

MOLECULAR CHARACTERIZATION OF CSF B CELL RESPONSE IN MULTIPLE SCLEROSIS

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INTRODUCTION

There is increasing evidence that B cells may play an important pathogenic role in multiple sclerosis (MS). Oligoclonal band analysis has not helped to define the underlying basis for the B cell response in MS. Molecular comparative analysis of single CSF B cells by its highly specific nature allows for identification of clonally expanded B cells. If clonal expansion is shown to persist over time, it is plausible that further investigation of these select clones may help to identify important antigenic targets.

OBJECTIVE

To sequence analyze immunoglobulin heavy and light chain variable regions from single B cells from MS CSF, and to characterize and investigate clonal expansion and clonal persistence over time.

DESIGN AND METHODS

Cerebrospinal fluid (CSF) was obtained from 36 clinically definite MS patients with informed consent and IRB approval. After centrifugation, B cells were stained with anti-CD19 and anti-CD138 antibodies and FACS sorted into single cell well plates. After cell lysis and reverse transcription, nested polymerase chain reactions (PCR) were performed using heavy and light chain primers. Amplified products were cloned and sequence analyzed. Identical clones were noted. Repeat CSF samples and analysis, enabled the identification of persistent clones. The variable heavy and light chain regions of identical and persistent clones were expressed as a recombinant antibody for screening against neural and viral antigens.

RESULTS

CSF B-cell analysis showed that clonal expansion was present in 16 of 36 patients (44%). However, due to various factors such as low CSF B cell numbers, sort efficiency, PCR success rate, and limitations on CSF volumes; the extent of clonal expansion is probably underestimated. Using a minimum criteria of 5 detectable heavy or 5 detectable light chain sequences to eliminate samples due to these factors, 16 of 21 patients (76%) show clonal expansion. Setting this criteria however, may be biasing results toward patients with a more B cell pathology.

In one patient, we have identified a clonally expanded and persistent clone. This clonal expansion and persistence has been established over a course of 10 months. In the patient's first CSF sample (A1577), 4 of 6 B cells from the first analysis (Figure 1) were clonally identical. This result also validates our technique. Cells that show clonal expansion (2, 4, 6, and 8) in the heavy chain also show clonal expansion of the light chain. This is indicative of single cell analysis.

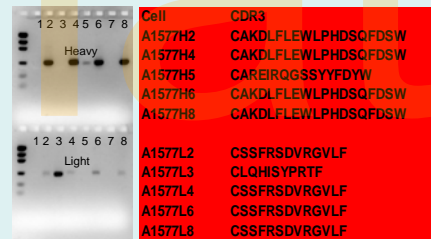


Figure 1: (left) Heavy and light variable chain nested PCR results from 1st CSF sample. Heavy chains were detected in cells 2, 4, 5, 6, and 8. Light chains were detected in cells 2, 3, 4, 6, and 8. (right) Sequence results show identical heavy and light CDR3 variable regions in cells 2, 4, 6, and 8; indicative of clonal expansion.

In a subsequent CSF sample (D1645) from the same patient, 22 cells with detectable variable chains were sequenced analyzed (Table 1). Of these 22 cells, 16 cells show clonal relationship to the initial expanded cell from A1577. Nine cells had identical heavy and light chain sequences while 7 cells show replacement mutations in the CDR3 regions; perhaps indicative of affinity maturation. However, VDJ and VJ rearrangements in the heavy and light chains show all cells has identical rearrangements and therefore were all clonally related to the initial A1577 clone.

In another subsequent CSF sample (C1849) from the same patient 10 months from the initial sample, the same identical clone was detected in 14 of 38 cells (sequence data not shown). No clones with replacement mutations were detected.

Table 2 shows a summary of this clonally persistent clone spanning a period of 10 months.

Table 1: (left) Heavy CDR3 variable chain sequences from 2nd CSF sample. Eighteen heavy chains were detected. (right) Light CDR3 variable chains from 2nd CSF sample. Nineteen light chains were detected. Replacement mutations in clonally related cells are highlighted in yellow.

Cell	CDR3	Cell	CDR3
D1645_H1	CAKDLFLEWLPHDSQFDSW	D1645_L1	CSSFRSDVRGVLF
D1645_H3	TYRPLTNCYNIS'DFKSPGLRIPW	D1645_L2	CQQYGGSPPTVF
D1645_H4	CAKDLFLEWLPHDS'FDSW	D1645_L4	CSSFRSDVRGVLF
D1645_H5	CAKDLFLEWLPHDSQFDSW	D1645_L5	CSSFRSDVRGVLF
D1645_H6	CAKDLFLEWLPH'CSQFDSW	D1645_L6	CSSFR'CD'RGVLF
D1645_H7	CAKDLFLEWLPHDSQFDSW	D1645_L7	CSSFRSDVRGVLF
D1645_H8	CAKDLFLEWLPH'CSQFDSW	D1645_L8	CSSFR'CD'RGVLF
D1645_H10	CAKDLFLEWLPHDSQFDSW	D1645_L10	CSSFRSDVRGVLF
D1645_H11	CARARAYFDSASVHYRPL'HCDFW	D1645_L11	CQQYGGSPPTVF
D1645_H13	CAKDLFLEWLPH'CSQFDSW	D1645_L12	CQQYGGSPPTVF
D1645_H14	CAKDLFLEWLPH'CSQFDSW	D1645_L13	CSSFR'CD'RGVLF
D1645_H16	CAKDLFLEWLPH'CSQFDSW	D1645_L14	CSSFR'CD'RGVLF
D1645_H17	CAKDLFLEWLPHDSQFDSW	D1645_L15	CQQYS'LPWTF
D1645_H18	CAKDLFLEWLPHDSQFDSW	D1645_L17	CSSFRSDVRGVLF
D1645_H19	CAKDLFLEWLPHDSQFDSW	D1645_L18	CSSFRSDVRGVLF
D1645_H20	CAKDLFLEWLPHDSQFDSW	D1645_L19	CSSFRSDVRGVLF
D1645_H21	CAKDLFLEWLPH'CSQFDSW	D1645_L20	CSSFRSDVRGVLF
D1645_H23	CAKDLFLEWLPHDSQFDSW	D1645_L23	CSSFRSDVRGVLF
		D1645_L24	GSSYAGNNLLF

Table 2: Summary of the persistent clone identified in a MS patient. Clonal persistence was detected over a period of 10 months.

Sample	CDR3	V-GENE	D-GENE	J-GENE	Frequency
Oct-07	H:CAKDLFLEWLPHDSQFDSW	IGHV3-30*03	IGHD3-3*01	IGHJ5*01(a)	4/6 Total 4/6
	L:CSSFRSDVRGVLF	IGLV2-23*01		IGLJ2*01	
Dec-07	H:CAKDLFLEWLPHDSQFDSW	IGHV3-30*03	IGHD3-3*01	IGHJ5*01(b)	9/22 1/22 6/22 Total 16/22
	L:CSSFRSDVRGVLF	IGLV2-23*01		IGLJ2*01	
	H:CAKDLFLEWLPHDS'FDSW	IGHV3-30*03	IGHD3-3*01	IGHJ5*01(b)	
	L:CSSFRSDVRGVLF	IGLV2-23*01		IGLJ2*01	
Jul-08	H:CAKDLFLEWLPHDSQFDSW	IGHV3-30*03	IGHD3-3*01	IGHJ5*01(b)	14/38 Total 14/38
	L:CSSFRSDVRGVLF	IGLV2-23*01		IGLJ2*01	

We have expressed this expanded and persistent clone as a recombinant IgG₁ antibody. Current and future studies are aimed at finding its reactive antigen. Candidate antigens to screen against include brain and spinal cord lysates, Epstein-Barr viral lysates and primary brain cell lysates. Immunohistochemistry studies will also be performed to determine the antibody's reactivity against MS brain lesions. Brain and spinal cord cDNA libraries will also be screened using this antibody for potential antigenic targets.

CONCLUSIONS

We have established by single cell molecular analysis that clonal expansion is present in the B cell response seen in MS patients. We have also detected a clonal expanded and persistent clone in one MS patient. This suggests that the antibody response is antigen driven and not a bystander event. Current and future antigenic screening studies may further elucidate the basis of the antibody response in MS.