ID No.	K2102
Project Title	Multiple sclerosis: analysis of the T and B cell receptor repertoire in Mexican patients
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Report

The aim of this project is to analyze the T and B cell receptor repertoire (TCR and BCR) of patients with multiple sclerosis (MS) during relapse and remission using next generation sequencing (NGS).

Preparation of libraries and NGS were performed at the Japanese Foundation for Cancer Research during a research stay from October 1st to 31st, 2018. The study population incuded 15 MS patients during relapse, 10 of which were also analyzed during remission. In addition, 6 patients with other inflammatory neurological diseases (OIND) were included as controls. For BCR repertoire analysis, RNA from peripheral blood mononuclear cells (PBMC) of each patient was used for the synthesis of cDNA and the subsequent generation of heavy and light chain (kappa and lambda) libraries. TCR repertoire analysis was performed through the amplification of the TCR beta chain from total PBMC. Additionally, for 4 patients, we separated subpopulations of $CD4^+$, $CD8^+$ and $CD25^+$ using magnetic beads coupled to specific antibodies, and amplified both alpha and beta chains of the TCR). Sequencing was performed in an Illumina MiSeq system with a MiSeq reagent v2 kit (300 cycles).

A second research stay was scheduled from January 25 to February 17 to acquire the skills for BCR and TCR sequencing data analysis. In a very fruitful stay, students learned how to apply Tcrip and Bcrip pipelines from the data obtained from Mexican patients to reference TCR and BCR sequences from IMGT (www.imgt.org). Thus, the clonotypes of each patient was obtained. In addition, the diversity index (DI) of unique combinations of V (D) J within CDR3 sequences was calculated from the inverse of the Simpson diversity index.

Preliminary results of BCR and TCR repertoire analysis

- Diversity Index

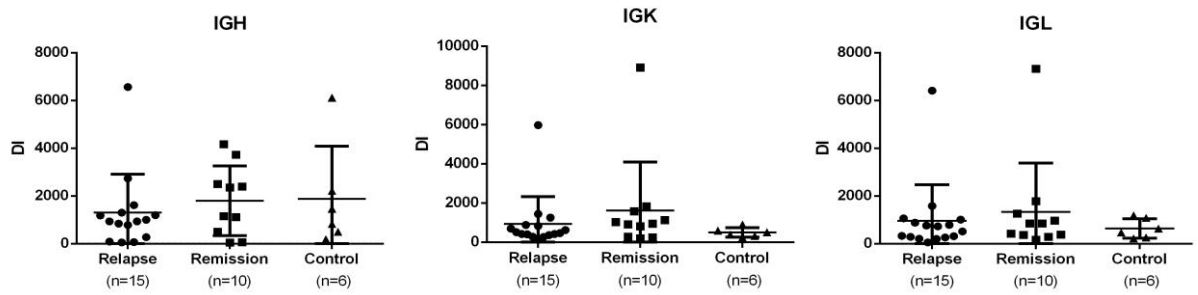

Fig. 1. Diversity index (DI) of IGH, IGK and IGL. A Mann-Whitney test did not reveal significant differences between the three groups.

- Shared clonotypes

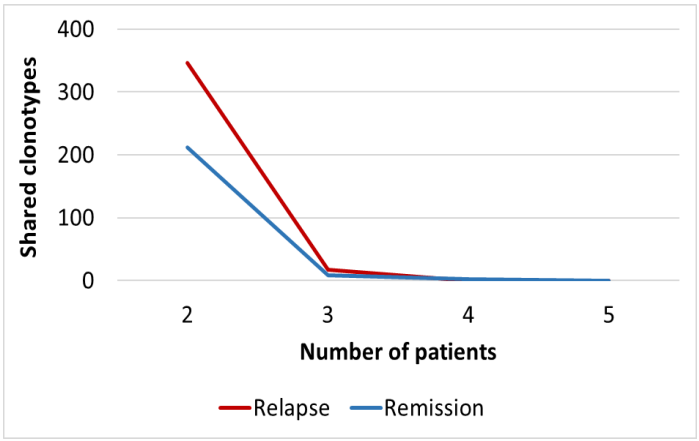

Fig. 2. Shared IGH clonotypes at relapse and remission of MS patients. Since patients during remission showed greater diversity, a higher probability of finding shared clonotypes between two or more patients was expected. Interestingly, shared clonotypes were more abundant among patients during relapse.

- IGHV gene usage

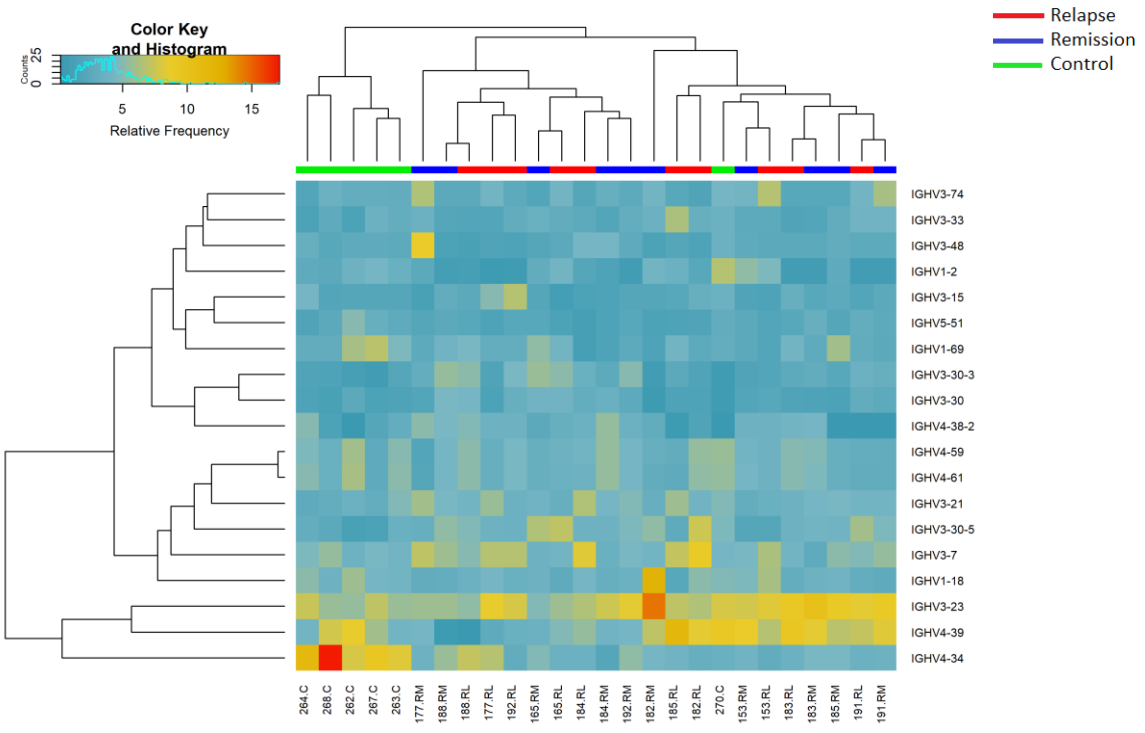

Fig. 3. IGHV gene usage. According to the V-segment use profile, controls were mainly clustered with a higher predominance the IGHV4-34 gene. MS patients, however, tended to favor usage of IGHV3-23 and, less frequently, IGHV4-39 genes.

- Diversity Index

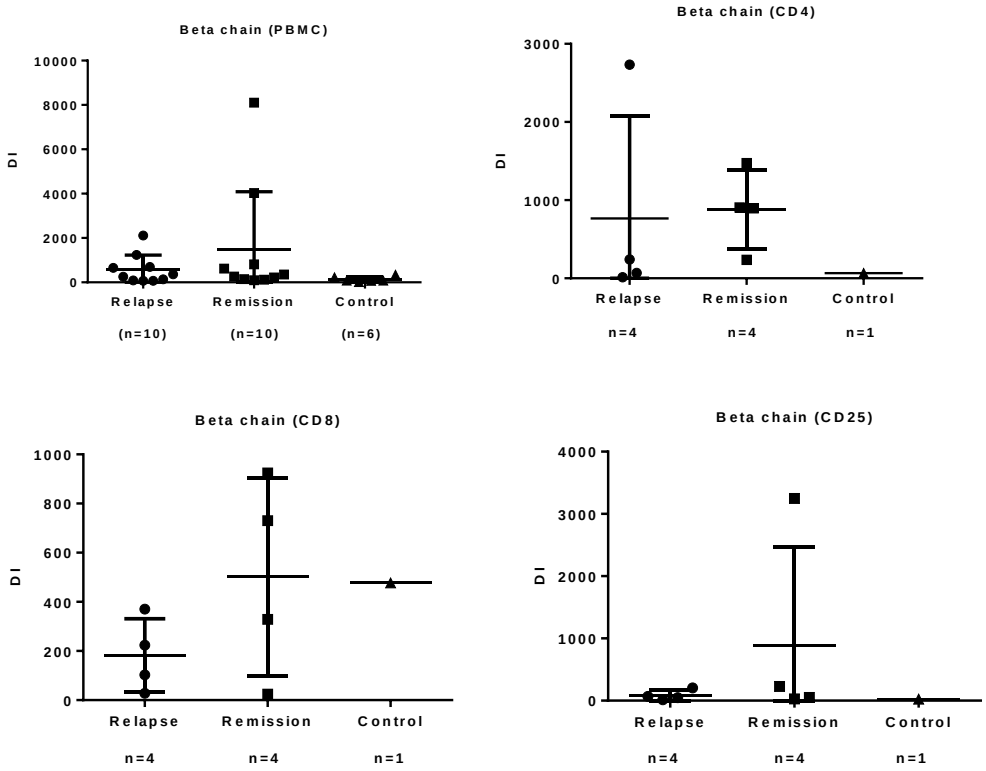

Fig. 4. Diversity index (DI) of PBMC, $CD4^+$, $CD8^+$ and $CD25^+$ Beta chain. A lower diversity index tended to be observed in the relapse phase of MS patients compared to remission and controls, but this did not reach statistical significance according to the Mann-Whitney test.

- Shared clonotypes

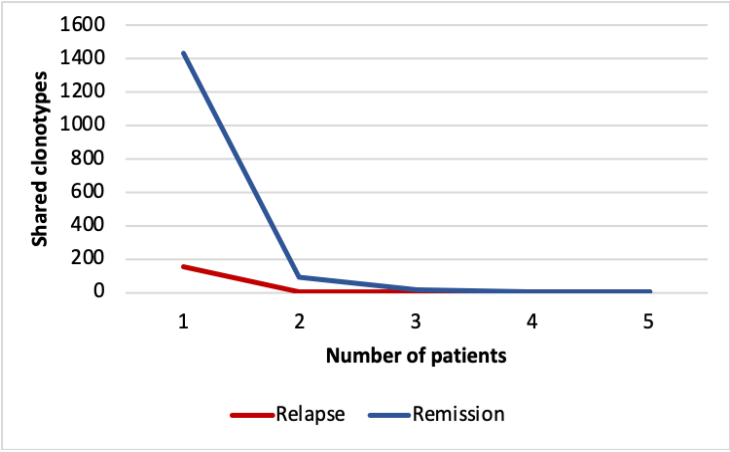

Fig. 5. TCR shared clonotypes between relapse and remission phases. Contrary to the results obtained with the BCR repertoire, when TCRs were analyzed a greater number of clonotypes were shared in the remission phase, which showed also a higher diversity index.

- Sequence logos.

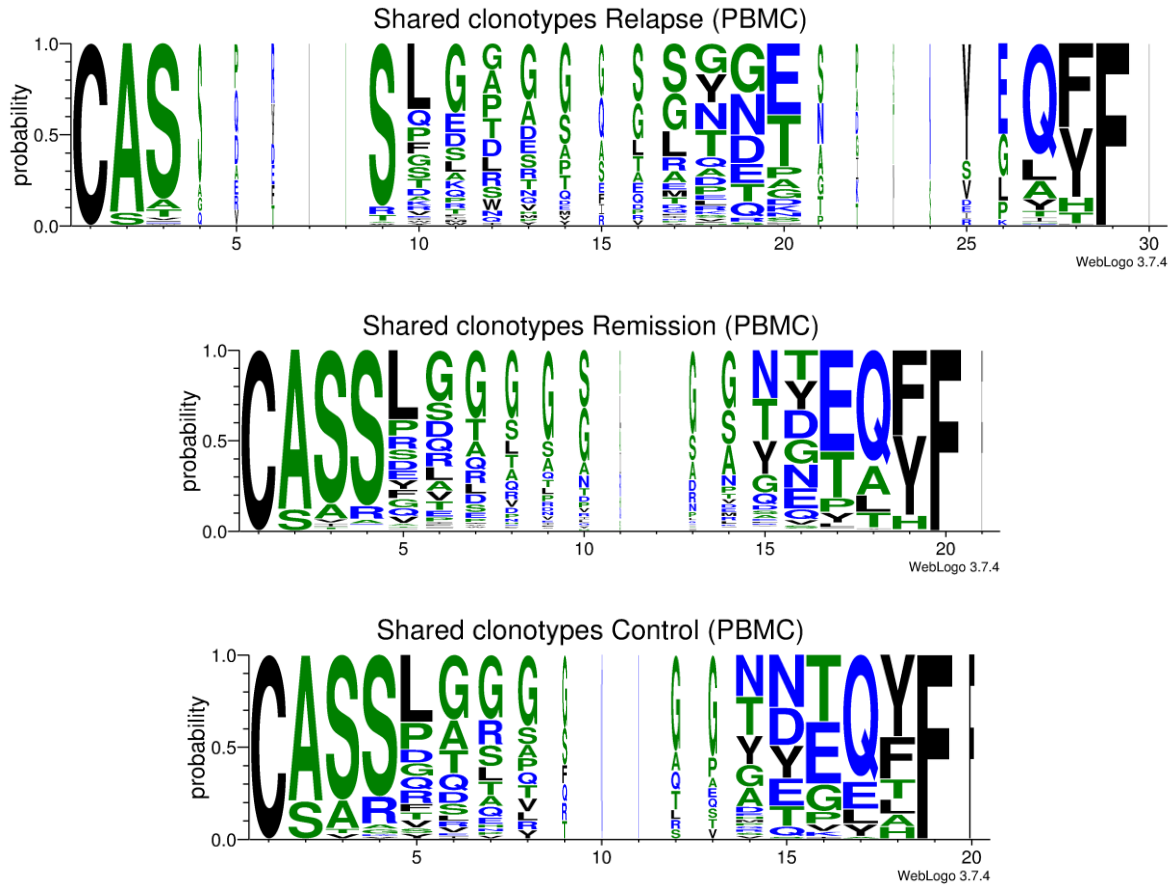

Fig 6. CDR3 sequence alignment. Logos from shared clonotypes between phases were observed. Similar logos were found in the remission phase and controls in terms of length and amino acid position, while the CDR3 in relapse phase was longer and showed different distribution of amino acids.

- TRBV gene usage

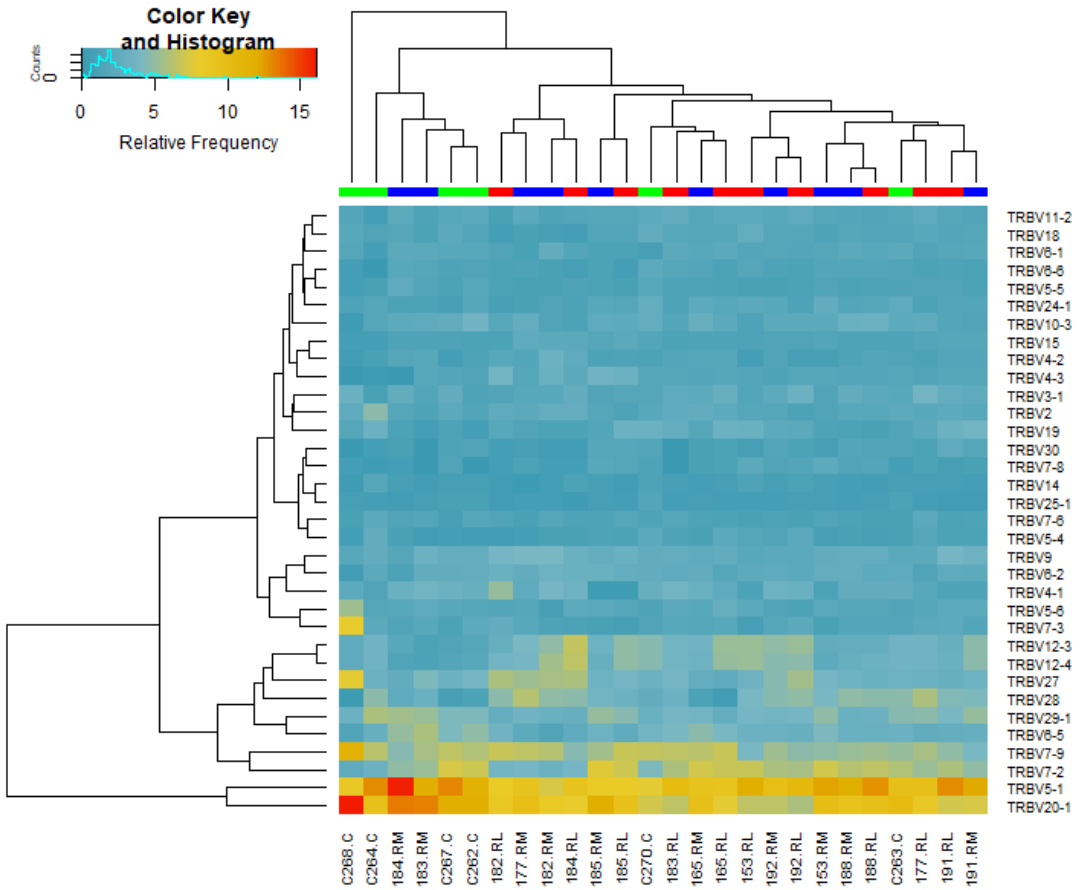

Fig. 7. TRBV gene usage. For both MS patients and controls, a predominance of TRBV20-1 and TRBV5-1 gene usage was observed.

Preliminary conclusions

BCR:

A clonal expansion in the BCR repertoire of MS patients during relapse is suggested

IGHV3-23 gene usage was identified as predominant in Mexican patients with MS, which could serve as a molecular marker in the future.

TCR:

A higher diversity index was observed in MS patients in the remission compared to the relapse phase in PBMCs and T cell subpopulations

According to the logos analysis, clonotypes present in the remission phase are similar to those of control patients, while those observed during relapse differ in length and composition, suggesting the participation of these clonotypes in the acute phase of the disease

From these preliminary results, we can conclude that there is no common BCR or TCR clonotype among MS patients, neither during relapse nor during remission, indicating that the response of each patient is activated by a different antigen. Therefore, the next step in this study is to identify predominant clonotypes in each patient and model the peptide that it may recognize.