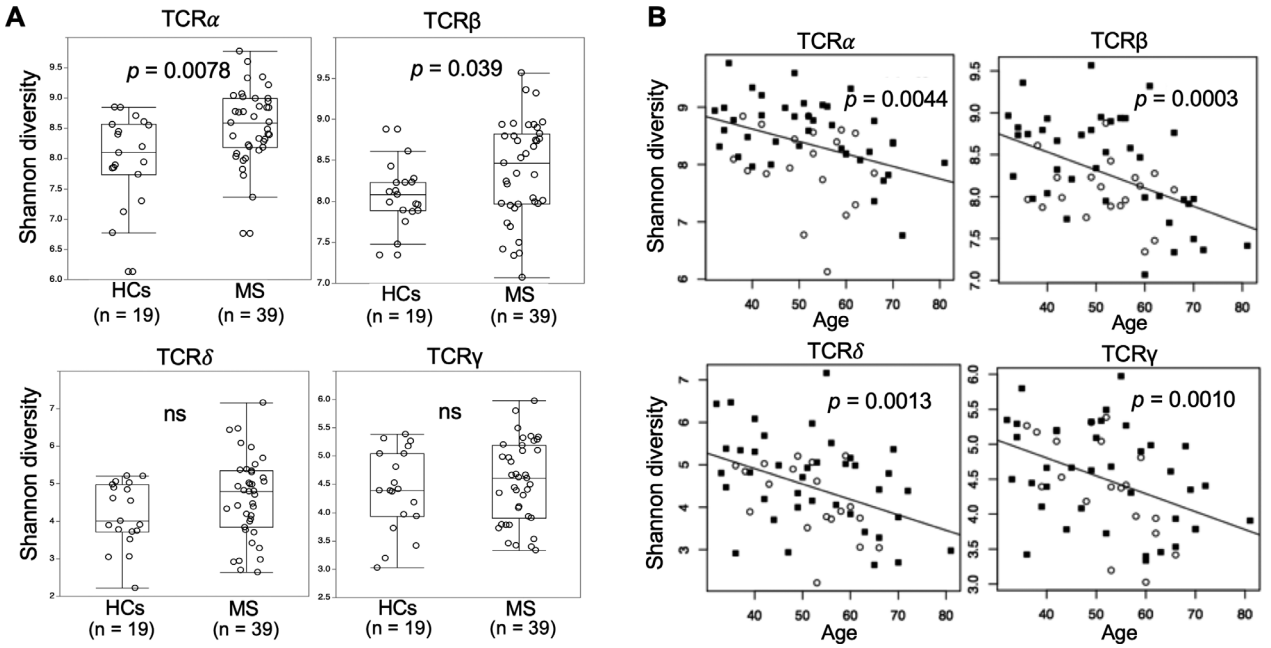

Figure 1. T-cell receptor (TCR) repertoire diversity. (A) Comparison of TCR repertoire diversity between healthy controls (HCs) (n = 19) and multiple sclerosis (MS) patients (n = 39). MS patients had higher TCRα and TCRβ diversity than HCs. (B) Association between TCR repertoire diversity and age. Closed squares represent MS patients, whereas open circles represent HCs. ns = not significant (P > 0.1).

applied to obtain corrected P-values (P^{corr}) from uncorrected P-values (P^{uncorr}) after multiplication by the number of comparisons per region. To assess the susceptibility effect of CMV-IgG, logistic regression analysis was conducted. All statistical analyses, including power calculations, were performed using JMP® Pro version 14.1 (SAS Institute Inc., Cary, NC, USA) or R version 3.4.1 (www.r-project.org).

Results

Demographic features of study participants

The demographic features of the study participants for the TCR repertoire analysis are summarized in Table 1. There were no significant differences in sex, age at examination, or frequencies of *HLA* alleles between MS patients and HCs.

Higher TCR repertoire diversity in MS patients

Diversities of TCRα and TCRβ were higher in MS patients compared with HCs (P = 0.0078 and 0.039, respectively, Fig. 1A). Higher TCRα and TCRβ diversities in MS patients remained significant even after correction for age (P = 0.0015 and 0.024, respectively). No significant differences in TCRδ and TCRγ diversities were observed between MS patients and HCs. Among all participants, higher age was associated with lower TCRα/β/δ/γ diversity (P = 0.0044, 0.0003, 0.0013, and 0.0010, respectively, Fig. 1B). Bootstrapping was conducted to remove the possible impact of different sample sizes between MS patients and HCs, by randomly selecting 19 samples from MS patients, the same number as the HCs. This approach confirmed the higher TCRα/β diversity in MS patients compared with HCs even with consideration of the possible impact of statistical power differences (Fig. S1). When the relationship between TCR diversities and age was compared between MS patients and HCs, all four chains showed no difference in terms of the slopes or rates of change by age (interaction P-values> 0.05 for all chains). Comparison of TCRβ diversity with susceptibility *HLA* allele status revealed no significant differences.

Limited differences in TCR V and J gene usage between MS patients and controls

We compared usage of the TCR V and J repertoire between MS patients and HCs in all four TCR chains. In *TRA*, there were no significant differences between MS patients and HCs in all 41 *TRAV* genes (Table S1), whereas only *TRAJ56* was increased in MS patients (P^{corr} = 0.027, Table S2). In *TRB*, *TRBV4-3* was increased

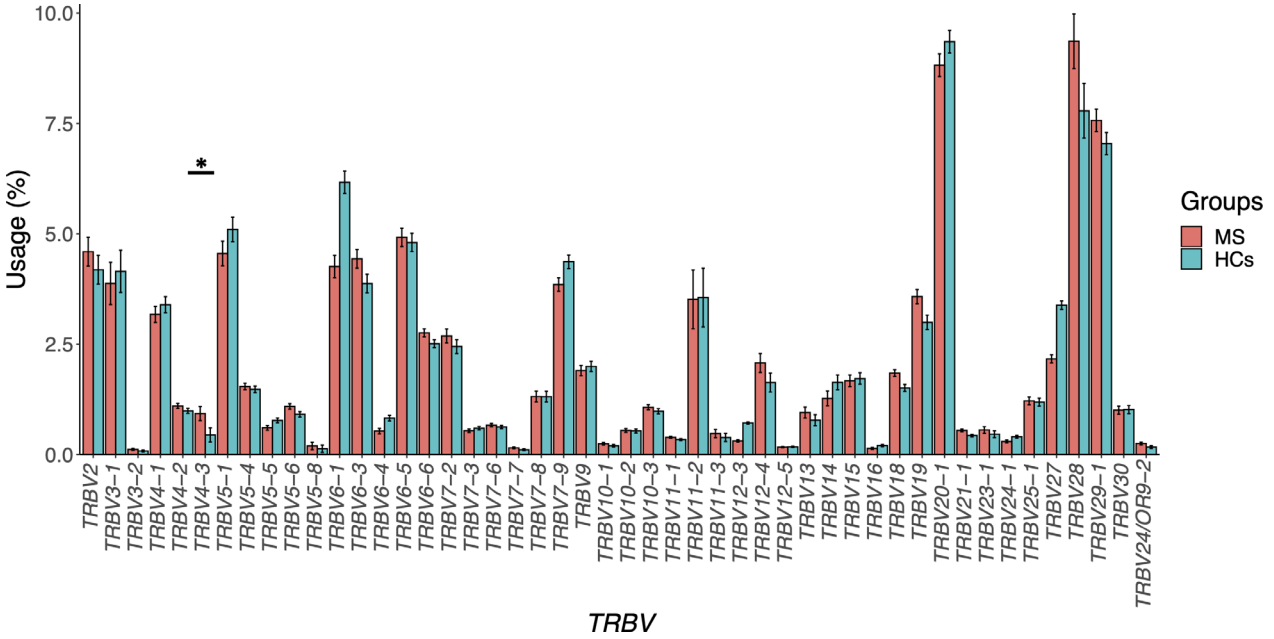

Figure 2. T-cell receptor Vβ gene usage distribution. Vβ genes with $p < 0.05$ between multiple sclerosis (MS) patients and healthy controls (HCs) are indicated by *. In *TRB*, only *TRBV4-3* was increased in MS patients ($P^{corr} = 0.040$).

in MS patients ($P^{corr} = 0.040$, Fig. 2 and Table S3), but there were no significant differences in all 13 *TRBJ* genes (Table S4). In *TRD* and *TRG*, there were no significant differences in TCR V and J gene usages (Tables S5 and S6).

Identification of disease-specific TCRs by GLIPH

Given the importance of CDR3β compared with CDR3α in terms of the epitope contact site,[14] we focused on TCRβ in the search for disease-specific TCRs by GLIPH. The study flow of the GLIPH analysis is described in Figure 3. GLIPH consolidated all 208,674 TCR clonotypes from MS patients into 1,294 clusters. We then narrowed down the clusters based on two criteria. The first criterion was whether TCR clusters were dominantly possessed by MS patients with specific susceptibility *HLA* alleles with *HLA* enrichment $P < 0.05$. For the second criterion, we looked more closely at each cluster and checked whether the positions of the significant motifs were aligned with respect to the N-terminus. The first step extracted 66 TCR clusters enriched with *HLA-DRB1*04:05* and 55 clusters enriched with *HLA-DRB1*15:01*. The second step identified two clusters with representative CDR3 amino acids sequences, CASSSRQGLQPRHF and CASSPQRNTEAFF, and the total summary scores of the two clusters were 4.46×10^{-18} and 8.02×10^{-18}, respectively. Both clusters were enriched in MS patients carrying *HLA-DRB1*04:05* ($P = 0.045$ and 0.018, respectively) and the V usage in the identified clusters was *TRBV6-6* and *TRBV4-3*, respectively.

Next, VDJdb database software was used to search for known antigen epitopes to which the identified TCRs were predicted to bind. Although the CDR3 sequences in the former candidate cluster did not match any known epitopes (designated unknown-TCR), one of the CDR3 sequences in the latter cluster (CASSPQRNTEAFF) recognized a CMV peptide, RPHERNGFTVL, located within phosphoprotein 65 (pp65), using *TRBV4-3* in VDJdb. We named the identified TCR as CMV-TCR. The CDR3 motifs included in the CMV-TCR cluster were seen in 8 of the 39 MS patients but none of the HCs, which indicate that CMV-TCR was significantly highly prevalent in MS (MS patients: 8/39 (20.5%) vs. HCs: 0/19 (0%), $P = 0.032$) (Tables S7 and S8). No significant difference was observed in TCRβ diversity between CMV-TCR-positive and CMV-TCR-negative MS patients. To assess whether any additional TCR clusters could fulfill the criteria for sufficient motif alignment, irrespective of the *HLA-DRB1* alleles, we further explored the top 154 TCR clusters that had total significance summary scores of $< 1.0 \times 10^{-17}$. However, no additional cluster had sufficient motif alignment to be considered a candidate.

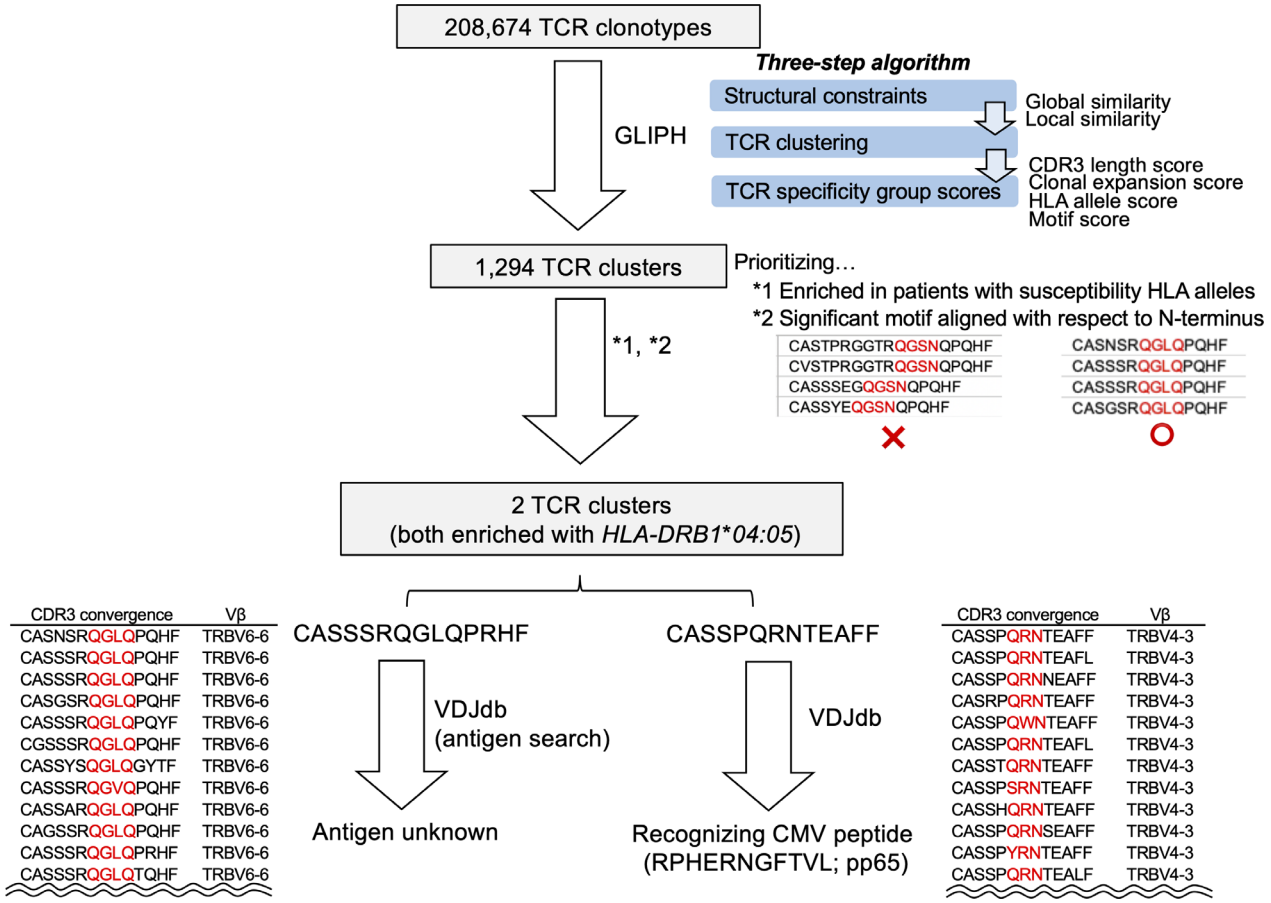

Figure 3. Study flow for the Grouping of Lymphocyte Interactions by Paratope Hotspots (GLIPH) analysis. In the three-step GLIPH algorithm, first, global similarity and local similarity were searched for. Second, all identified global and local similarities were used to construct clusters of T-cell receptor (TCR) specificity groups. Third, each specificity group was analyzed for enrichment of a complementarity-determining region 3 (CDR3) length distribution, clonal expansions, shared *HLA* alleles in subjects, motif significance, and cluster size, and a total summary score was obtained by combining the above probabilities into a single score by conflation. GLIPH consolidated all 208,674 TCR clonotypes from multiple sclerosis (MS) patients into 1294 clusters. Based on two criteria, comprising enrichment in MS patients with susceptibility *human leukocyte antigen* (*HLA*) alleles ($P < 0.05$) and positions of significant alignment, the 1294 clusters were then narrowed down to two clusters. One of the clusters recognized a cytomegalovirus (CMV) peptide. In the alignments, significant motif residues are highlighted in red.

CMV-IgG and CMV-DNA in the original cohort

In the original cohort, CMV-IgG was positive in 34/39 (87.2%) MS patients and 18/19 (94.7%) HCs ($P = 0.65$) (Table S9). The CMV-IgG positivity rates were then compared by the carrier status of *HLA-DRB1*04:05* within MS patients and HCs. In MS patients, there was no significant difference in CMV-IgG positivity between the *HLA-DRB1*04:05*-positive group (18/19, 94.7%) and *HLA-DRB1*04:05*-negative group (16/20, 80.0%) ($P = 0.34$). Similarly, when the analysis was performed in HCs, there was no significant difference between the two groups (*HLA-DRB1*04:05*-positive group: 9/10 vs. *HLA-DRB1*04:05*-negative group: 9/9, $P = 1.0$). Thus, the infection rate of CMV did not differ according to *HLA-DRB1*04:05* positivity in either MS patients or HCs. Regarding the CMV-IgG positivity rate according to CMV-TCR status, CMV-IgG was detected in 8/8 (100%) CMV-TCR-positive MS patients and 26/31 (83.9%) CMV-TCR-negative MS patients ($P = 0.56$). To assess the CMV infection status, we measured CMV-DNA by real-time PCR in all eight patients with CMV-TCR. As a result, no CMV-TCR-positive individuals carried CMV-DNA, which suggests that CMV-TCR was not caused by active CMV infection, but rather expanded in the latent inactive infection status.

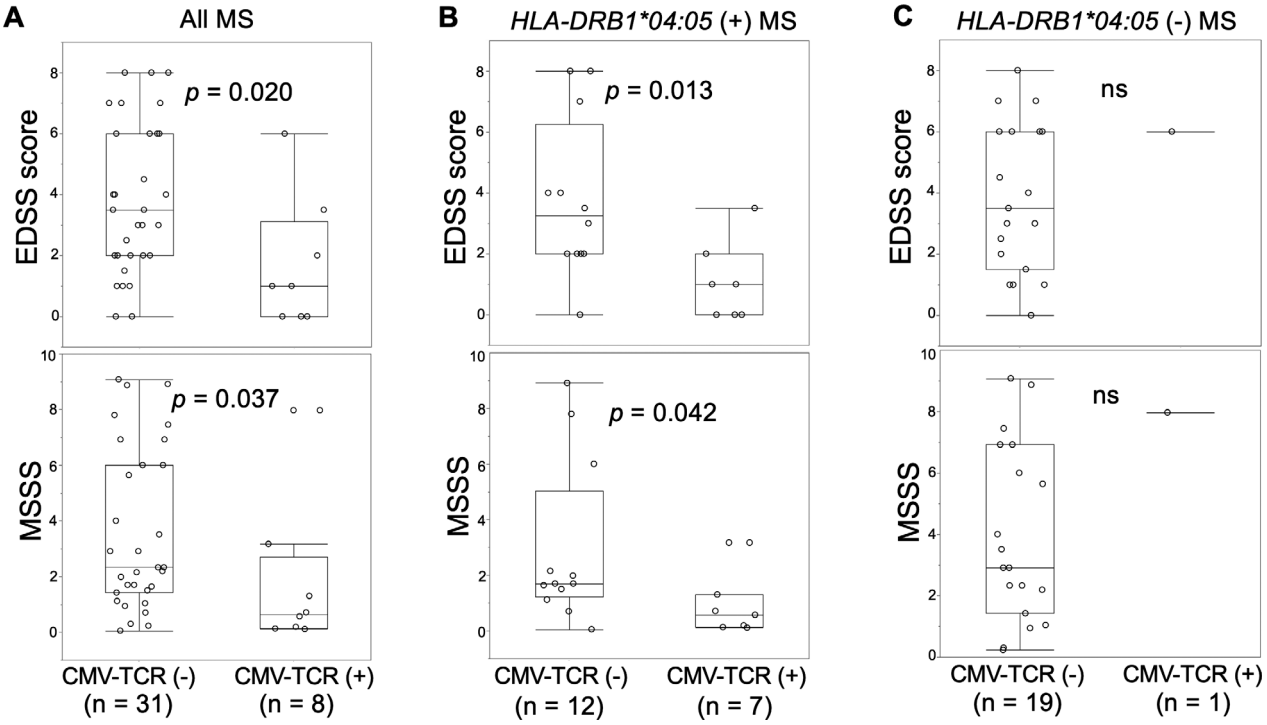

Figure 4. Comparison of disease severity among multiple sclerosis (MS) patients with distinct positivity status for T-cell receptors against cytomegalovirus (CMV-TCR). Expanded Disability Status Scale (EDSS) scores and MS Severity Scores (MSSS) were lower in patients with CMV-TCR than in patients without CMV-TCR (P = 0.020, 0.037, 0.013, and 0.042, respectively). (A) All MS patients. (B) *Human leukocyte antigen* (*HLA*)-*DRB1*04:05*-positive MS patients. (C) *HLA-DRB1*04:05*-negative MS patients. ns = not significant (P > 0.1).

Relationship between CMV-TCR and HLA-DRB1*04:05

From the perspective of the genetic background, *HLA-DRB1*04:05*-positive MS patients demonstrated a higher frequency of CMV-TCR positivity than *HLA-DRB1*04:05*-negative MS patients (7/19 (36.8%) vs. 1/20 (5.0%), P = 0.018) (Table S10). Even when the analysis was limited to CMV-IgG-positive patients, the higher CMV-TCR frequency in *HLA-DRB1*04:05*-positive MS patients compared with *HLA-DRB1*04:05*-negative MS patients remained significant (7/18 (38.9%) vs. 1/16 (6.3%), P = 0.031). As none of the HCs had CMV-TCR, we could not analyze the relationship between CMV-TCR and CMV-IgG or *HLA-DRB1*04:05* in HCs.

Effects of CMV-TCR on clinical features in the original MS Cohort

When the clinical features of MS patients were compared according to CMV-TCR positivity (8 CMV-TCR-positive patients vs. 31 CMV-TCR-negative patients), no significant differences were observed with regard to sex, age at examination, age at onset, and cerebrospinal fluid parameters (Table S11). Interestingly, EDSS scores and MSSS were lower in CMV-TCR-positive patients than in CMV-TCR-negative patients (P = 0.020 and 0.037, respectively, Fig. 4A). *HLA-DRB1*04:05* has been linked to a milder disease course than other *HLA-DRB1* alleles.[6] To clarify whether the lower MSSS and EDSS scores in the CMV-TCR-positive group simply arose because of the higher proportion of MS patients with *HLA-DRB1*04:05* rather than CMV-TCR positivity itself, we stratified MS patients by *HLA-DRB1*04:05* status and compared their disease progression according to CMV-TCR positivity. Even in *HLA-DRB1*04:05*-positive MS patients, EDSS scores and MSSS were still lower in the CMV-TCR-positive group than in the CMV-TCR-negative group (P = 0.013 and 0.042, respectively, Fig. 4B). Such a difference by CMV-TCR positivity was not clear in *HLA-DRB1*04:05*-negative MS patients because of a lack of statistical power (Fig. 4C). These findings indicate that CMV plays a protective role against MS progression, at least in *HLA-DRB1*04:05*-positive patients.

Reduced MS progression associated with CMV in HLA-DRB1*04:05 carriers in a replication cohort

To verify the role of CMV-TCR in MS, we further conducted a replication study using an independent cohort. Sex

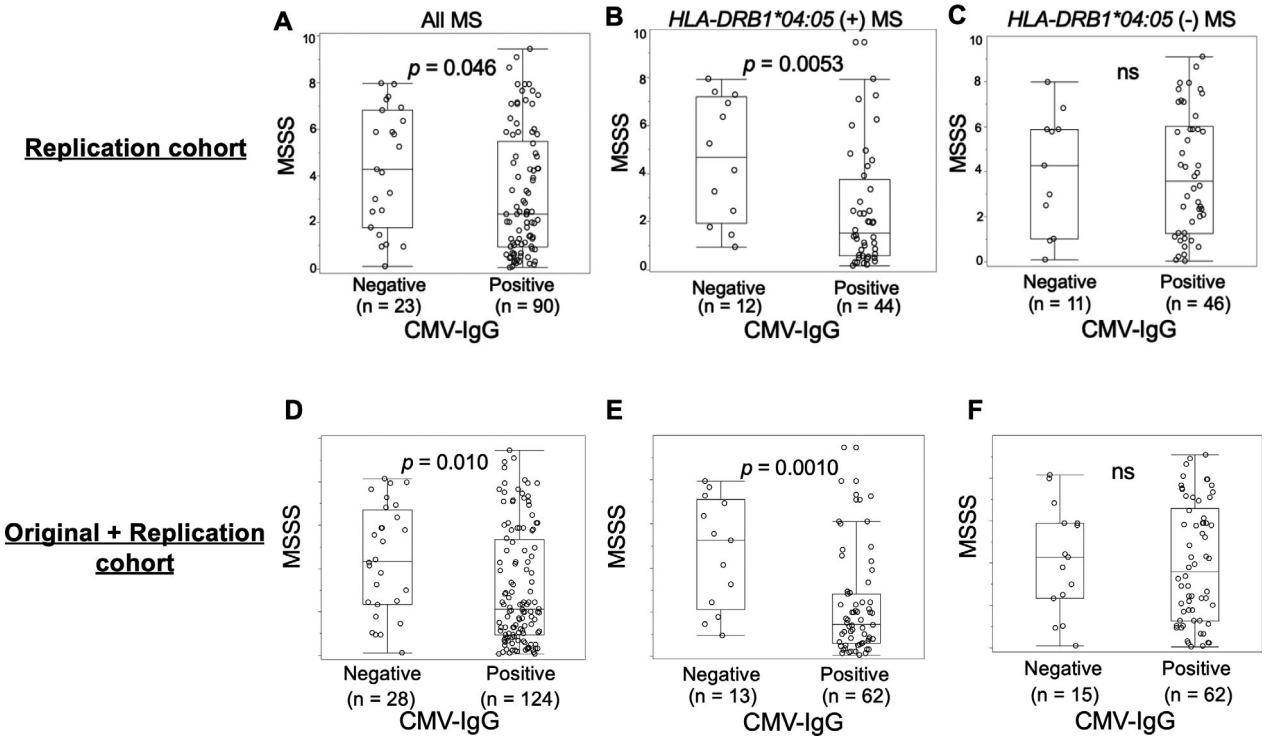

Figure 5. Comparison of multiple sclerosis severity scores (MSSS) among multiple sclerosis (MS) patients with distinct cytomegalovirus (CMV) serostatus in a replication cohort. CMV-IgG-positive MS patients had higher MSSS in the *human leukocyte antigen* (*HLA*)-*DRB1*04:05*-positive group ($P = 0.0053$), but not in the *HLA-DRB1*04:05*-negative group. When analyzed in the combined cohort (original + replication), MSSS was significantly decreased in the CMV-IgG-positive group, especially in *HLA-DRB1*04:05*-positive patients ($P = 0.0010$). (A–C) Replication cohort. (D–F) Original + replication cohort. (A, D) All MS patients. (B, E) *HLA-DRB1*04:05*-positive MS patients. (C, F) *HLA-DRB1*04:05*-negative MS patients. ns = not significant ($P > 0.1$).

composition and age at examination did not differ between MS patients and HCs in the replication case-control cohort (Table S12). The carrier frequencies of the Japanese susceptibility *HLA* alleles were higher in MS patients than in HCs, as expected ($P = 0.0003$ for *HLA-DRB1*04:05* and $P = 0.018$ for *HLA-DRB1*15:01*). CMV-IgG-positive MS patients had lower MSSS than CMV-IgG-negative MS patients ($P = 0.046$, Fig. 5A). Furthermore, after stratification by *HLA-DRB1*04:05* positivity, the *HLA-DRB1*04:05*-positive group demonstrated significantly lower MSSS in CMV-IgG-positive patients than in CMV-IgG-negative patients ($P = 0.0053$, Fig. 5B), whereas the *HLA-DRB1*04:05*-negative group did not (Fig. 5C). When analyzed in the combined cohort (original + replication), MSSS was significantly lower in CMV-IgG-positive patients, particularly in the *HLA-DRB1*04:05*-positive group ($P = 0.0010$, Fig. 5D–F). In multivariate analysis with age at examination, sex, age at onset, disease duration, and treatment status as covariates, the association between CMV-IgG positivity and decreased MSSS remained significant ($P = 0.014$, Table S13). The CMV-IgG positivity rates did not differ significantly between MS patients and HCs ($P = 1.0$, Supplementary Table 14). Even when we stratified the case-control cohort by *HLA-DRB1*04:05* positivity, CMV-IgG positivity was not significantly linked to MS susceptibility.

Stronger humoral immune response to CMV in HLA-DRB1*04:05-positive Individuals

To confirm whether *HLA-DRB1*04:05* specifically acts on CMV, we compared CMV-IgG titers between *HLA-DRB1*04:05*-positive and *HLA-DRB1*04:05*-negative groups among all participants. The results revealed that CMV-IgG positivity was comparable between the two groups (*HLA-DRB1*04:05*-positive group: 84.1% vs. *HLA-DRB1*04:05*-negative group: 80.5%, $P = 0.51$), whereas the CMV-IgG titer was significantly higher in the *HLA-DRB1*04:05*-positive group than in the *HLA-DRB1*04:05*-negative group ($p = 0.017$, Fig 6).

Restoration of Treg Frequency in CMV-IgG-positive HLA-DRB1*04:05-positive MS Patients

CMV infection has been reported to increase Treg numbers, which downregulate immune responses.[23,24]

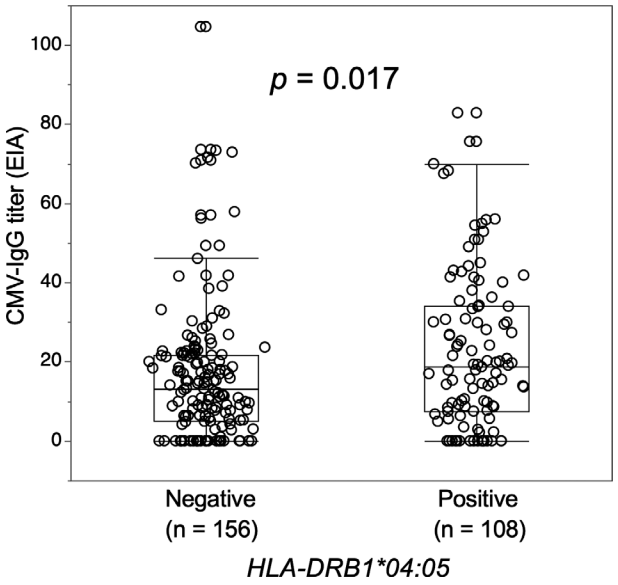

Figure 6. Comparison of cytomegalovirus-IgG (CMV-IgG) titer among all participants with distinct *human leukocyte antigen* (*HLA*)-*DRB1*04:05* status. CMV-IgG titer was significantly higher in the *HLA-DRB1*04:05*-positive group than in the *HLA-DRB1*04:05*-negative group ($P = 0.017$). EIA = enzyme immunoassay.

Therefore, we measured the percentage of Tregs in $CD4^+$ T cells (Treg%) in our participants, using the gating strategy shown in Fig. 7A. Treg% was lower in total MS patients than in HCs ($P = 0.0055$, Fig. 7B). In MS patients, no association was observed between CMV-TCR and Treg% (5.0% in CMV-TCR-positive MS patients vs. 4.4% in CMV-TCR-negative MS patients, $P = 1.0$). Regarding CMV-IgG positivity status, Treg% tended to be higher in CMV-IgG-positive MS patients than in CMV-IgG-negative MS patients (mean: 4.4% vs. 3.3%, $P = 0.054$, Fig. 7C). HCs demonstrated no differences in Treg% with respect to CMV-IgG positivity status (mean: 6.2% in CMV-IgG-positive HCs vs. 5.9% in CMV-IgG-negative HCs, $P = 0.62$). Compared with HCs, Treg% was significantly decreased in CMV-IgG-negative and *HLA-DRB1*04:05*-negative MS patients ($P = 0.022$) but not in CMV-IgG-positive and *HLA-DRB1*04:05*-positive MS patients (Fig. 7D), which indicate a partial restoration of Tregs in MS patients with CMV-IgG and *HLA-DRB1*04:05*. Concerning the usage of treatments for MS (interferon-beta, low-dose prednisolone, and azathioprine), we found no significant associations of treatment usage with CMV-IgG positivity, CMV-IgG titer, and %Treg (Table S15).

Effects of unknown-TCR on MS

Finally, we assessed the possible effects of the unknown-TCR cluster on clinical features, immune response against CMV, and Treg%. No clear correlations were found between unknown-TCR positivity and these parameters (Table S16).

Discussion

The present TCR repertoire study shows that MS patients have higher diversity for TCRα and TCRβ but not for TCRδ and TCRγ, even after correction for age, and more frequent use of *TRBV4-3* and *TRAJ56* compared with HCs. Furthermore, we successfully identified two TCR clusters that were highly expressed in MS patients with *HLA-DRB1*04:05* compared with HCs by GLIPH; one of these clusters, using *TRBV4-3*, recognized a CMV pp65 peptide. The TCR against the CMV pp65 peptide and CMV-IgG positivity were associated with milder disability in *HLA-DRB1*04:05*-positive patients.

The trend of decreasing TCRα/β/δ/γ repertoire diversity with aging in both HCs and MS patients demonstrated in the present study extends previous findings that TCRβ repertoire diversity decreased with age in HCs.[25] The reasons for the decreasing trend of TCR repertoire diversity with aging can be explained by the following: (i) decreased supply of naïve T cells from the thymus because of thymic senescence, (ii) reduction in the number of naïve T cells through telomere attrition, and (iii) decreased numbers of naïve T cells because of age-related DNA damage.[25–27] The finding that age-related changes are seen in TCRα/β/δ/γ diversity, irrespective of affectation status, indicates that aging needs to be taken into account when TCR diversity is discussed in the context of MS.

Importantly, TCRα and TCRβ repertoire diversity were significantly greater in MS patients than in HCs, even after adjustment for age. This finding is in line with a previous study showing that MS patients had higher TCRβ diversity than control subjects.[13] Diseases caused by a single known pathogen, such as HTLV-1-associated myelopathy, demonstrate decreased TCR repertoire diversity;[13] therefore, MS, a disease with high TCR diversity, is less likely to be caused by any single infectious species. In patients with systemic autoimmune diseases, such as systemic lupus erythematosus[28] and rheumatoid arthritis,[29] TCR diversity is also lower than that in controls. Therefore, although MS is currently regarded as an autoimmune disease with unproven autoantigens,[30] MS is unique in terms of TCR diversity for a disease involving autoimmune processes.

TCR diversity was correlated with the percentage of naïve T cells, as determined by $CD45RA^{high}/CD27^{high}$ T cells.[25] In general, the naïve TCR pool in healthy individuals is mainly produced by the thymus,[26] whereas long-lived naïve T cells outside the thymus constitute a minor naïve TCR pool.[31] As thymic function declines with age, naïve T cells also decrease in number and variety, which

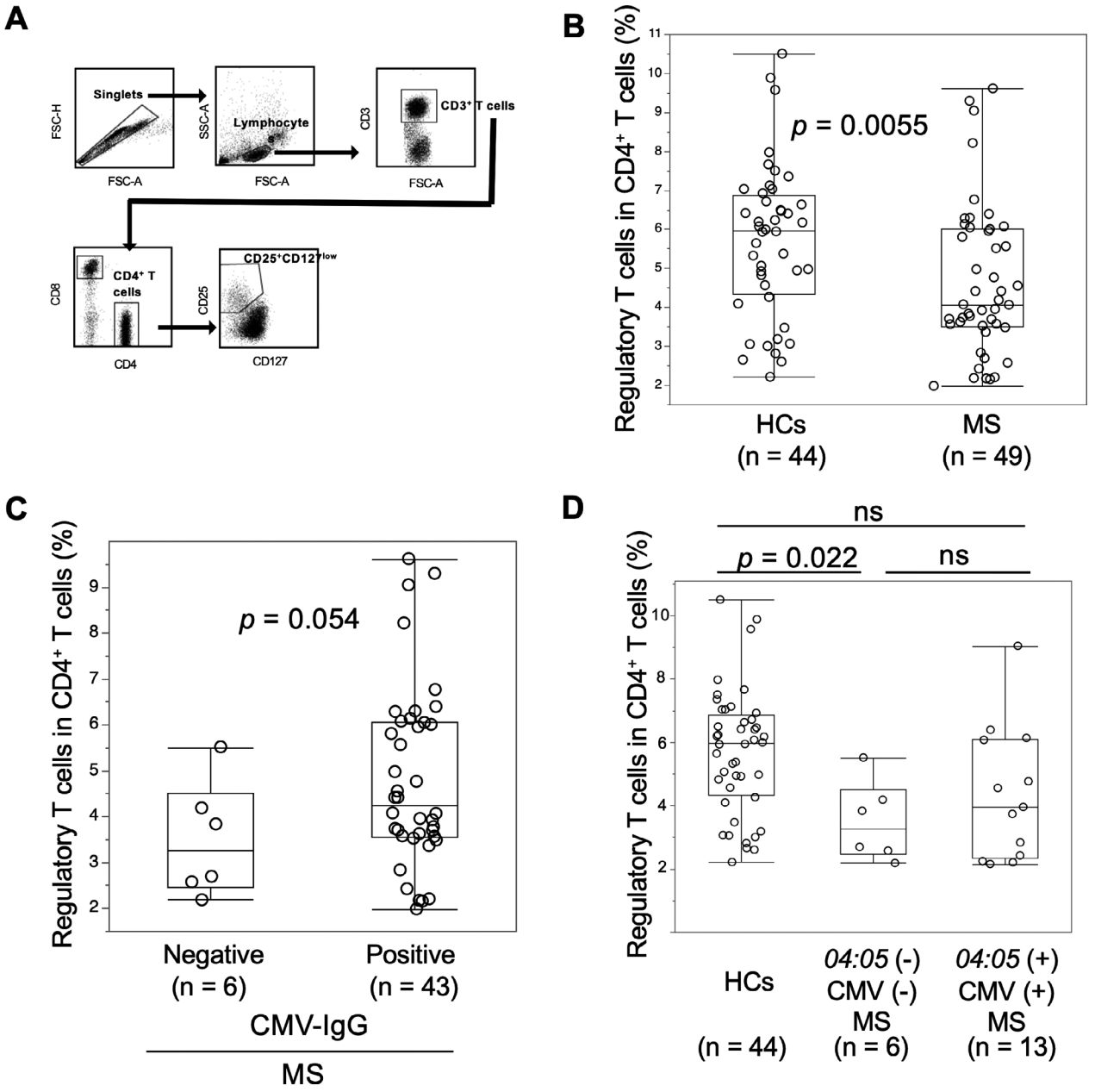

Figure 7. Immunophenotyping gating strategy for regulatory T cells (Tregs). A: Tregs were defined as $CD4^+CD25^+CD127^{low}$ based on the indicated gating strategy. B–D: The percentage of Tregs among $CD4^+$ T cells was compared between healthy controls (HCs) and multiple sclerosis (MS) patients (B), between MS patients with different cytomegalovirus (CMV)-IgG serostatus (C), and between HCs and MS patients carrying neither human leukocyte antigen (*HLA*)-*DRB1*04:05* nor CMV-IgG or MS patients carrying both *HLA-DRB1*04:05* and CMV-IgG (D). The *P*-values after multiple correction are provided. ns = not significant ($P > 0.1$).

result in reduced TCR diversity. Patients with MS are suggested to have a peripheral (nonthymic or postthymic) mechanism that enhances naïve T-cell homeostatic proliferation because of the lower levels of signal joint T-cell receptor excision circles, a marker of recent thymic emigrants, and higher expression levels of Ki-67 proliferation antigen in naïve T cells.[26] Therefore, we assume that the increased TCR diversity in MS, resulting from an increase in naïve T-cell species, could be caused by such nonthymic mechanisms.

We did not find a significant difference in TCRδ or TCRγ repertoire diversity in MS patients compared with

HCs. Thus, the peripheral mechanism may not involve γδ T cells. Alternatively, the Shannon index, which we used as an index of diversity, shows a larger value as the number of TCR species increases. Because TCRα and TCRβ have overwhelmingly larger numbers of TCR species compared with TCRδ and TCRγ (in the order of 10^{14} vs. $10^{12–13}$), the values and ranges of the Shannon index for TCRα and TCRβ become higher than those for TCRδ and TCRγ. Thus, the statistical power may not be sufficient in terms of TCRδ and TCRγ repertoire diversity to reach significant differences.

Despite the increased TCR diversity and limited differences in TCR V and J gene usage between MS patients and HCs, we successfully identified disease-specific TCRs that were significantly enriched in MS patients by GLIPH. One of the two TCR clusters corresponding to increased use of *TRBV4-3* in MS was highly shared by *HLA-DRB1*04:05*-positive MS patients and was predicted to recognize the CMV pp65 peptide. CMV pp65, one of the most abundantly expressed viral proteins, has a critical role for entry into the latent infection phase through phosphorylation of viral immediate early gene products, which prevent recognition of infected cells by cytotoxic T cells.[32] The relationship between MS and CMV has long been controversial, ranging from CMV being a risk factor for MS to it being protective.[1,33–37] Few previous studies have taken the *HLA* genetic background into account when assessing the relationship between MS and CMV. Although one study reported that *HLA* class I molecules, especially *HLA-B7*, determine the T-cell repertoire against CMV,[38] the relationship between CMV and *HLA* class II genotype remains unclear. However, some studies found no associations between *HLA-DRB1*15:01* or *HLA-DRB1*15:03* positivity and CMV-IgG titers.[39,40] Our study is the first to show that a particular virus, CMV, plays a protective role in MS patients with a certain *HLA* class II allele, *HLA-DRB1*04:05*, which confers a humoral immune response to the virus. In MS, *HLA-DRB1*15:01* was reported to potentiate a humoral immune response to Epstein–Barr virus,[39] which is known to increase MS susceptibility.[41] Thus, it appears to be important to take the *HLA* background into account when investigating the role of a particular virus in MS.

The significant expansion of CMV-TCR in *HLA-DRB1*04:05*-positive patients even in the absence of active viral replication suggests that the immune response to CMV differs between *HLA-DRB1*04:05*-positive and *HLA-DRB1*04:05*-negative MS patients. It is also possible that some host peptides with molecular mimicry to CMV pp65 may partly facilitate the expansion of T cells bearing CMV-TCR. We revealed that *HLA-DRB1*04:05* increased the CMV-IgG titer, but did not affect the CMV-IgG positivity rate, which indicates that *HLA-DRB1*04:05* only influences the humoral immune response to CMV, and not susceptibility to the virus. The humoral immune response to certain viruses, such as JC virus, is well known to change according to the *HLA* allele.[42–44] CMV itself exerts immunosuppressive effects in hosts to promote virus survival.[45,46] In a latent infection, CMV is known to use IL-10-producing Tregs to prevent latently infected cells from being recognized by the immune system.[47,48] Treg% was decreased in MS patients in the present study, in accordance with a previous study,[49] but was partially restored in CMV-IgG-positive, *HLA-DRB1*04:05*-positive MS patients. CMV infection induces Tregs that downregulate CMV-specific as well as non-specific immune responses in CMV-infected humans and in a mouse model.[23,24,50] It is therefore possible that CMV infection protects against disability progression by counteracting the decrease in Tregs inherent to MS.[49] Our cohort did not contain a sufficient number of MS patients who were *HLA-DRB1*04:05*-negative but CMV-IgG-positive to enable robust statistical significance to be achieved in the Treg assay. Therefore, it remains uncertain whether the partial Treg restoration by CMV is unique to *HLA-DRB1*04:05*-positive MS patients. Alternatively, an $NKG2C^+$ subset of natural killer (NK) cells may expand in acute CMV infection and remain overrepresented in seroconverted individuals with latent infection.[51–56] In some CMV-infected MS patients, increased numbers of $NKG2C^+$ NK cells were associated with lower disability in early MS.[54] The beneficial effects of daclizumab, an anti-CD25 monoclonal antibody drug for MS, are attributed to increased numbers of circulating NK cells;[57] therefore, NK cells, including $NKG2C^+$ NK cells, may contribute to slower disability progression in CMV-infected MS patients with *HLA-DRB1*04:05*.

Unfortunately, no information is available for the candidate antigens recognized by the other TCR (using the CDR3 cluster; CASSSRQGLQPRHF) that was enriched in *HLA-DRB1*04:05*-positive MS patients. Apparently, this unknown-TCR cluster was not associated with CMV-IgG positivity, CMV-IgG titer, or Treg%. The cluster was restricted to *TRBV6-6*, which was also predominantly expressed on T cells in the peripheral blood of patients with psoriasis compared with HCs.[58] As bioinformatics tools develop, the possible antigens for this CDR3 cluster and the mechanisms by which these antigens act in MS pathogenesis may be revealed in the future.

Several limitations require consideration. First, the TCR repertoire was analyzed using whole peripheral blood mononuclear cells and the phenotype of CMV-TCR-bearing cells was not characterized. Second, the T-cell response to CMV was not compared between CMV-TCR-positive and CMV-TCR-negative MS patients, and should be determined in future studies. Finally, the

dynamics of NKG2C$^+$ NK cells were not assessed in our cohort and it remains unclear whether NKG2C$^+$ NK cells as well as Tregs contribute to the milder disease course in CMV-IgG-positive MS patients with *HLA-DRB1*04:05*. In the future, the percentages of NKG2C$^+$ NK cells should also be examined according to *HLA-DRB1*04:05* status in MS patients with and without CMV infection.

In conclusion, higher TCR diversity is inherent in MS irrespective of age. The GLIPH identification of enriched TCRs recognizing CMV epitopes in MS patients with risk allele *HLA-DRB1*04:05* indicates viral modulation of the disease course, which underscores the usefulness of this methodology.

Acknowledgments

This study was supported by grants from the Japan Society for the Promotion of Science [Grant Nos. 16H02657 (to J.K.), 16K09694 (to K.M), 18K07529 (to N.I.), 19H01045 (to J.K.), 19K07995 (to M.W.), and 19K07997 (to T.M., M.W., and N.I.)], and a Health and Labour Sciences Research Grant on Intractable Diseases [H29-Nanchitou (Nan)-Ippan-043 and 20FC1030 (to J.K.)] from the Ministry of Health, Labour, and Welfare, Japan. We thank Edanz Group (https://en-author-services.edanzgroup.com/ac) for editing a draft of this manuscript.

Authors' contributions

F.H., N.I., and J.K. conceived the experiments. All authors contributed to the experimental design. F.H., N.I., G.M., and M.W. performed the experiments. F.H. and N.I. analyzed the results. J.G. and T.M. provided technical advice for the analyses. F.H., N.I., J.G., and J.K. were involved in the interpretation of the results. F.H., N.I., and J.K. drafted the manuscript. All authors reviewed the manuscript.

Conflicts of Interest

N.I. received grant support from Mitsubishi Tanabe Pharma, Osoegawa Neurology Clinic, Bayer Yakuhin Ltd., and Japan Blood Products Organization. T.M. received speaker honoraria payments from Takeda Pharmaceutical Company and Biogen Japan. M.W. received speaker honoraria and consultant fees from Novartis Pharma. J.K. is a consultant for Biogen Japan and Medical Review, and has received honoraria from Bayer Healthcare, Mitsubishi Tanabe Pharma, Nobelpharma, Otsuka Pharmaceutical, Sanofi K.K., Chugai Pharmaceutical Co. Ltd., Teijin Pharma, Novartis Pharma, and Medical Review.

References

1. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nat Rev Neurol 2017;13:25–36.
2. Piccolo L, Kumar G, Nakashima I, et al. Multiple sclerosis in Japan appears to be a milder disease compared to the UK. J Neurol 2015;262:831–836.
3. Nakashima I, Fujihara K, Okita N, et al. Clinical and MRI study of brain stem and cerebellar involvement in Japanese patients with multiple sclerosis. J Neurol Neurosurg Psychiatry 1999;67:153–157.
4. Nakamura Y, Matsushita T, Sato S, et al. Latitude and HLA-DRB1*04:05 independently influence disease severity in Japanese multiple sclerosis: a cross-sectional study. J Neuroinflammation 2016;13:239.
5. Briggs FBS, Yu JC, Davis MF, et al. Multiple sclerosis risk factors contribute to onset heterogeneity. Mult Scler Relat Disord 2019;28:11–16.
6. Yoshimura S, Isobe N, Matsushita T, et al. Genetic and infectious profiles influence cerebrospinal fluid IgG abnormality in Japanese multiple sclerosis patients. PLoS One 2014;9:e95367.
7. Shinoda K, Matsushita T, Nakamura Y, et al. HLA-DRB1*04:05 allele is associated with intracortical lesions on three-dimensional double inversion recovery images in Japanese patients with multiple sclerosis. Mult Scler J 2018;24:710–720.
8. Hohlfeld R, Dornmair K, Meinl E, Wekerle H. The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research. Lancet Neurol 2016;15:317–331.
9. Dornmair K, Goebels N, Weltzien HU, et al. T-cell-mediated autoimmunity: Novel techniques to characterize autoreactive T-cell receptors. Am J Pathol 2003;163:1215–1226.
10. Calis JJA, Rosenberg BR. Characterizing immune repertoires by high throughput sequencing: strategies and applications. Trends Immunol 2014;35:581–590.
11. Greiff V, Miho E, Menzel U, Reddy ST. Bioinformatic and statistical analysis of adaptive immune repertoires. Trends Immunol 2015;36:738–749.
12. Miho E, Yermanos A, Weber CR, et al. Computational strategies for dissecting the high-dimensional complexity of adaptive immune repertoires. Front Immunol 2018;9:224.
13. de Alves Sousa A, Johnson KR, Ohayon J, et al. Comprehensive Analysis of TCR-β Repertoire in Patients with Neurological Immune-mediated Disorders. Sci Rep 2019;9:344.
14. Glanville J, Huang H, Nau A, et al. Identifying specificity groups in the T cell receptor repertoire. Nature 2017;547:94–98.

15. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann Neurol 2011;69:292–302.
16. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology 1983;33:1444–1452.
17. Roxburgh RHSR, Seaman SR, Masterman T, et al. Multiple Sclerosis Severity Score: Using disability and disease duration to rate disease severity. Neurology 2005;64:1144–1151.
18. Ryuji S, Tadasu S. T cell receptor and B cell receptor repertoire analysis system, and use of same in treatment and diagnosis. Pat Appl Publ 2016;US2016/0289760.
19. Kitaura K, Shini T, Matsutani T, Suzuki R. A new high-throughput sequencing method for determining diversity and similarity of T cell receptor (TCR) α and β repertoires and identifying potential new invariant TCR α chains. BMC Immunol 2016;17:1–16.
20. Shugay M, Bagaev DV, Zvyagin IV, et al. VDJdb: a curated database of T-cell receptor sequences with known antigen specificity. Nucleic Acids Res 2018;46:D419–D427.
21. Maimaitijiang G, Shinoda K, Nakamura Y, et al. Association of decreased percentage of Vδ2+Vγ9+ γδ T cells with disease severity in multiple sclerosis. Front Immunol 2018;9:748.
22. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003;4:330–336.
23. Almanan M, Raynor J, Sholl A, et al. Tissue-specific control of latent CMV reactivation by regulatory T cells. PLoS Pathog 2017;13:1–22.
24. Tovar-Salazar A, Weinberg A. Cytomegalovirus infection in HIV-infected and uninfected individuals is characterized by circulating regulatory T cells of unconstrained antigenic specificity. PLoS One 2017;12:1–14.
25. Britanova OV, Putintseva EV, Shugay M, et al. Age-related decrease in TCR repertoire diversity measured with deep and normalized sequence profiling. J Immunol 2014;192:2689–2698.
26. Duszczyszyn DA, Beck JD, Antel J, et al. Altered naive CD4 and CD8 T cell homeostasis in patients with relapsing-remitting multiple sclerosis: Thymic versus peripheral (non-thymic) mechanisms. Clin Exp Immunol 2006;143:305–313.
27. Goronzy JJ, Fang F, Cavanagh MM, et al. Naive T cell maintenance and function in human aging. J Immunol 2015;194:4073–4080.
28. Thapa DR, Tonikian R, Sun C, et al. Longitudinal analysis of peripheral blood T cell receptor diversity in patients with systemic lupus erythematosus by next-generation sequencing. Arthritis Res Ther 2015;17:1–12.
29. Liu X, Zhang W, Zhao M, et al. T cell receptor β repertoires as novel diagnostic markers for systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis 2019;78:1070–1078.
30. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol 2015;15:545–558.
31. Kohler S, Thiel A. Life after the thymus: CD31+ and CD31 human naive CD4+ T-cell subsets. Blood 2009;113:769–774.
32. Gilbert MJ, Riddell SR, Plachter B, Greenberg PD. Cytomegalovirus selectively blocks antigen processing and presentation of its immediate-early gene product. Nature 1996;383:720–722.
33. Milovanovic J, Popovic B, Milovanovic M, et al. Murine cytomegalovirus infection induces susceptibility to EAE in resistant BALB/c mice. Front Immunol 2017;8:192.
34. Vanheusden M, Broux B, Welten SPM, et al. Cytomegalovirus infection exacerbates autoimmune mediated neuroinflammation. Sci Rep 2017;7:1–11.
35. Sanadgol N, Ramroodi N, Ahmadi GA, et al. Prevalence of cytomegalovirus infection and its role in total immunoglobulin pattern in Iranian patients with different subtypes of multiple sclerosis. New Microbiol 2011;34:263–274.
36. Makhani N, Banwell B, Tellier R, et al. Viral exposures and MS outcome in a prospective cohort of children with acquired demyelination. Mult Scler J 2016;22:385–388.
37. Vanheusden M, Stinissen P, 't Hart BA, Hellings N. Cytomegalovirus: A culprit or protector in multiple sclerosis? Trends Mol Med 2015;21:16–23.
38. Emerson RO, DeWitt WS, Vignali M, et al. Immunosequencing identifies signatures of cytomegalovirus exposure history and HLA-mediated effects on the T cell repertoire. Nat Genet 2017;49:659–665.
39. Waubant E, Mowry EM, Krupp L, et al. Antibody response to common viruses and human leukocyte antigen-DRB1 in pediatric multiple sclerosis. Mult Scler J 2013;19:891–895.
40. Nourbakhsh B, Rutatangwa A, Waltz M, et al. Heterogeneity in association of remote herpesvirus infections and pediatric MS. Ann Clin Transl Neurol 2018;5:1222–1228.
41. Waubant E, Lucas R, Mowry E, et al. Environmental and genetic risk factors for MS: an integrated review. Ann Clin Transl Neurol 2019;6:1905–1922.
42. Sundqvist E, Buck D, Warnke C, et al. JC polyomavirus infection is strongly controlled by human leucocyte antigen class II variants. PLoS Pathog 2014;10:e1004084.
43. Jelcic I, Aly L, Binder TMC, et al. T cell epitope mapping of JC polyoma virus-encoded proteome reveals reduced T cell responses in HLA-DRB1*04:01+ donors. J Virol 2013;87:3393–3408.
44. Watanabe M, Nakamura Y, Isobe N, et al. Two susceptible HLA-DRB1 alleles for multiple sclerosis differentially regulate anti-JC virus antibody serostatus along with fingolimod. J Neuroinflammation 2020;17:1–10.

45. Söderberg-Nauclér C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? J Intern Med 2006;259:219–246.
46. Litjens NHR, van der Wagen L, Kuball J, Kwekkeboom J. Potential beneficial effects of cytomegalovirus infection after transplantation. Front Immunol 2018;9:389.
47. Humphreys IR, De Trez C, Kinkade A, et al. Cytomegalovirus exploits IL-10-mediated immune regulation in the salivary glands. J Exp Med 2007;204:1217–1225.
48. Jones M, Ladell K, Wynn KK, et al. IL-10 restricts memory T cell inflation during cytomegalovirus infection. J Immunol 2010;185:3583–3592.
49. Kouchaki E, Salehi M, Reza Sharif M, et al. Numerical status of CD4(+)CD25(+)FoxP3(+) and CD8(+)CD28(-) regulatory T cells in multiple sclerosis. Iran J Basic Med Sci 2014;17:250–255.
50. Tovar-Salazar A, Patterson-Bartlett J, Jesser R, Weinberg A. Regulatory function of cytomegalovirus-specific CD4+CD27−CD28− T cells. Virology 2010;398:158–167.
51. Trautmann L, Rimbert M, Echasserieau K, Saulquin X. Selection of T cell clones expressing high-affinity public TCRs within human cytomegalovirus-specific CD8 T cell responses. J Immunol 2005;175:6123–6132.
52. Day EK, Carmichael AJ, ten Berge IJM, et al. Rapid CD8 + T cell repertoire focusing and selection of high-affinity clones into memory following primary infection with a persistent human virus: human cytomegalovirus. J Immunol 2007;179:3203–3213.
53. López-Botet M, Muntasell A, Vilches C. The CD94/NKG2C+ NK-cell subset on the edge of innate and adaptive immunity to human cytomegalovirus infection. Semin Immunol 2014;26:145–151.
54. Martínez-Rodríguez JE, Cobo-Calvo A, Villar LM, et al. Adaptive natural killer cell response to cytomegalovirus and disability progression in multiple sclerosis. Mult Scler 2016;22:741–752.
55. Alari-Pahissa E, Moreira A, Zabalza A, et al. Low cytomegalovirus seroprevalence in early multiple sclerosis: a case for the 'hygiene hypothesis'? Eur J Neurol 2018;25:925–933.
56. Luo XH, Meng Q, Rao M, et al. The impact of inflationary cytomegalovirus-specific memory T cells on anti-tumour immune responses in patients with cancer. Immunol 2018;155:294–308.
57. Baldassari LE, Rose JW. Daclizumab: development, clinical trials, and practical aspects of use in multiple sclerosis. Neurotherapeutics 2017;14:842–858.
58. Yin G, Li X, Li J, et al. Screening of differentially expressed genes and predominant expression of β variable region of T cell receptor in peripheral T cells of psoriatic patients. Eur J Dermatology 2011;21:938–944.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Comparison of *TRAV* usage in the case-control cohort.
Table S2. Comparison of *TRAJ* usage in the case-control cohort
Table S3. Comparison of *TRBV* usage in the case-control cohort.
Table S4. Comparison of *TRBJ* usage in the case-control cohort.
Table S5. Comparison of *TRDV/J* usage in the case-control cohort.
Table S6. Comparison of *TRGV/J* usage in the case-control cohort.
Table S7. List of CDR3 motifs categorized in the CMV-TCR cluster with carrier frequencies in each affectation status.
Table S8. List of study participants with numbers of unique CDR3 motifs included in CMV-TCR and total read counts for carriers of CDR3 motifs in CMV-TCR.
Table S9. CMV-IgG positivity rate in MS patients and HCs according to *HLA-DRB1*04:05* and CMV-TCR status.
Table S10. CMV-TCR positivity rate in MS patients according to CMV-IgG and *HLA-DRB1*04:05* status.
Table S11. Clinical characteristics of MS patients with distinct CMV-TCR positivity.
Table S12. Demographic and clinical characteristics of the replication cohort.
Table S13. Multivariate analysis between CMV-IgG positivity and MSSS in *HLA-DRB1*04:05*-positive MS patients.
Table S14. Comparison of CMV-IgG positivity and CMV-IgG titer by affectation status and relationship of CMV-IgG positivity with MS susceptibility.
Table S15. Immunological characteristics of MS patients with different usage of treatments.
Table S16. Immunological characteristics of MS patients with distinct positivity for the identified TCR cluster with unknown target antigens (unknown-TCR).
Figure S1. Distribution of mean T-cell receptor (TCR) diversity indexes obtained by 1,000 times bootstrapping of samples from multiple sclerosis (MS) patients.

