The intrathecal syntheses of CXCL13, BAFF and IgGOB probably reflect different phases of B-cell activation.

A study in multiple sclerosis at clinical onset.

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Background. Several lines of evidence suggest that B cells play a central role in the pathogenesis and/or the clinical evolution of multiple sclerosis (MS). The identification of meningeal FLS has stimulated the hypothesis of a 'germinal centre reaction' within the CNS of MS.

Objective. We studied serum and CSF levels of soluble factors involved in naïve B-cell recruitment (CXCL13), in follicular reaction (IL 21) and intrathecal B cell survival (BAFF) in MS patients at clinical onset and correlated their intrathecal synthesis with local Ig production and with magnetic resonance imaging (MRI) parameters of white and grey matter damage.

Materials an Methods. Paired serum and CSF were obtained from 40 patients with CIS/eRRMS and 18 healthy controls (HC). Routine examination of CSF and serum included Intrathecal IgG synthesis evaluation by means of quantitative formulae (IgG Index, IgG Hyp. Function for IgG intrathecal synthesis fraction (IgGIF) and Local Production (IgGLoc)) and demonstration of IgG oligoclonal bands (IgGOB). CXCL13, IL 21 and BAFF were detected by means of a highly sensitive ELISA. Cytokine ratio (CSF-cytokine/serum-cytokine, $Q_{cytokine}$) and Indexes ($Q_{cytokine}/Q_{Alb}$, Cytokine Index) were evaluated. Global Cortical Thickness (gCTh) were also calculated on 3D-T1 sequences by means of Freesurfer.

Results. 1. Intrathecal cytokine and IgG synthesis in HC and CIS/eRRMS. The 40 CIS/eRRMS and the 17 controls did not differ in age and gender (p=0.4) (Table 1). Table 1 summarizes routine serum and CSF findings in HC and CIS/eRRMS for IgG synthesis. **CXCL13.** In HC, CXCL13 was found in all sera and, at very low levels (0.9±1.5 pg/mL), in 8/17 (47%) CSF, but no correlation was observed between QCXCL13 and QAlb (r: 0.2, p=0.4), even when only CXCL13-positive CSF were included in the analysis (r: 0.7, p=0.1). Since these data indicated that CXCL13 was intrathecally produced, rather than derived from passive filtration from blood, only the CSF concentrations of this cytokine were considered in further analyses. CXCL13 was detected more frequently (75%, p<0.05) and at higher concentration (19.7±29.3 pg/mL, p<0.05) in the CSF of CIS/eRRMS than HC. **IL 21.** Detectable IL 21 was found in all CSF (28.1±29.1 pg/mL) and in only one serum of HC, thus suggesting that CSF IL 21 was intrathecally produced. No difference in CSF IL 21 levels was observed between HC (28.1±29.1 pg/mL) and CIS/eRRMS (20.7±22.8 pg/mL, p=0.3). **BAFF.** BAFF was detected in all the sera and CSF of HC and CIS/eRRMS. In HC QBAFF mildly correlated to QAlb (r: 0.4, p=0.1), indicating that CSF BAFF may results from both choroid plexus filtration and active production within the CNS. The BAFF Index was lower in CIS/eRRMS (12.4±5.5 pg/mL) than in HC (17.5±5.2 pg/mL, p<0.005).

	HC	CIS/eRRMS
Gender (F/M)	13/4	26/14
Age at LP (y)	43.2 ± 9.2	37.8 ± 10.0
Disease Duration (y)	n.a.	0.5 ± 0.9
CSF [Alb] (mg/dL)	17.0 ± 5.1	22.5 ± 9.8
Serum [Alb] (mg/dL)	4370.6 ± 379.0	4260.0 ± 333.6
Q_{alb} (10^{-3})	3.9 ± 1.2	5.3 ± 2.3*
CSF [IgG] (mg/dL)	2.2 ± 0.9	3.7 ± 1.8***
Serum [IgG] (mg/dL)	1141.2 ± 215.2	1042.0 ± 197.7
Q_{IgG} (10^{-3})	1.9 ± 0.6	3.6 ± 1.7****
IgG Index	0.5 ± 0.1	0.7 ± 0.3***
IgG Loc (mg/dL)	0.1 ± 0.3	5.9 ± 10.2*
IgIF (%)	0 ± 0	11 ± 17*
IgGOB (%)	0%	65%****
Leucocytes count (/µL)	2.1 ± 1.1	6.9 ± 7.7*

Table 1. Standard serum and CSF parameters in HC and CIS/eRRMS. *:<0.5; ** p<0.01; *** p<0.005; **** p<0.001

3. BOIgG-based stratification of CIS/eRRMS . Based on the presence of IgGOB in the CSF, CIS/eRRMS patients were divided in IgGOB positive (IgGOB+, 26 patients) and in IgGOB negative (IgGOB-, 14 patients) (Table 2). CSF CXCL13 concentrations were higher in IgGOB+ compared to both IgGOB- (p<0.05) and HC (p<0.005) (Figure 1B). No difference in CSF IL 21 levels was observed between HC, IgGOB- and IgGOB+ (Figure 1C). As expected on the base of previous results, CSF BAFF levels and Index were significantly lower in IgGOB+ (50.7±20.5 pg/ml, p<0.05, and 11.9±6.1, p<0.005, respectively) but not in IgGOB- (69.9 ± 27.3 pg/ml and 13.2 ± 5.1, respectively) compared to HC (Figure 1D).

	HC	IgGOB-	IgGOB+
Gender (F/M)	13/4	9/5	17/9
Age at LP (y)	43.2 ± 9.2	37.2 ± 10.7	38.2 ± 9.7
Disease Duration (y)	n.a.	0.3 ± 0.6	0.6 ± 1.1
csf-[Alb] (mg/dL)	17.0 ± 5.1	21.2 ± 8.7	23.1 ± 10.4
s-[Alb] (mg/dL)	4370.6 ± 379.0	4225.0 ± 297.7	4278.8 ± 355.6
Qalb (10^{-3})	3.9 ± 1.2	5.0 ± 2.0	5.4 ± 2.4*
csf-[IgG] (mg/dL)	2.2 ± 0.9	2.4 ± 0.8	4.5 ± 1.8**** °°°°
s-[IgG] (mg/dL)	1141.2 ± 215.2	944.6 ± 124.2**	1094.3 ± 211.6°
QIgG (10^{-3})	1.9 ± 0.6	2.6 ± 1.0	4.2 ± 1.8**** °°
IgG Index	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.3**** °°°
IgG Loc (mg/dL)	0.1 ± 0.3	0.2 ± 0.6	9.0 ± 11.5** °
IgIF (%)	0 ± 0	1.1 ± 2.7	16.4 ± 18.5*** °°
Leucocyte's count (/µL)	2.1 ± 1.1	2.9 ± 2.4	9.0 ± 8.6*** °
csf -[BAFF] (pg/mL)	67.3 ± 21.6	69.9 ± 27.3	50.7 ± 20.5* °
BAFF Index	17.5 ± 5.2	13.2 ± 5.1	11.9 ± 6.1**
csf-[CXCL13]	0.9 ± 1.5	5.0 ± 8.2	27.7 ± 33.5*** °
csf-[IL21] (pg/mL)	28.1 ± 29.1	21.6 ± 25.3	20.2 ± 21.9

Table 2. Serum and CSF parameters in HC, BOIgG- and BOIgG+ patients. BOIgG+ differed from both BOIgG- and GC for intrathecal IgG syntesis parametrs, CSF-CXCL13 concentrations and BAFF Index values. P-values compared to HC:*<0.5;**<0.01;***<0.005;****<0.001; p-values compared to IgGOB-:°<0.5;°°p<0.01;°°°p<0.005;°°°° p<0.001;

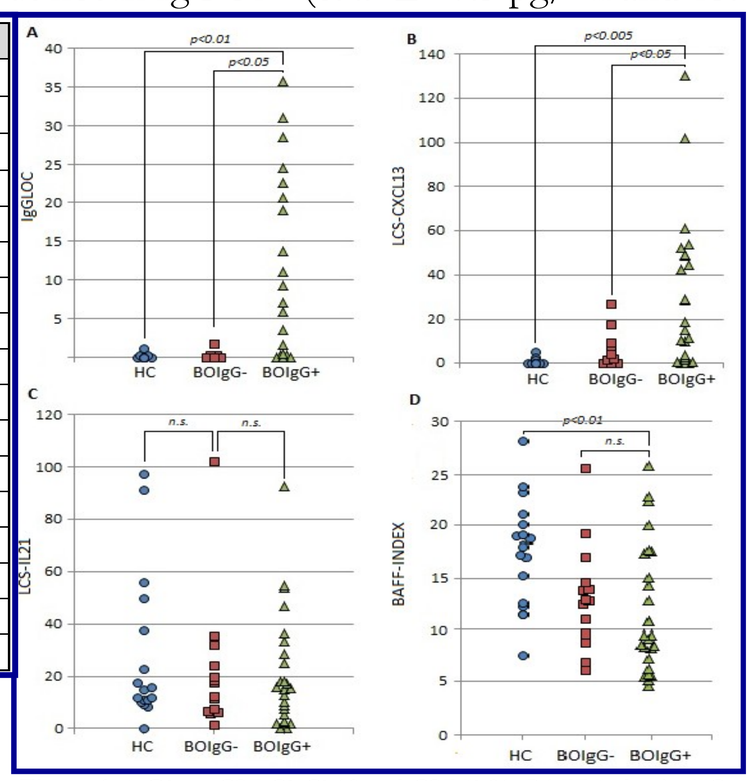

Figure 1. IgGLoc, CSF-CXCL13 and CSF-IL21, BAFF Index in HC, BOIgG- and BOIgG+ patients.

4. Correlation analysis. To avoid the influence of null-values on a direct correlation, (Table 3A), we considered only patients with quantitatively increased intrathecal IgG synthesis (Table 3B). In these 15 patients only BAFF Indexcorrelated to all these parameters.

A) All patients	BAFF-INDEX	LCS-CXCL13	B) only IS+	BAFF-INDEX	LCS-CXCL13
Index	-0,38	0,48 *	Index	-0,64 *	0,43
IgG Loc mg/dL	-0,41 *	0,59 ***	IgG Loc mg/dL	-0,56 *	0,57 *
IgIF (%)	-0,36	0,45 *	IgIF (%)	-0,52 *	0,35

Table 3 Correlation between intrathecal IgG synthesis parameters and BAFF-Index or CXCL13. The correlation was calculating on overall population of BOIgG+ patients (A) or including only patients with the presence of intrathecal IgG synthesis (IS+, B).

5. MRI parameters in CIS/eRRMS. To dichotomize CSF CXCL13, we identified a CXCL13 cut-off value based on HC CXCL13 CSF concentrations. This cut-off value was calculated as µ+4δ and resulted in 6.8 pg/mL. All HC and 20 CIS/eRRMS (50%, p<0.001) had CXCL13 values above this limit. CIS/eRRMS patients were divided into CXCL13- (below the cut-off value) and CXCL13+ (over the cut-off value). While no difference for any MRI parameter was observed between IgGOB+ and IgGOB-, CXCL13+ presented a thinning of gCTh (2.41±0.09mm) compared to CXCL13- (2.49±0.06mm, p<0.05, Figure 2).

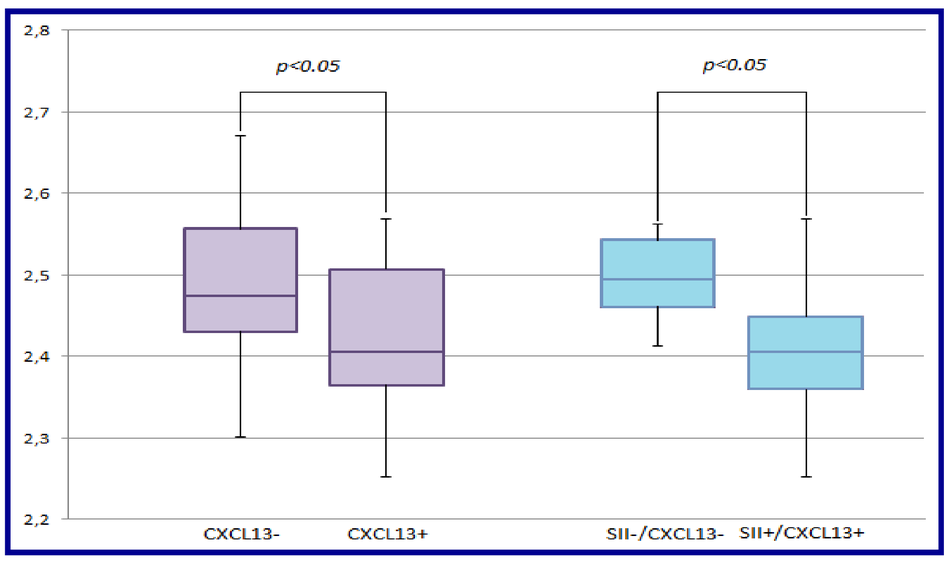

Figure 2. CTh in CIS/eRRMS.

Conclusions. Our study supports the hypothesis that CSF IgG are produced by peripheral PC that enter the CNS from the peripheral blood at a given time and then remain as long-term long-lasting PC. A role for FLS as source of intrathecal IgG seems unlikely, at least in early disease phases. Finally, our study seems to identify, at clinical onset, a subgroup of MS patients characterized by the association of an early B-cell recruitment in the CNS and evidence of cortical thinning.

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